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Ultra-Fast Total Petroleum Hydrocarbons (TPH) Analysis with Agilent Low Thermal Mass (LTM) GC and Simultaneous Dual-Tower Injection

Application Note

Environmental

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Abstract

This application note is targeted for ultra-high productivity of total petroleum hydrocarbons (TPH) analysis in environmental laboratories. Agilent's Low Thermal Mass (LTM) technology is employed here to perform ultra-fast gas chromatographic (GC) separations. The LTM technology uses a column module combining a fused silica capillary column with heating and temperature-sensing components wound around it, which can be heated and cooled very efficiently. In this application note, the speed of analysis for the hydrocarbon group eluting between C_{10} and C_{44} can be dramatically increased to about 13 times faster than a conventional method. In addition, the ultra-fast cooling function of an LTM module can reduce the total GC cycle time to 5.1 minutes. The simultaneous dual-tower injection from Agilent is used to further double productivity. The final result for TPH analysis productivity is 5.1 minutes per two samples.

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Introduction

Total petroleum hydrocarbons (TPH) is a term used to describe a large family of several hundred chemical compounds that originally came from crude oil. Many environmental laboratories in the world are analyzing the total amount of TPH at a site to evaluate the water or soil contamination by TPH, such as oil, gasoline, diesel fuel, etc.

The Agilent Low Thermal Mass (LTM) system (except for an external power supply) is built into a replacement GC oven door, which is mounted as an add-on to an Agilent 7890A GC. A version is also available for the Agilent 6890 GC. The key component of LTM system is the LTM column module combining a fused silica capillary column with heating and temperature-sensing components wound around it. The LTM system can heat and cool the column very efficiently for significantly shorter analytical cycle times as compared to conventional air bath GC oven techniques involving much higher thermal mass.

The GC method translation software from Agilent is a calculator used to scale a method between different column dimensions with equal or increased speed. In this application note, a 40-minute separation with a 30-meter column is translated into a 20-minute separation with a 15-meter column at first, without LTM technology. Then the method is further translated for LTM use with a 5-meter column within 3.1 minutes.

As a base for the LTM system, the Agilent 7890A can provide dual complete analysis channels. With a configuration of dual injection towers, single sample tray, dual split/splitless inlets, and dual detectors, the simultaneous TPH analysis can be accomplished to double lab productivity, in addition to the speed gains realized with LTM.

Experimental

Standard Preparation

The custom alkanes mix (cus-908) from Ultra Scientific (North Kingstown, Rhode Island, U.S.) contains n-alkanes from n-decane (C_{10}) to n-tetratetracontane (C_{44}) in hexane at the concentration listed in Table 1. Dilutions in dichloromethane are made up at 1.0, 5.0, 10.0, 50.0, and 100.0 µg/mL concentrations.

Table 1. Custom Alkanes Mix

Component	Concentration, mg/mL	Component	Concentration, mg/mL
n-decane	0.2	n-tetracosane	0.1
n-dodecane	0.1	n-hexacosane	0.1
n-tetradecane	0.2	n-octacosane	0.1
n-hexadecane	0.1	n-triacontane	0.1
n-octadecane	0.1	n-dotriacontane	0.1
n-eicosane	0.1	n-hexatriacontane	0.1
n-docosane	0.1	n-tetracontane	0.1
n-tricosane	0.2	n-tetratetracontane	0.1

Sample Preparation

Soil samples are mixed with sodium sulfate to remove excess moisture and then sonicated with 60-mL aliquots of dichloromethane, three times. Water samples are placed in a 2-L separate funnel. A 100-mL aliquot of dichloromethane is added and the mixture is shaken automatically for about 2 minutes. The liquid-liquid extraction is repeated two more times. For both matrices, the extract is concentrated on a steam bath to either 5 mL for a soil sample or 1 mL for a water sample. The extracts are not routinely treated with silica gel, unless specified.

Instrumentation and Conditions

Agilent 7890A GC with LTM system, consisting of:

G3440A	7890A Series GC system
#112	Split/splitless inlet with EPC (2)
#211	Capillary FID with EPC (2)
	Autoinjector modules (2)
	Autosampler tray module
G6579A	LTM system bundle for 2-channel LTM operation, for use with standard size LTM column modules (100–2000LTM DB-5 5 M × 0.32 mm id, 1.0 µm standard 5-inch LTM column module)

ChemStation 32-bit version B.04.01

Table 2. Gas Chromatograph Conditions

	Original 1X Method	2X Method	LTM Method
GC			
Agilent Technologies 7890A			
Inlet	EPC split/splitless	EPC split/splitless	EPC split/splitless
Mode	Constant pressure	Constant pressure	Ramp pressure
Injection type	Split	Split	Split
Injection volume (µL)	1.0	1.0	1.0
Inlet temp (°C)	300	300	300
Pressure, nominal (psig)	30	14.319	13.1 (0.1 min), 11.27 psi/min to 30 (1.5 min)
Liner	Helix liner, open ended, deactivated (p/n 5188-5396)	Helix liner, open ended, deactivated (p/n 5188-5396)	Helix liner, open ended, deactivated (p/n 5188-5396)
Split ratio	2:1	2:1	2:1
Gas saver	20 mL/min after 2 min	20 mL/min after 2 min	20 mL/min after 2 min
Gas type	Helium	Helium	Helium
Sample overlap	2 min after end of GC run	2 min after end of GC run	2 min after end of GC run
Oven	GC Oven	GC Oven	LTM module (p/n G6579A) with GC oven 300 °C for 3.1 min
Initial oven temp (°C)	40	40	40
Initial oven hold (min)	1	0.5	0.1
Ramp rate (°C/min)	10	20	200
Final temp (°C)	320	320	340
Final hold (min)	11	6.5	1.5
Run time (min)	40	21	3.1
Cooldown time (min)	5.4	5.4	2
Cycle time (min)	45.4	25.4	5
Column			
Type	DB-5 (p/n 123-5032)	DB-5 (p/n 123-5012)	DB-5 (p/n*)
Length (m)	30	15	5
Diameter (mm)	0.32	0.32	0.32
Film thickness (um)	0.25	0.25	1.0
FID			
Telperature (°C)	300	300	300
H_2 flow (mL/min)	30	30	30
Air flow (mL/min)	400	400	400
Makeup flow (mL/min)	25	25	25
Sampling rate (Hz)	50	50	50

*100–2000LTM DB-5 5M x 0.32 mm id, 1.0 µm standard 5-inch LTM column module

Results and Discussion

Ultra-Fast Separation of n-alkanes Mixture with LTM System and Scale-Up Using the GC Method Translator

The application is started with the analysis of a standard mixture of n-alkanes, containing n-C_{10}, n-C_{12}, up to n-C_{44}. Figure 1 compares the chromatogram of the standard mixture using three different methods in the same time scale. With the LTM system, the GC run time can be more than 10 times faster than conventional methods. In terms of cooling down, the classical GC oven such as 7890 fast oven will take about 5.4 minutes from 320 to 40 °C. Relatively, the LTM system has a much lower thermal mass, which can perform ultra-fast cooling. In this case, the LTM system will take about 2 minutes from 340 to 40 °C, for dual parallel LTM modules. In addition, sample overlap of the 7890 sample tray can prepare the sample after the end of the last GC run parallel with GC oven cooldown. The resulting cycle time for LTM is 5.1 minutes, which means about nine times faster than the conventional method.

Resolution is also a concern with fast analysis. Figure 2 is the expanded view of Figure 1 with the nominal time scale, which demonstrates that all the peaks of n-alkanes are baseline separated, even with the nine-times-faster LTM method (speed calculated by total cycle time). The result is calculated by total amount of TPH, not by the individual peak amount; peak-grouping of ChemStation is employed here. The calibration is checked by injecting the standard mixture in different concentration levels, ranging from 1 to 100 µg/mL. The calibration curve of the LTM method is displayed in Figure 3, with average n-alkanes response factor by peak-grouping.

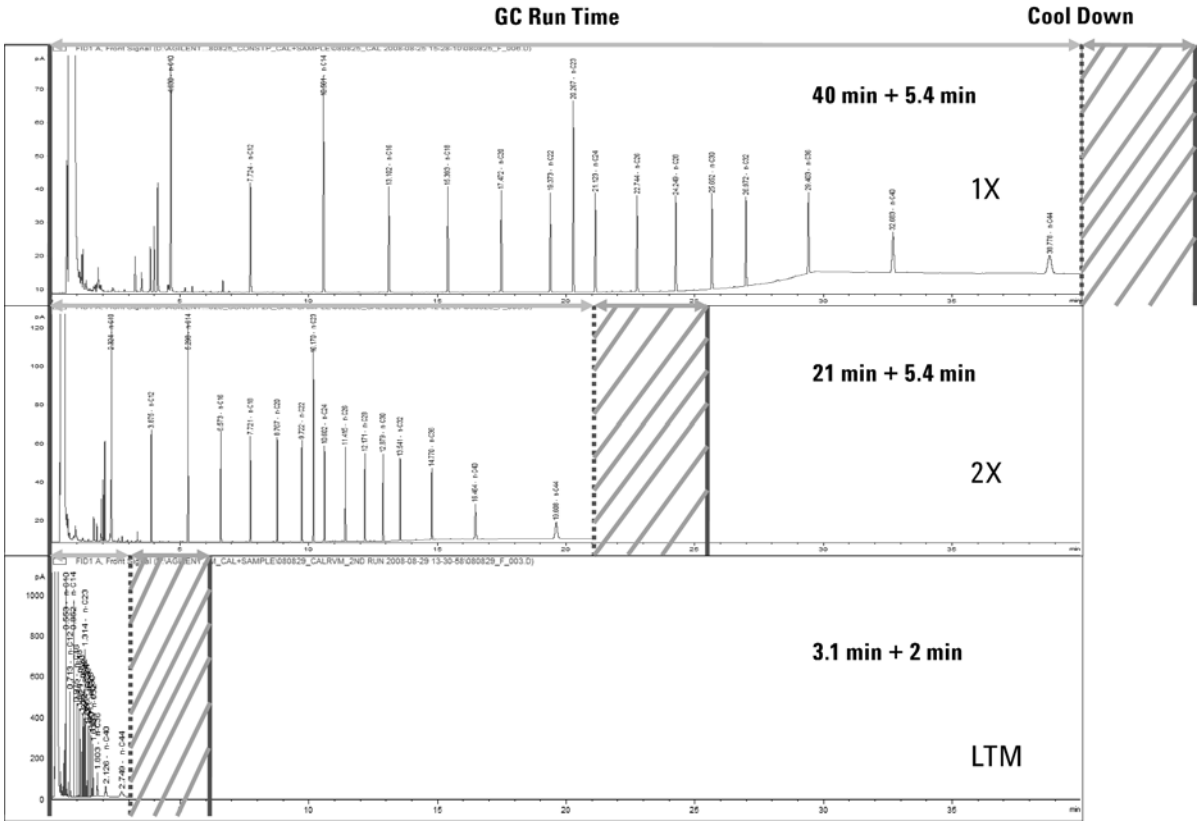

Figure 1. Comparison of conventional method and LTM method.

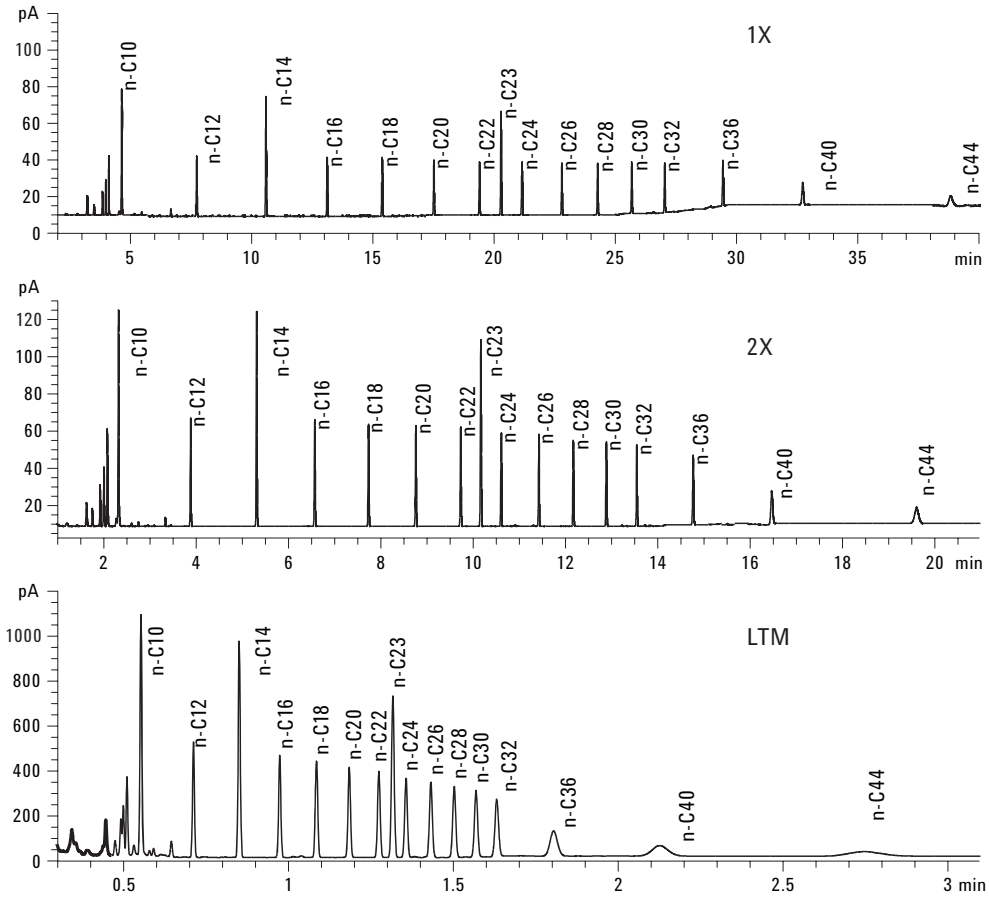

Figure 2. Expanded view of Figure 1, with the nominal time scale.

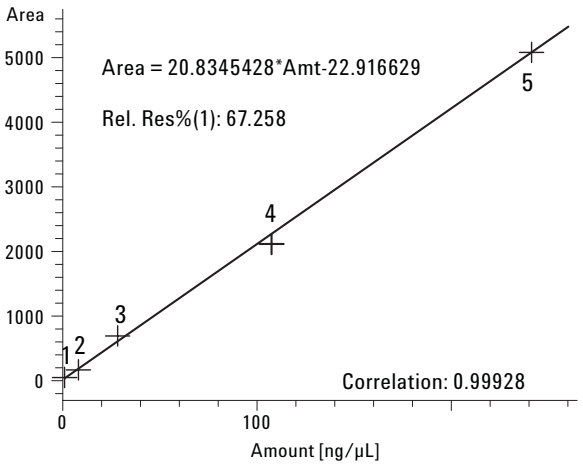

Figure 3. Calibration curve of LTM method after peak-grouping.

Simultaneous Dual-Channel Analysis with Agilent Dual-Tower Injection

Agilent 7890A and 6890 GCs make dual-channel analysis possible, with the configuration of a single sample tray and dual injection towers, inlets, columns, and detectors. Typically, a dual-channel configuration is used to identify target compounds in one GC run, using different retention time in columns of different polarity. The purpose here is to double lab productivity using dual identical channels at a much lower cost compared to two single-channel instruments. ChemStation can provide different choices for final data file generation. Figure 4 shows one option of detection signal setting for separating the dual-tower injection into two individual data files. Figure 5 is the chromatogram of two real samples with simultaneous dual-tower injection.

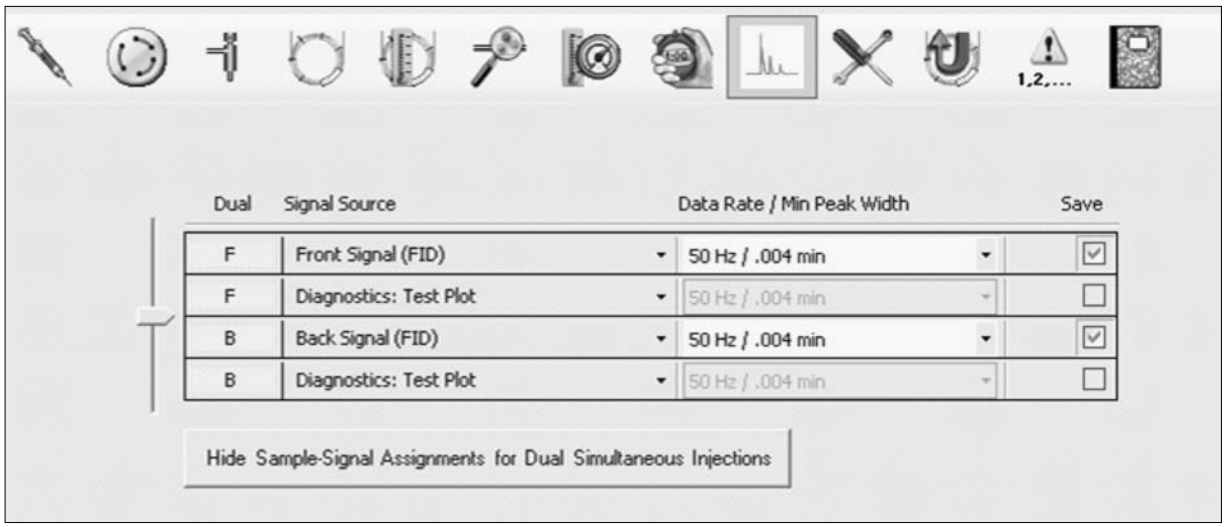

Figure 4. Signal setting for dual-tower injection to generate two individual data files.

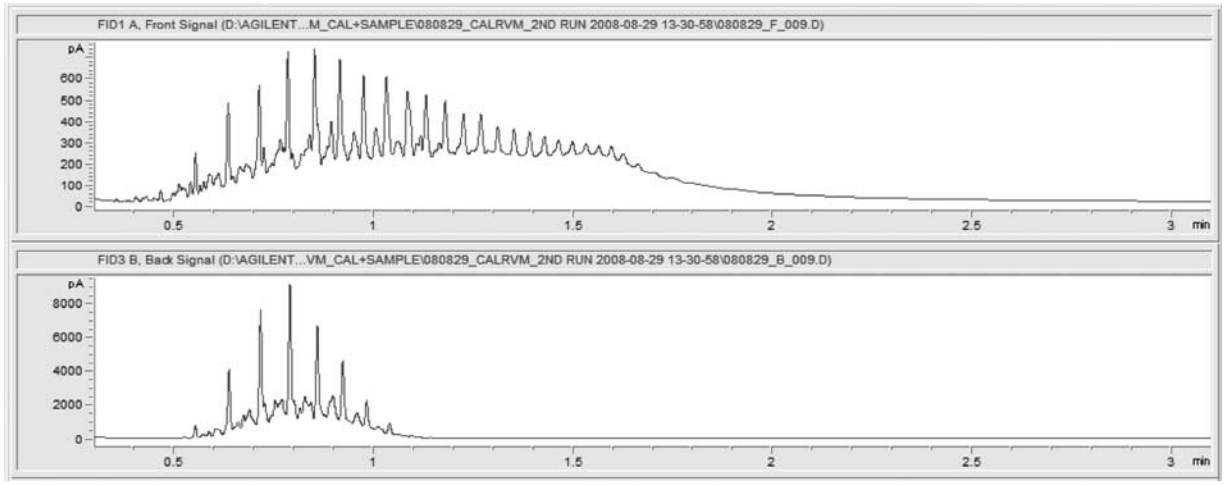

Figure 5. Chromatogram of two real samples with simultaneous dual-tower injection.

Quantitative Analysis of TPH with Peak-Grouping and Peak-Summing

Peak-grouping is used to average each n-alkane response factor. With this average response factor, the nominal calibration curve can be used for quantitation of each peak, including unidentified peaks eluting between n-C_{10} and n-C_{44}. In this case, the compound peak-grouping details and unidentified peak calibration settings can be seen in Figure 6.

Another requirement for TPH analysis is quantitation across the whole eluting time range between n-C_{10} and n-C_{44} to calculate all petroleum hydrocarbons not only n-alkanes. Baseline-holding and peak-summing in the ChemStation integration events table are necessary to meet this requirement; the related setting can be seen in Figure 7. For example, the integration result of real sample is shown in Figure 8.

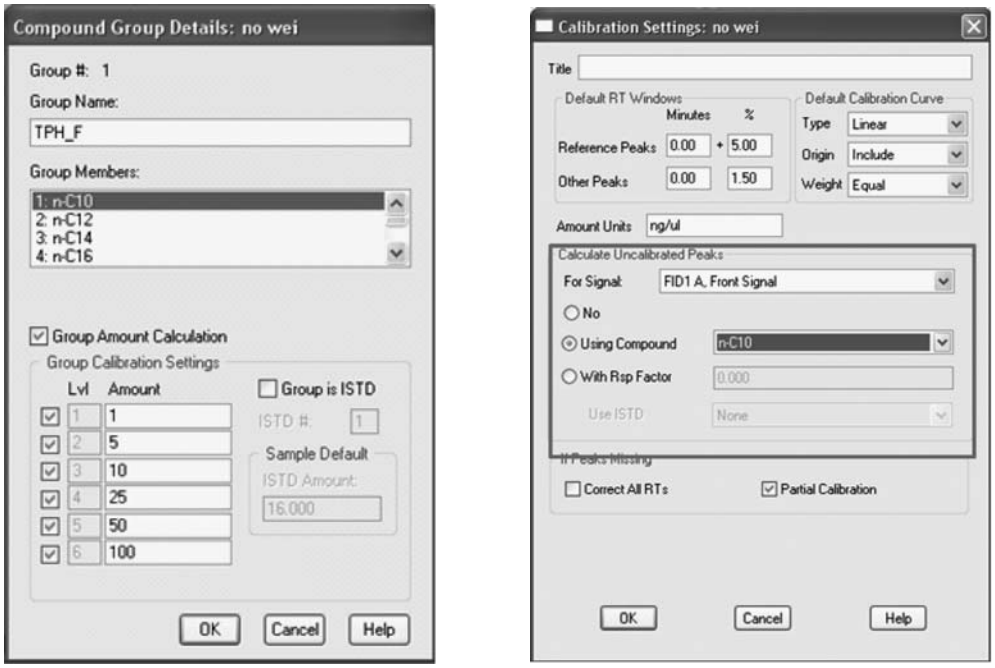

Figure 6. Peak-grouping (left) and unidentified peak calibration setting (right) in ChemStation.

Time	Integration Events	Value
Initial	Slope Sensitivity	10
Initial	Peak Width	0.04
Initial	Area Reject	1
Initial	Height Reject	1
Initial	Shoulders	OFF
0.000	Integration	OFF
4.560	Area Sum	ON
4.560	Baseline Hold	ON
4.560	Integration	ON
39.100	Integration	OFF
39.100	Area Sum	OFF

Figure 7. Baseline-holding and peak-summing setting in the ChemStation integration events table.

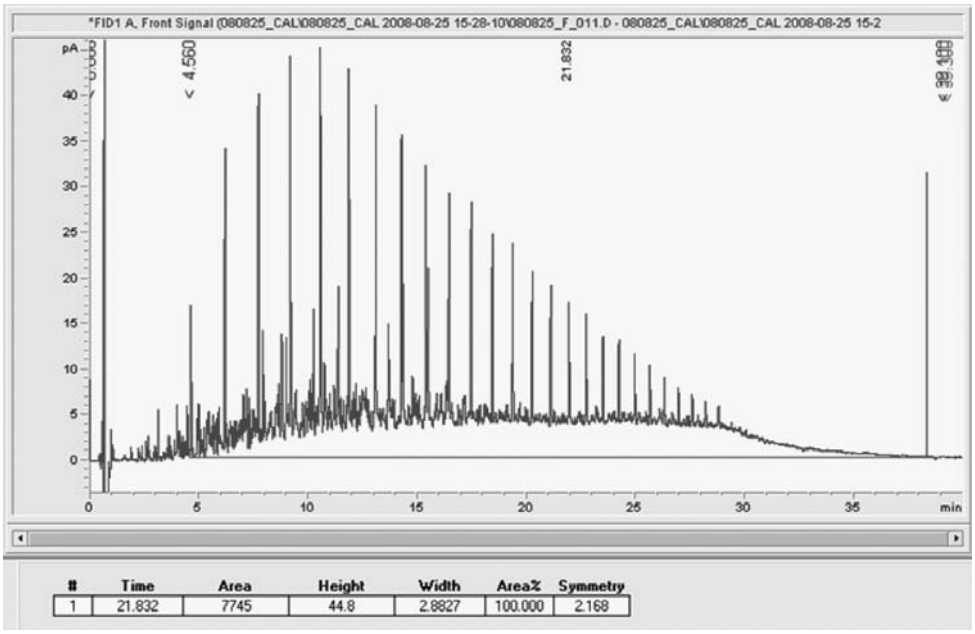

Figure 8. Integration result of real sample after baseline-holding and peak-summing.

Real Sample Analysis

After calibration by peak-grouping and integration through peak-summing, the quantitation result can be reported as the total amount of TPH in a real sample. As a comparison of quantitation results with three different acquired methods, Table 3 demonstrates that the real sample analysis result by the ultra-fast LTM method is comparable with conventional methods.

Table 3. Comparison of Quantitation Result with Three Different Acquired Methods

	TPH Concentration (µg/mL)
Original method (30 m)	1097
2X method (15 m)	920
LTM method (5 m)	909

Conclusions

The low thermal mass of the Agilent LTM system can perform very efficient column heating and cooling, and is used here to develop an ultra-fast TPH analysis to meet the requirement for high lab productivity. Dual-tower injection is also used to further double the productivity with much less cost. The final solution with the LTM system and dual-tower injection can perform TPH analyses at a rate of 5.1 minutes per two samples. The total productivity increase is 18x compared to a conventional analysis on a single-channel system.

References

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2. Wei Luan, Chuanhong Tu, and Michael Woodman, "Evaluation of Total Petroleum Hydrocarbon in Soil Using LC with Fraction Collector and GC/MS," Agilent Technologies publication 5989-6012EN, April 2007

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Published in the USA
November 18, 2008
5990-3201EN

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Chlorinated Solvents and Disinfection By-Product Analysis Using Agilent J&W HP-1ms Ultra Inert and DB-1301 Capillary GC Columns

Application Note

Environmental

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Abstract

Trace-level chlorinated hydrocarbon analyses using methods such as US EPA Method 551.1 are important tools for assessing organochlorine contamination in water. The wide diversity of target organochlorine compounds can prove chromatographically challenging due mainly to their high volatility and limited retention. This application note shows the benefits of using an Agilent J&W HP-1ms Ultra Inert Capillary GC column as the primary column for detection in this dual-column analysis.

Agilent Technologies

Introduction

The disinfection of water for safe human consumption is a critical process worldwide. Chlorination is an effective means of achieving water disinfection, but has been shown to produce a wide variety of disinfection byproducts (DBPs). These byproducts are formed when the chlorinated disinfectant reacts with naturally present organic matter. Some of the byproducts formed include trihalomethanes, haloacetonitriles, and chloropropanones. Many of the DBPs have been linked to adverse health effects, including birth defects, bladder and colon cancer [1–3]. Because of these health concerns, the levels of the by-products are monitored to ensure they are below safety standard limits.

US EPA Method 551.1 [4] is a commonly used method for detecting organochlorine compounds in water samples by GC/ECD. This method encompasses several classes of analytes: chlorinated organic solvents, trihalomethanes (THMs), haloacetonitriles, and other DBPs. The high volatility and limited retention of several of these analytes can prove problematic chromatographically. Reliable detection at very low levels is also a challenge for this analyte set. Active sites in the sample path can compromise an analyte's response. Minimizing activity in the GC column is essential to ensure accurate quantitation. Capillary GC column activity as a potential source of result uncertainty has been effectively eliminated with the Agilent J&W Ultra Inert series of columns.

Agilent Technologies, Inc. has implemented new testing procedures for the J&W Ultra Inert column series to more effectively evaluate GC column inertness performance. This testing procedure employs deliberately aggressive probes to thoroughly investigate column inertness performance on this new series of columns. These aggressive probes, including 1-propionic acid, 4-picoline, and trimethyl phosphate, are used to verify each column's inertness performance.

A standard preparation containing chlorinated solvents, THMs, and disinfection by-products (DBPs) was analyzed to evaluate column performance. This analysis used simultaneous primary and confirmation analysis from a single injection source through an Agilent Capillary Flow Technology two-way splitter without makeup device. The primary analysis column used was an Agilent J&W HP-1ms Ultra Inert 30 m × 0.25 mm × 1.0 µm and the confirmation column was an Agilent J&W DB-1301 30 m × 0.25 mm × 1.0 µm.

Experimental

An Agilent 7890A GC equipped with dual µECDs and an Agilent 7683B automatic liquid sampler was used for this series of experiments. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions for EPA Method 551.1 Calibration Standards

GC:	Agilent 7890A
Sampler:	Agilent 7683B, 5.0 µL syringe (Agilent p/n 5181-1273) 0.5 µL splitless injection
Carrier:	Helium 25 cm/s, constant flow
Inlet:	Splitless; 200 °C, Purge flow 20 mL/min at 0.25 min
Inlet liner:	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Retention gap:	1 m 0.32 mm id deactivated fused silica high-temperature tubing (Agilent p/n 160-2855-5)
Column 1:	Agilent J&W HP-1ms Ultra Inert 30 m × 0.25 mm × 1.0 µm (Agilent p/n 19091S-733UI)
Column 2:	Agilent J &W DB-1301 30 m × 0.25 mm × 1.0 µm (Agilent p/n 122-1333)
Oven:	33 °C (14 min) to 60 °C (5 °C/min), hold 5 min, 15 °C/min to 275 °C, hold 20 min
Detection:	Dual G2397A µECD; 300 °C, const col + makeup (N_2) = 30 mL/min

Table 2 Flow Path Supplies

CFT device:	Two-way splitter accessory without makeup gas (Agilent p/n G3181B)
	Alternative: Deactivated quartz y-splitter (Agilent p/n 5181-3398)
CFT fittings:	Internal nut (Agilent p/n G2855-20530)
	Swaging nut (Agilent p/n G2855-20555)
CFT ferrules:	SilTite ferrules, 0.32 mm id (Agilent p/n 5188-5362)
	SilTite ferrules, 0.25 mm id (Agilent p/n 5188-5361)
Vials:	Amber crimp cap glass vials (Agilent p/n 5183-4496)
Vial caps:	Crimp caps (Agilent p/n 5282-1210)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe:	5 µL (Agilent p/n 5181-1273)
Septum:	Advanced Green (Agilent p/n 5183-4759)
Inlet liners:	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
	0.5 mm id short; 85/15 Vespel/graphite (Agilent p/n 5062-3514)
20x magnifier:	20x magnifier Agilent p/n 430-1020)

Sample Preparation

EPA551.1 Standards

Two EPA551.1 standards containing chlorinated solvents, THMs, and DBPs were purchased from AccuStandard (New Haven, CT) and used to prepare a six-level calibration standard set. The stock solutions as delivered had a nominal concentration of 1000 µg/mL. The calibration standards were prepared at standard concentrations of 0.1, 0.05, 0.02, 0.01, 0.005, and 0.002 µg/mL. All solutions were prepared in MTBE using class A volumetric pipettes and flasks. MTBE used was Burdick and Jackson high-purity grade purchased thorough VWR International (West Chester, PA). MTBE was used as a reagent blank and syringe wash solvent.

Column Installation Using Two-Way Splitter Without Makeup Gas Capillary Flow Technology (CFT)

This analysis was performed using simultaneous confirmation from a single injector onto both the primary and confirmation columns. While a typical injector setup for dual column analysis uses a deactivated glass or quartz Y-splitter (Agilent p/n 5181-3398) to join the retention gap to the primary and confirmation columns, an Agilent Capillary Flow Technology two-way splitter without makeup gas (p/n G3181B) was employed. This device holds several advantages over the Y-splitter.

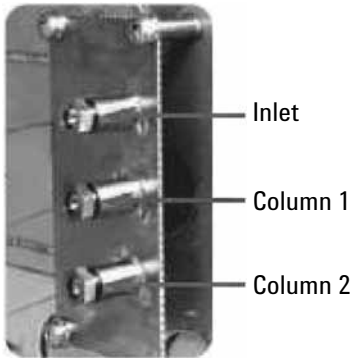
Correct assembly of a Y-splitter can be difficult, and detachment and/or leaks may occur upon thermal cycling of the oven. When using the Y-splitter, a periodic check of the connections is recommended. The Agilent CFT splitter uses SilTite metal ferrules that minimize the likelihood of leaks or detachment, even with thermal cycling as high as 350 °C. Installation of the retention gap and columns into the splitter module uses ferrules and internal nuts similar to a typical column installation. The CFT splitter is deactivated, yielding an inert sample path. The point-of-seal of the fittings design provides extremely low dead-volume column connections, improving optimal performance.

For this analysis, a 1 m, 0.32 mm id deactivated fused silica high-temperature tubing was installed into the inlet and into the top position of the two-way splitter. For the column connections to the splitter, the column end was threaded though the internal nut, SilTite ferrule, and swaging nut. The swaging nut was then tightened, seating the ferrule onto the column. Using a column cutter, the column end was trimmed to about 0.3 mm of column extending above the ferrule. The column was then connected to the two-way splitter. A diagram of the splitter and column setup is shown in Figure 1. Because the column connections are individually installed in the splitter, column maintenance can be done independent of the other column.

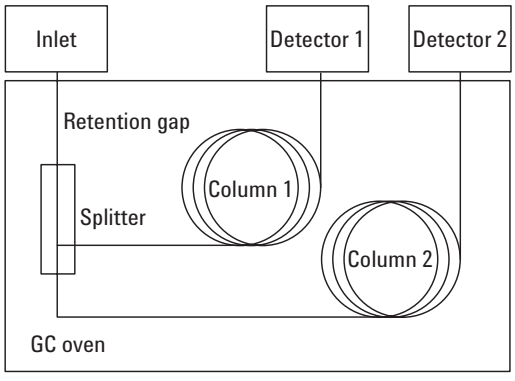
Results and Discussion

Baseline Inertness Profile for Ultra Inert Columns

The basic approach for inertness verification for the Agilent J&W Ultra Inert series of capillary GC columns is QC testing with aggressive active probes at low concentration and low temperature [5]. This is a rigorous approach that establishes



Two-way splitter without makeup gas connections



Plumbing diagram for the two-way splitter without makeup gas

Figure 1. Agilent Capillary Flow Technology two-way splitter without makeup gas (p/n G3181B) and diagram of instrument setup of simultaneous confirmation from a single injection onto both the primary and confirmation columns.

consistent baseline inertness profiles for each column. The baseline inertness profile then serves as a predictor for successful analysis of chemically active species that tend to adsorb onto active sites, particularly at trace level like the chlorinated species in this application example. Additional application examples can be found in references [6–10].

EPA 551.1 Analysis

In this application note a six-level calibration curve set was evaluated over the concentration range of 0.002 to 0.1 µg/mL using simultaneous confirmation of a single injection. A two-way splitter without makeup capillary flow device (p/n G3181B) was used in place of a y-splitter to split the sample onto the two columns. Figure 2 shows a chromatogram for the 5 pg on column loading from a single injection of the 551.1 standard on the primary and confirmation columns.

Excellent peak resolution and peak shape were obtained on both the J&W HP-1ms Ultra Inert and the J&W DB-1301 columns as shown in Figures 3 and 4. Chloral hydrate is unstable and, as is described in the EPA method, does not resolve as a discreet peak due to selectivity on a 1301 phase column. Figure 5 shows that chloral hydrate is well resolved and has symmetrical peak shape even at low levels on the J&W HP-1ms Ultra Inert primary column. One method criteria for primary column performance for this analysis is the resolution between bromodichloromethane and trichloroethylene. The acceptance criteria requires a resolution greater than 0.5 using the calculation described in the method. Figure 6 shows the resolution of bromodichloromethane and trichloroethylene in the 0.05 µg/mL EPA 551.1 standard on the J&W HP-1ms Ultra Inert primary analysis column. The resolution was found to be 0.787, well above the method criteria. This resolution was also determined at the lowest and highest level standards studied in this application. The resolution was 0.825 for the 0.002 µg/mL standard (0.5 pg on column) and 0.734 for the 0.1 µg/mL standard (25 pg on column) as can be seen in Figure 7.

Linearity was excellent across the range studied, giving R^2 values of 0.998 or greater for the chlorinated analytes on both the J&W HP-1ms Ultra Inert primary analysis column and also on the J&W DB-1301 confirmation column. Table 3 indicates the correlation coefficients for each component on both columns.

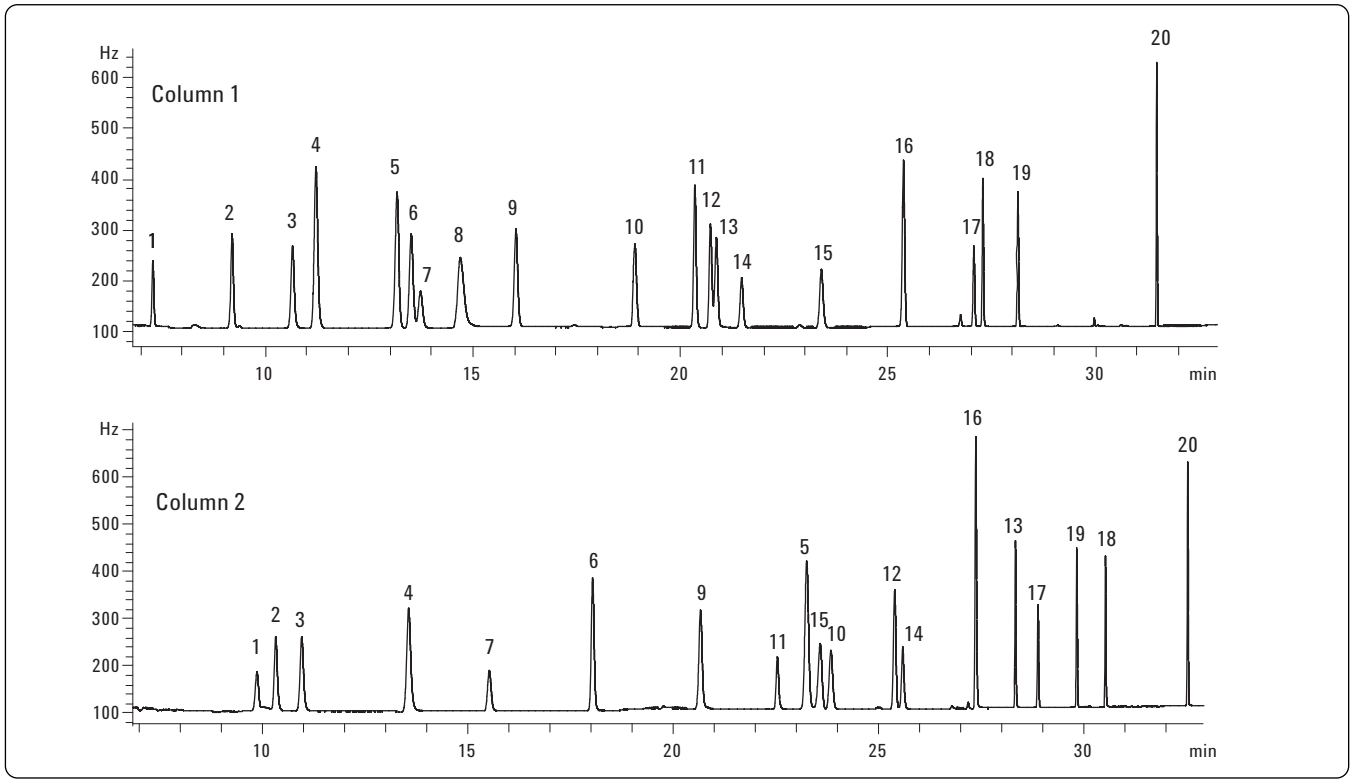


Figure 2. Single-injection chromatogram of the 5 pg on-column EPA551.1 standard solution loading on an Agilent J&W HP-1ms Ultra Inert 30 m × 0.25 mm × 1.0 µm capillary GC column (p/n 19091S-733UI) and J&W DB-1301 30 m × 0.25 mm × 1.0 µm capillary GC column (p/n 122-1333). Chromatographic conditions are listed in Table 1. Refer to Table 4 for a peak number key.

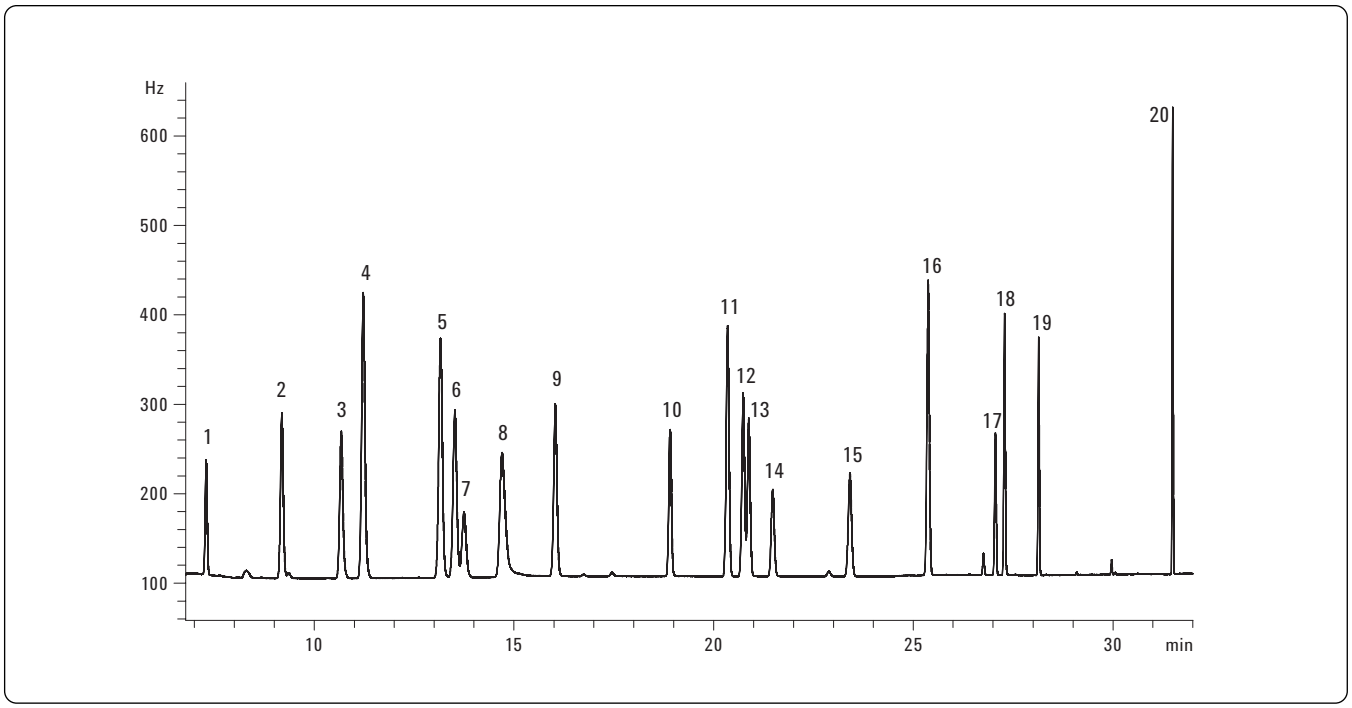


Figure 3. Enlarged chromatogram of the 5 pg on-column EPA551.1 standard solution loading on an Agilent J&W HP-1ms Ultra Inert 30 m × 0.25 mm × 1.0 µm capillary GC column (p/n 19091S-733UI). Chromatographic conditions are listed in Table 1. Refer to Table 4 for a peak number key.

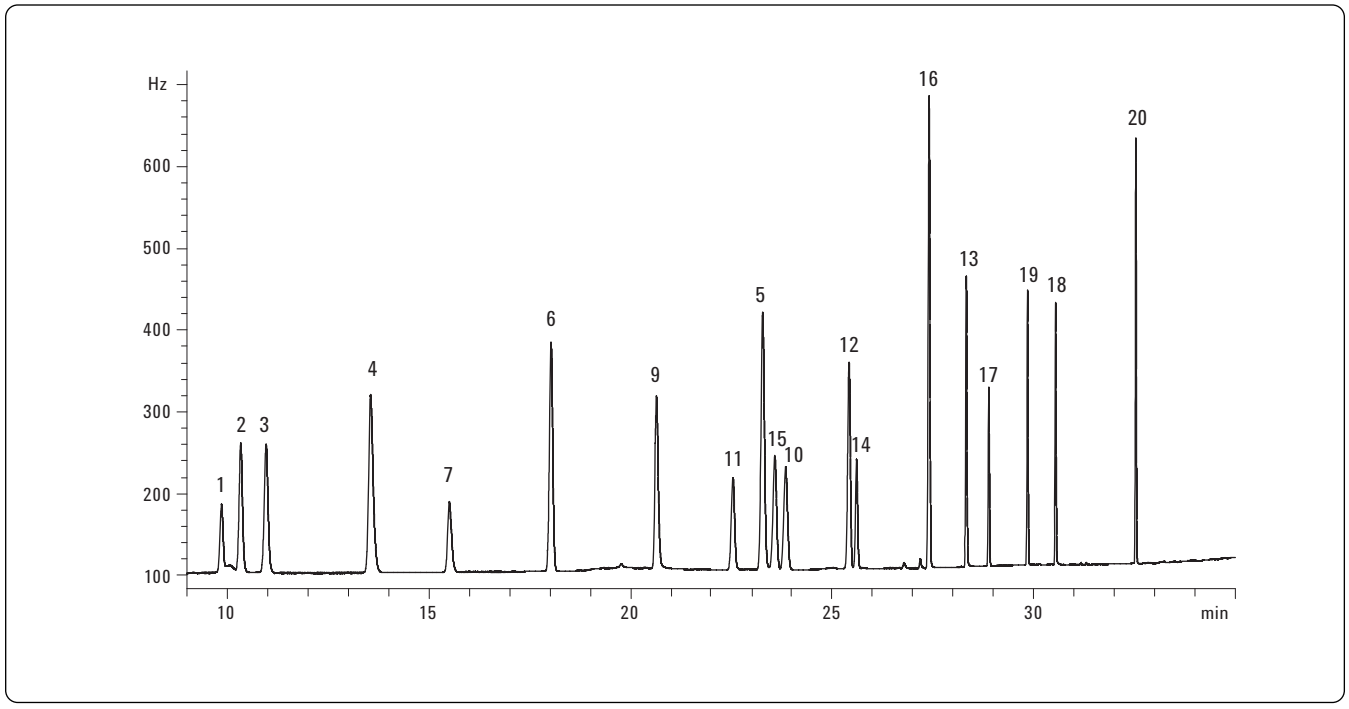


Figure 4. Enlarged chromatogram of the 5 pg on-column EPA551.1 standard solution loading on an Agilent J&W DB-1301 30 m × 0.25 mm × 1.0 µm capillary GC column (p/n 122-1333). Chloral hydrate (peak # 8) does not elute as a discreet peak on this column. Chromatographic conditions are listed in Table 1. Refer to Table 4 for a peak number key.

Table 3. Correlation Coefficients for the Analytes in the EPA Method 551.1 Standard Over the 0.002 to 0.1 µg/mL Range of This Study for a 0.5 µL Single Injection Loading onto the Dual Column System

Component	Agilent J&W HP-1ms UI R^2	Agilent J&W DB-1301 R^2
Chloroform	0.9997	0.9997
1,1,1-Trichloroethane	0.9999	0.9999
Carbon tetrachloride	0.9987	0.9988
Trichloroacetonitrile	0.9989	0.9979
Dichloroacetonitrile	0.9995	0.9993
Bromodichloromethane	0.9995	0.9994
Trichloroethylene	0.9998	0.9998
Chloral hydrate	0.9982	X
1,1-Dichloro-2-propanone	0.9999	0.9995
1,1,2-Trichloroethane	0.9998	0.9994
Chloropicrin	0.9995	0.9975
Dibromochloromethane	0.9995	0.9994
Bromochloroacetonitrile	0.9993	0.9981
1,2-Dibromoethane	0.9998	0.9999
Tetrachloroethlyene	0.9994	0.9999
1,1,1-Trichloro-2-propanone	0.9995	0.9992
Bromoform	1.0000	0.9998
Dibromoacetonitrile	0.9984	0.9975
1,2,3-Trichloropropane	0.9999	1.0000
1,2-Dibromo-3-chloropropane	0.9995	0.9998

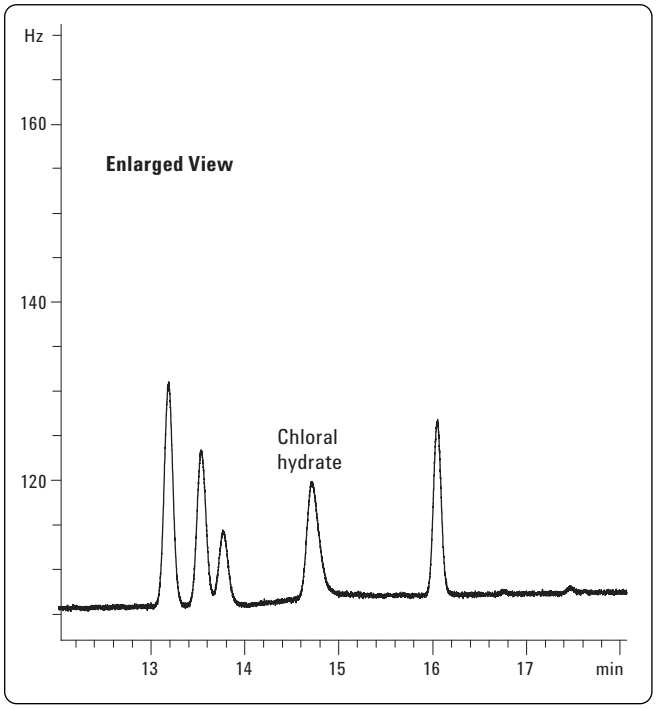


Figure 5. Enlarged chromatogram for a 0.5 µL injection of 0.002 µg/mL EPA 551.1 standard on the Agilent J&W HP-1ms Ultra Inert 30 m × 0.25 mm × 1.0 µm capillary GC column. Peak shape on the J&W HP-1ms Ultra Inert column is symmetrical and well resolved from the other components.

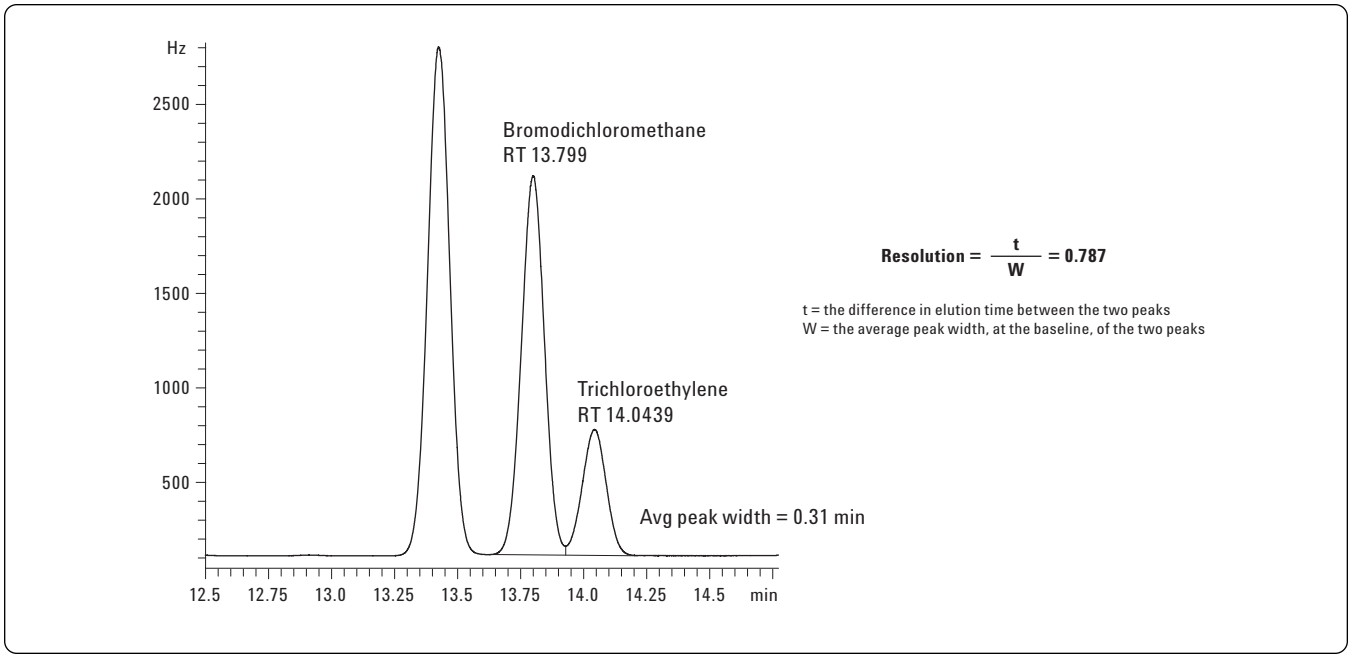


Figure 6. Enlarged chromatogram of 0.05 µg/mL EPA 551.1 standard on the Agilent J&W HP-1ms Ultra Inert 30 m × 0.25 mm × 1.0 µm capillary GC column. Method criteria for column performance is a resolution greater than 0.50 between bromodichloromethane and trichloroethylene.

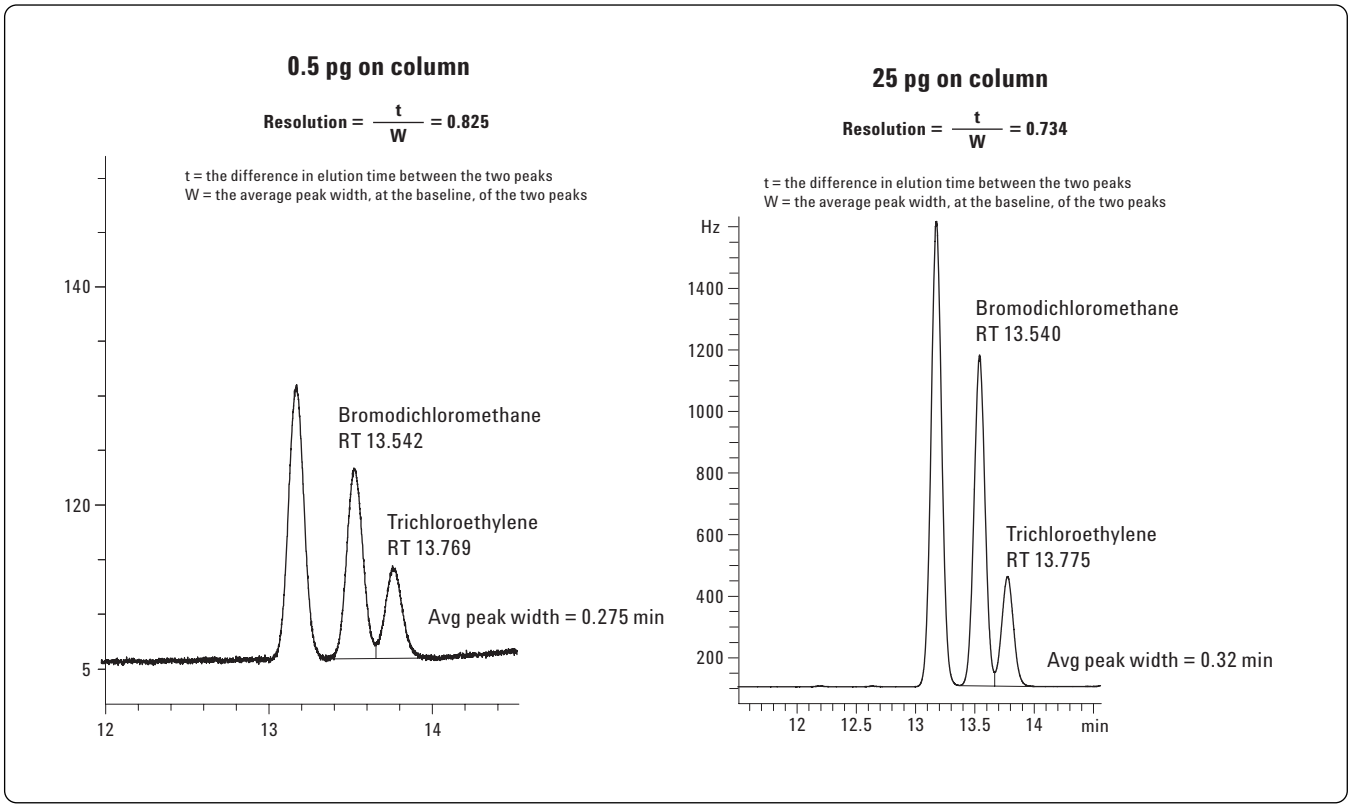


Figure 7. Enlarged chromatograms of the low and high range EPA551.1 standards on the Agilent J&W HP-1ms Ultra Inert 30 m × 0.25 mm × 1.0 µm capillary GC column. Method criteria for column performance is a resolution greater than 0.50 between bromodichloromethane and trichloroethylene.

Table 4. Peak Identification Table for EPA551.1 Chromatograms Shown in Figures 2 Through 4

Peak number	Peak name
1	Chloroform
2	1,1,1-Trichloroethane
3	Carbon tetrachloride
4	Trichloroacetonitrile
5	Dichloroacetonitrile
6	Bromodichloromethane
7	Trichloroethylene
8	Chloral hydrate
9	1,1-Dichloro-2-propanone
10	1,1,2-Trichloroethane
11	Chloropicrin
12	Dibromochloromethane
13	Bromochloroacetonitrile
14	1,2-Dibromoethane
15	Tetrachloroethlyene
16	1,1,1-Trichloro-2-propanone
17	Bromoform
18	Dibromoacetonitrile
19	1,2,3-Trichloropropane
20	1,2-Dibromo-3-chloropropane

Conclusions

This application successfully demonstrates the use of an Agilent J&W HP-1ms Ultra Inert capillary GC column for primary analysis of EPA 551.1 chlorinated solvents, trihalomethanes, and disinfection by-products. Linearity was excellent for all organochlorine analytes studied, yielding 0.998 or greater R^2 values down to a 0.5 pg on-column loading. One of the reasons for the excellent linearity and high R^2 values is the highly inert surface of the column. The excellent peak shape of the chloral hydrate and resolution between bromodichloromethane and trichloroethylene emphasize the advantage of the Agilent J&W HP-1ms Ultra Inert capillary GC column. The lack of chemically active sites makes this column an excellent choice for EPA Method 551.1 analysis.

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Printed in the USA
February 27, 2009
5990-3737EN

Agilent Technologies

Techniques for Optimizing the Analysis of Volatile Organic Compounds in Water Using Purge-and-Trap/GC/MS

Application

Environmental

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Abstract

The analysis of volatile organic compounds in water is normally accomplished by purge-and-trap/gas chromatography/mass spectrometry. U.S. EPA Method 8260B with purge and trap sample introduction is widely used for the analysis of aqueous samples other than drinking water. This application note discusses problems that can arise and some easy solutions for them. These techniques have resulted in robust calibrations that meet Method 8260B calibration requirements over the range of 1–200 µg/L.

Introduction

U.S. EPA Method 8260B [1] is a general purpose method for the analysis of volatile organic compounds (VOCs) in matrices such as ground and surface water, sludges, soils and sediments, filter cakes, spent carbons, and spent catalysts. This method is only used for the analyses of target VOCs by gas chromatography with mass spectral detection (GC/MS). It refers analysts to other U.S. EPA sample introduction methods that are appropriate for the matrix to be analyzed. This paper focuses on the analysis of VOCs in water using purge and trap (P&T) sample introduction according to U.S. EPA Method 5030C [2] coupled to GC/MS for separation and analysis (P&T/GC/MS). For simplicity, the combination of Methods 5030C with 8260B is referred to as just Method 8260B.

This P&T/GC/MS procedure is widely used in environmental laboratories for the analysis of VOCs in surface, ground, and wastewater samples. A similar method for the analysis of drinking water is described in EPA Method 524.2 [3]. Though well established, P&T/GC/MS methods can be a challenge to run successfully. There are numerous P&T, GC, and MS variables to optimize in order to obtain good recoveries for the target VOCs without undo disturbance from water and methanol that are inevitably transferred to the GC during trap desorption.

This application note describes techniques for optimizing Method 8260B using the Agilent 6890N GC and new 5973 inert mass selective detector (MSD) coupled to the new Teledyne Tekmar Velocity XPT P&T system. Included, in the paper, are suggestions for MSD tuning, sample preparation, instrument setpoints, and maintenance techniques that lead to a robust method for the analysis of VOCs in water. The discussion is applicable to most other P&T/GC/MS methods.

Experimental

Chemical Standards, Reagents, and Vials

High purity B&J brand methanol was obtained from Honeywell Burdick & Jackson Co. (Muskegon, MI). Standard mixtures used for the preparation of calibration samples, spiking solutions, tune evaluation, and stability test samples were purchased from AccuStandard (New Haven, CT). These include the following: Part no. M-502-10X-Pak containing 60 VOC target analytes (54 liquids and 6 gases) at 2000 µg/mL each in methanol; Part no. M-8260A/B-IS/SS-10X-PAK containing p-bromofluorobenzene (BFB), chlorobenzene-d_5, dibromofluoromethane, 1,4-dichlorobenzene-d_4 (DCB-d_4) , 1,2-dichloroethane-d_4, fluorobenzene (FBz), and toluene-d_8 at 2000 µg/mL each in methanol; and part no. M-524-FS-PAK containing BFB, 1,2-dichlorobenzene-d_4, and fluorobenzene (FBz) at 2000 µg/mL each in methanol.

VOC-free water was used for the preparation of standards and test samples. TraceClean 40-mL (nominal volume, actual volume is 43 mL) VOA vials (part no. 15900-022) were purchased from VWR Scientific (West Chester, PA).

Preparation of Calibration and Spiking Solutions

Secondary spiking solutions were prepared in methanol for each calibration level so that each 43-mL water sample could be spiked with 10 µL of the calibration solution (containing 60 VOCs) and 10 µL of the internal standard/surrogate mixture. Table 1 provides details on how the eight calibration standards were prepared.

Table 1. Procedure for Preparing Calibration Samples

A	B	C	D	E
Calibration level (µg/L)	Volume of 2000 µg/mL VOC Standard (µL)	Diluted to this volume in methanol (mL)	Results in this secondary standard concentration (µg/L)	Amount to spike into 43-mL vial (µL)
1	53.75	25.00	4.3	10.00
2	43.00	10.00	8.6	10.00
5	53.75	5.00	21.5	10.00
20	43.00	1.00	86	10.00
50	43.00	0.40	215	10.00
100	43.00	0.20	430	10.00
200	43.00	0.10	860	10.00
300	*	*	2000*	6.45**

Column A. Concentration of each analyte in the final aqueous calibration solution.

Column B. Volume of the 2000 µg/mL 60-component VOC standard solution which was diluted to the volume shown in column C.

Column C. Final volume of VOC solution after dilution in methanol.

Column D. Concentration of the calibration spiking solution prepared by diluting the amount of 2000 µg/mL standard in column B to the volume shown in column C.

Column E. Amount of the secondary standard solution (column D) added to 43 mL of water to prepare the calibration standard at the level shown in column A.

*The undiluted VOC standard (2000 µg/mL) was used for spiking.

**The 300 µg/L aqueous calibration standard was prepared by adding 6.45 µL of the 2000 µg/mL AccuStandard VOC solution and 3.55 µL of methanol to 43 mL of water in a VOA vial.

As discussed below, containers for storing the secondary standards (column C, Table 1) were chosen to minimize the headspace. Larger volumes were transferred to 2-mL screw top vials, while smaller volumes were transferred to crimp cap microvials of the appropriate size.

A solution of the internal standards (ISTDs) and surrogates was prepared at 215 ppm in methanol by diluting 43 µL of the 2000-µg/mL AccuStandard solution to a volume of 400 µL. Each 43-mL water sample was spiked with 10 µL of this solution so that all samples and standards contained 50 µg/L of each compound.

Preparation of Solutions for Repeatability Studies

Two kinds of spiked water samples were prepared for use in repeatability studies.

- System blanks consisted of clean water spiked with fluorobenzene, BFB, and 1,2-dichlorobenzene-d_4 at 10 µg/L each.
- VOC spikes consisted of clean water with fluorobenzene, BFB, and 1,2-dichlorobenzene-d_4 at 10 µg/L and the 60 VOC target compounds at 20 µg/L each.

Replicate samples were prepared as follows.

- Secondary dilution standards containing fluorobenzene, BFB, and 1,2-dichlorobenzene-d_4 at 50.0 µg/mL were prepared in 2-mL autosampler vials by diluting 25 µL of the 2000-µg/mL AccuStandard solution with 975 µL of methanol.
- Secondary dilution standards of the 60-component VOC solution were prepared at 100 µg/mL in 2-mL autosampler vials by diluting 50 µL of the 2000 µg/mL AccuStandard solution with 950 µL of methanol.

System blanks were prepared by adding 100 µL of the 50.0 µg/mL three component solution and 100-µL methanol to 500 mL of water in a 1.0-L screw-cap bottle. After inverting to mix thoroughly, this bottle was attached to the apparatus shown in Figure 1 and 11 VOA vials were filled by transferring the spiked water solution under nitrogen pressure.

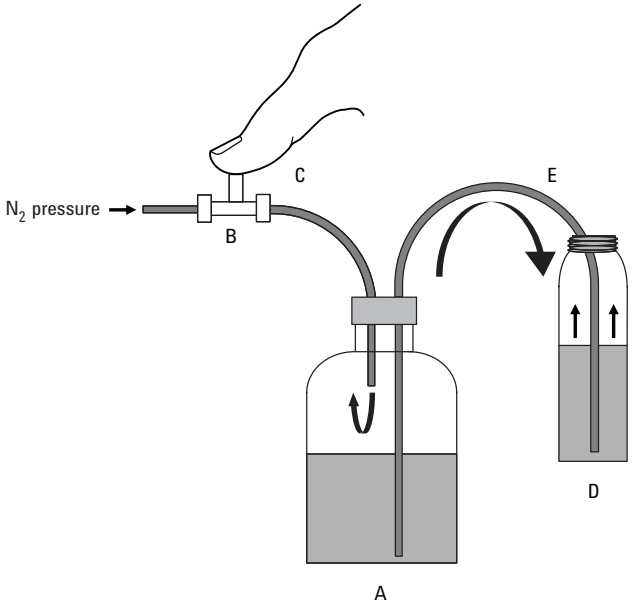

Figure 1. Apparatus used to fill multiple VOA vials with the same spiked water solution.
A) 1-L liquid chromatography solvent bottle
B) Swagelok Tee with nothing connected to one fitting
C) Finger used to cap fitting in order to pressurize the reservoir bottle
D) VOA vial
E) 1/8-inch PTFE tubing

VOA spiked samples were prepared by adding 100 µL of the 50.0-µg/mL three component solution and 100 µL of the 100-µg/mL 60-component VOC standard to 500 mL of water in a 1.0-L screw cap bottle. After inverting to mix thoroughly, this bottle was attached to the apparatus shown in Figure 1 and 11 VOA vials were filled by transferring the spiked water solution under nitrogen pressure.

Instrumentation and Analytical Conditions

The P&T instrumentation and setpoints are listed in Table 2. The following P&T options were not used: DryFlow trap, automatic ISTD addition, sample heating, dry purging, and sample cryofocusing. The method shown in Table 2 was derived using the wizard that is provided in the TekLink 2.2 P&T control software.

Table 2. Purge and Trap Instrumentation and Setpoints

P&T Instrument	**Teledyne Tekmar Velocity XPT**
Automatic sampler	**Teledyne Tekmar Aquatek 70**
Software control	Teledyne Tekmar VOC Teklink version 2.2
Trap	Vocarb 3000
P&T-GC interface	P&T transfer line spliced into the GC split/splitless inlet carrier gas line and GC carrier gas plumbed to the Velocity XPT
Sample size	5 mL
Valve oven temperature	150 °C
Transfer line temperature	150 °C
Sample mount temp	90 °C
Purge ready temp	45 °C
DryFlow standby temperature	175 °C
Standby flow	10 mL/min
Pressurize time	0.25 min
Fill I.S. time	0.00 (ISTDs added by hand)
Sample transfer time	0.25 min
Pre-purge time	0.00 min
Pre-purge flow	40 mL/min
Sample heater	Off (Samples not heated)
Sample preheat time	1.00 min
Preheat temperature	40 °C
Purge time	11.00 min
Purge temperature	0 °C (That is, less than the purge ready temp of 45 °C)
Purge flow	40 mL/min
Purge rinse time	0.25 min
Purge line time	0.25 min
Dry purge time	0.00 min (Dry purge not used)
Dry purge temp	40 °C
Dry purge flow	200 mL/min
GC start	Start of desorb
Desorb preheat temperature	245 °C
Desorb drain	On
Desorb time	1.00 min
Desorb temperature	250 °C
Desorb flow	200 mL/min
Bake rinse	On
Number of bake rinses	3
Bake drain time	0.50 min
Bake drain flow	400 mL/min
Bake time	3.00 min
Bake temperature	270 °C
Dry flow bake temperature	300 °C
Bake flow	400 mL/min
Focus temperature	Not used
Inject time	1.00 min
Inject temperature	180 °C
Standby temperature	100 °C

Table 3. GC/MS Instrumentation and Setpoints

Gas Chromatograph	**Agilent 6890N**
Inlet	Split/Splitless
Inlet liner	Single taper, deactivated (Agilent part no. 5181-3316)
Inlet temperature	250 °C
Split ratio	50:1
Column	20 m × 0.18 mm × 1.0 µm DB-VRX (Agilent part no. 121-1524)
Carrier gas	Helium at 1.0 mL/min constant flow
Oven temperature program	40 °C (3 min), 10 °C/min to 100 °C (0 min), 25 °C/min to 225 °C (3 min)
Mass Spectrometer	**Agilent 5973 Inert MSD**
Transfer line temperature	260 °C
Quad temperature	150 °C
Source temperature	230 °C
EM voltage	2035 volts
Scan range	35–260 *m/z*
Threshold	0
Samples	3
Solvent delay	0 min
Software	MSD Productivity ChemStation Software (Part no. G1701DA version D.01.00)

Results and Discussion

Section 1.3 of Method 8260B can be used to quantitate most VOCs that have boiling points below 200 °C. It lists 123 compounds that can be determined by the method using various sample prep and sample introduction methods. Of these, seven are ISTDs or surrogates, nine are not recommended for P&T sample introduction, and three must be purged at 80 °C for efficient recovery. The remaining analytes vary considerably in their water solubility and volatility making this a challenging method to optimize. The intent of this application note is to share several techniques that one can use to optimize Method 8260B or any other P&T/GC/MSD method employed for water analysis.

For this study, the 60 VOCs listed in EPA Method 502.2 were analyzed along with three ISTDs and four surrogates (Table 4).

Table 4. Compound List with Average Response Factors (RF) and the RF %RSDs for Two Calibration Ranges: 1–300 and 1–200 µg/L

Type*	Compound	Retention time (min)	Minimum average response factor**	Maximum %RSD of calibration response factors***	Average RF 1–300 µg/L	RF %RSD 1–300 µg/L	Average RF 1–200 µg/L	RF %RSD 1–200 µg/L
T	Dichlorodifluoromethane	1.25		15	0.283	8.21	0.289	5.44
T,SPCC	Chloromethane	1.34	0.1	15	0.324	9.62	0.328	9.38
T,CCC	Vinyl chloride	1.42		30	0.220	2.47	0.220	2.66
T	Bromomethane	1.60		15	0.099	14.11	0.096	12.30
T	Ethyl chloride	1.67		15	0.152	5.57	0.154	4.27
T	Trichloromonofluoromethane	1.97		15	0.372	11.38	0.386	3.49
T,CCC	1,1-Dichloroethene	2.29		30	0.330	5.31	0.336	1.45
T	Methylene chloride	2.40		15	0.299	5.02	0.301	4.95
T	*trans*-1,2-Dichloro-ethene (E)	2.92		15	0.323	2.54	0.325	1.36
T,SPCC	1,1-Dichloroethane	3.14	0.1	15	0.444	4.93	0.446	5.22
T	*cis*-1,2-Dichloroethene (Z)	3.68		15	0.360	1.28	0.361	1.17
T	Bromochloromethane,	3.83		15	0.234	1.82	0.234	1.84
T,CCC	Chloroform	3.89		30	0.442	0.92	0.443	0.60
T	2,2-Dichloropropane	3.96		15	0.202	9.87	0.209	4.19
Sur	Dibromofluoromethane	4.01		15	0.248	0.83	0.248	0.89
Sur	1,2-Dichloroethane-d_4	4.47		15	0.298	1.76	0.299	1.79
T	1,2-Dichloroethane	4.55		15	0.359	1.57	0.359	1.66
T	1,1,1-Trichloroethane	4.64		15	0.388	7.99	0.398	1.43
T	1,1-Dichloropropene	4.86		15	0.336	12.44	0.351	3.16
T	Carbon tetrachloride	5.01		15	0.309	13.88	0.322	7.66
T	Benzene	5.08		15	1.063	7.10	1.077	6.52
ISTD	Fluorobenzene	5.34		15		1.34		1.41
T	Dibromomethane	5.68		15	0.198	1.86	0.198	2.01
T,CCC	1,2-Dichloropropane	5.75		30	0.266	1.58	0.268	0.77
T	Trichloroethylene	5.81		15	0.288	6.79	0.295	2.14
T	Bromodichloromethane	5.85		15	0.334	5.47	0.331	5.60
T	1,3-Dichloropropene (Z)	6.64		15	0.383	5.49	0.381	5.74
T	1,3-Dichloropropene (E)	7.18		15	0.322	8.76	0.318	8.93
T	1,1,2-Trichloroethane	7.32		15	0.236	1.57	0.237	1.67
Sur	Toluene-d_8	7.47		15	0.945	0.50	0.945	0.51
T,CCC	Toluene	7.55		30	1.098	7.47	1.126	2.07
T	1,3-Dichloropropane	7.62		15	0.428	1.28	0.428	1.20
T	Dibromochloromethane	7.86		15	0.254	12.10	0.249	11.88
T	1,2-Dibromoethane	8.15		15	0.244	1.88	0.244	2.03
T	Tetrachloroethylene	8.40		15	0.307	18.72	0.327	5.07
T	1,1,1,2-Tetrachloroethane	9.15		15	0.254	8.79	0.254	9.49
ISTD	Chlorobenzene-d_5	9.19		15		0.98		0.81
T,SPCC	Chlorobenzene	9.22	0.3	15	0.981	5.00	0.997	2.14
T,CCC	Ethylbenzene	9.51		30	1.559	11.66	1.623	1.90
T,SPCC	Bromoform	9.72	0.1	15	0.246	14.57	0.242	15.08
T	m- & p-Xylene	9.73		15	2.510	11.97	2.614	2.75
T	Styrene	10.03		15	1.008	5.68	1.022	4.25
T,SPCC	1,1,2,2-Tetrachloroethane	10.08	0.3	15	0.395	3.41	0.394	3.46
T	o-Xylene	10.10		15	1.289	9.27	1.330	1.89
T	1,2,3-Trichloropropane	10.21		15	0.347	2.90	0.346	2.94
Sur	BFB	10.44		15	0.381	0.93	0.382	0.82
T	Isopropylbenzene	10.44		15	1.474	17.44	1.562	4.13
T	Bromobenzene	10.58		15	0.643	5.20	0.653	3.12
T	n-propylbenzene	10.82		15	1.840	17.38	1.950	3.60
T	2-Chlorotoluene	10.85		15	1.124	10.66	1.166	1.93
T	4-Chlorotoluene	10.92		15	1.184	10.23	1.224	3.75

Table 4. Compound List with Average Response Factors (RF) and the RF %RSDs for Two Calibration Ranges: 1–300 and 1–200 µg/L (Continued)

Type*	Compound	Retention time (min)	Minimum average response factor**	Maximum %RSD of calibration response factors***	Average RF 1–300 µg/L	RF %RSD 1–300 µg/L	Average RF 1–200 µg/L	RF %RSD 1–200 µg/L
T	1,3,5-Trimethylbenzene	11.08		15	1.275	14.63	1.340	3.02
T	Tertbutylbenzene	11.26		15	1.196	18.98	1.274	4.24
T	1,2,4-Trimethylbenzene	11.36		15	1.353	12.22	1.411	2.35
T	sec-Butylbenzene	11.43		15	1.729	21.91	1.858	5.67
T	1,3-Dichlorobenzene	11.44		15	1.529	10.75	1.579	5.61
T	1,4-Dichlorobenzene	11.49		15	1.597	9.97	1.643	5.99
ISTD	1,4-Dichlorobenzene-d_4	11.47		15		1.09		1.17
T	p-Isopropyltoluene	11.58		15	2.587	19.00	2.757	3.52
T	1,2-Dichlorobenzene	11.73		15	1.485	6.33	1.516	2.74
T	Butylbenzene	11.87		15	2.355	20.68	2.522	4.81
T	1,2-Dibromo-3-chloropropane	12.06		15	0.186	13.90	0.180	11.56
T	1,2,4-Trichlorobenzene	12.95		15	1.211	12.42	1.250	8.76
T	Naphthalene	13.10		15	2.879	5.54	2.852	5.32
T	Hexachlorobutadiene	13.16		15	0.750	24.53	0.809	10.56
T	1,2,3-Trichlorobenzene	13.22		15	1.196	11.09	1.226	9.06
	Average %RSD of targets				9.07		4.60	
	Average %RSD of all compounds					8.22		4.23

*Compound designations as follows: T (target); SPCC (system performance check compound); CCC (calibration check compound); Surr (surrogate); ISTD (internal standard). Target compounds may also be designated as SPCCs or CCCs.

**The minimum average RF that must be met for the SPCCs.

***The maximum %RSD of the RFs. If any one or more of the CCC RF RSDs exceeds 30%, instrument maintenance is required. If the RF %RSD for any target compound exceeds 15%, other curve fits must be substituted for the average RF.

Method 8260B Requirements

Below is a summary of the most significant requirements of Method 8260B. If you are already very familiar with this method, you may want to skip this section.

ISTDs and surrogates: The ISTDs and surrogates listed in Table 4 are the recommended compounds for this method, although other compounds may be used instead.

Tuning requirements: Prior to running samples, the MSD must be adjusted so as to pass Method 8260B's BFB tuning specifications [1]. However, the method allows users to substitute CLP [4], Method 524.2 [3] or manufacturers' instructions for the specified BFB ion ratios. Table 5 lists the BFB tuning specifications for all three EPA methods. A scan range of 35–260 m/z is recommended.

Table 5. Criteria for BFB Tuning for Three Capillary GC/MS Volatiles Methods

Mass (*m/z*)	Relative abundance criteria Method 524.2	Method 8260B*	CLP-SOW
50	15%–40% of 95	Same**	8%–40% of 95
75	30%–80% of 95	30%–60% of 95	30%–66 % of 95
95	Base Peak, 100%	Same	Same**
96	5%–9% of 95	Same	Same
173	<2% of 174	Same	Same
174	>50% of 95	Same	50%–120% of 95
175	5%–9% of 174	Same	4%–9% of 174
176	>95% but <101% of 174	Same	93%–101% of 174
177	5%–9% of 176	Same	Same

*Alternative tuning criteria may be used (for example, CLP or Method 524.2) including manufacturer's instructions provided that method performance is not adversely affected.

**"Same" implies that this requirement is the same as that shown for Method 524.2. Note, however, that alternative tuning criteria may be used for Method 8260B (see previous footnote).

System Performance Check Compounds (SPCCs): The SPCCs are used to check the performance of the system after calibration and before analysis of samples. These compounds are known to be sensitive to active sites and instrument contamination. They must meet a minimum RF that is specified in Table 4.

Calibration Requirements: As a minimum, Method 8260B requires a five-point calibration curve. In order to assume linearity of the calibration curve, the RF RSD of all target compounds must be less than or equal to 15%. Six analytes are designated as Calibration Check Compounds (CCCs) (Table 4). If the RF RSDs for any of these compounds exceeds 30%, it is indicative of instrument problems and repairs must be made. Compounds that exceed 15% RSD for their RFs can use alternative curve fitting methods as specified in EPA Method 8000B [5].

GC/MS Calibration Verification for Each 12-hour Shift: The P&T/GC/MSD performance must be re-evaluated every 12 hours. The most significant requirements are:

- The BFB tune must be rechecked and pass the original tuning requirements.
- A sample near the midpoint of the calibration curve must be analyzed using P&T sample introduction, demonstrating that:
  - Each SPCC meets its minimum RF.
  - The percent difference (between current and original response) must be less than 20% for each CCC.
  - The retention time of each ISTD must not drift by more than 30 s.
  - The ISTD areas must not change by more than a factor of 2 from the original mid-point calibration level (50% to 200%).
  - A method blank must be run to show that there is no carryover or contamination of the system.

Calibration Results

Many laboratories employing Method 8260B generate five-point calibration curves between 5 and 200 µg/L. Knowing that laboratories often try to extend this range at both ends, an eight-point calibration was run at 1, 2, 5, 20, 50, 100, 200, and 300 µg/L. The signals for all analytes at 1 µg/L were sufficient to allow calibration at even lower levels. However, the lowest calibration level run for this work was 1 µg/L. Figure 2 shows a chromatogram of the targets, surrogates, and ISTDs at 50 µg/L each.

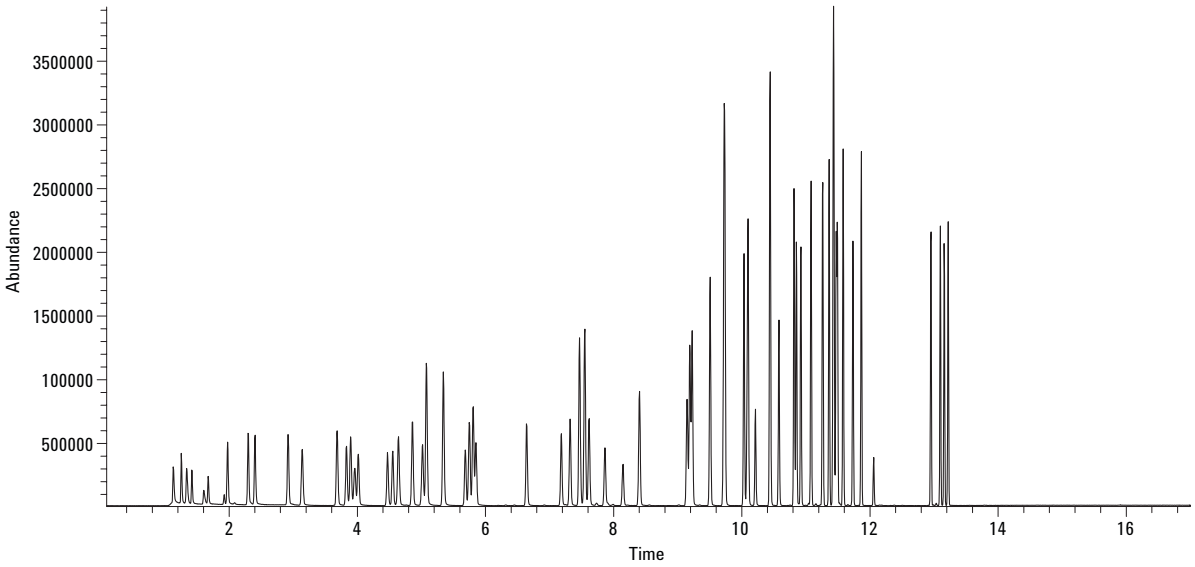

Figure 2. P&T/GC/MS analysis of a standard containing all of the compounds listed in Table 4, each at 50 µg/L in VOC-free water.

The average RF and %RSD of the RFs were calculated for each compound over the 1–300 µg/L and 1–200 µg/L ranges. As seen in Table 4, all five of the SPCCs exceeded their minimum RFs by a comfortable margin for both calibration ranges.

As mentioned above, the CCC RF RSDs must not exceed 30%. Table 4 shows that all six CCCs were significantly less than this for both calibration ranges. In fact, the average %RSD of the CCCs was only 4.90% for the 1–300 µg/L calibration and a remarkably small 1.58% in the narrower 1–200 µg/L range.

Only eight compounds exceeded the 15% RSD requirement in the 1–300 µg/L calibration range. In all cases, the RF fell off significantly for the 300 µg/L standard, suggesting that the strong target ion response overloaded the MSD at that very high concentration.

In the 1–200 µg/L calibration range, the average RF could be used for all targets except, perhaps, bromoform which exceeded the 15% limit by 0.08%. If one justifies only two significant figures, even bromoform could use an average RF for calibrations. The average of the %RSDs for all targets was 8.9% for the 1–300 µg/L calibration and only 4.5% for the 1–200 µg/L range (Table 4). Figure 3 shows a plot of the RFs for each target compound over the 1–200 µg/L calibration range.

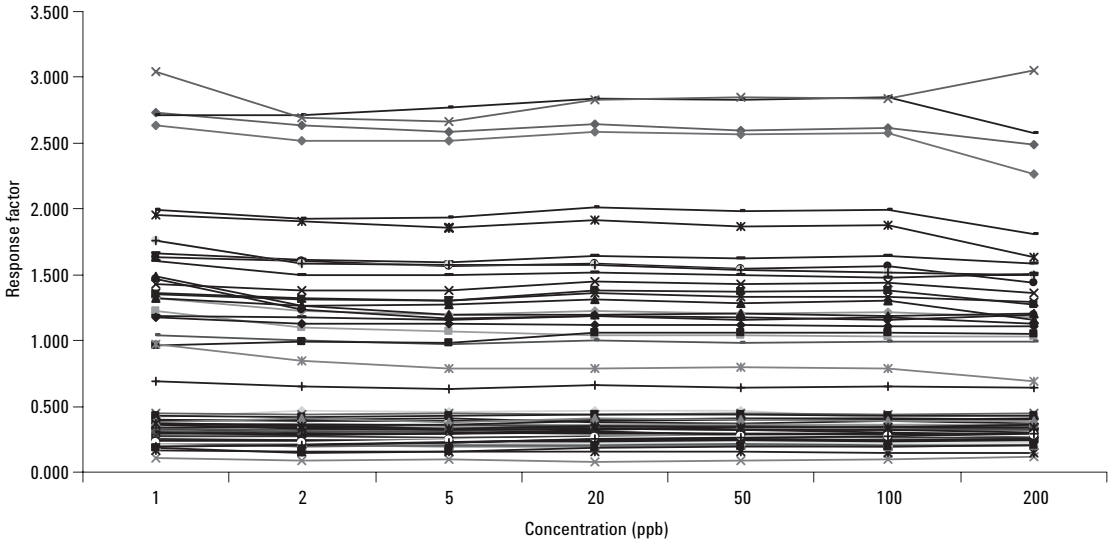

Figure 3. Plot of the RFs from a seven-level calibration for all of the target compounds listed in Table 4. Concentrations were at 1, 2, 5, 20, 50, 100, and 200 µg/L.

Figure 4 plots a distribution of the RF %RSD values for the 59 calibrated peaks (m- and p-Xylene were not resolved). It shows that most compounds have RFs over the 1–200 µg/L calibration range with less than six percent RSD. More than 91% of the compounds have RSD values of 10% or less.

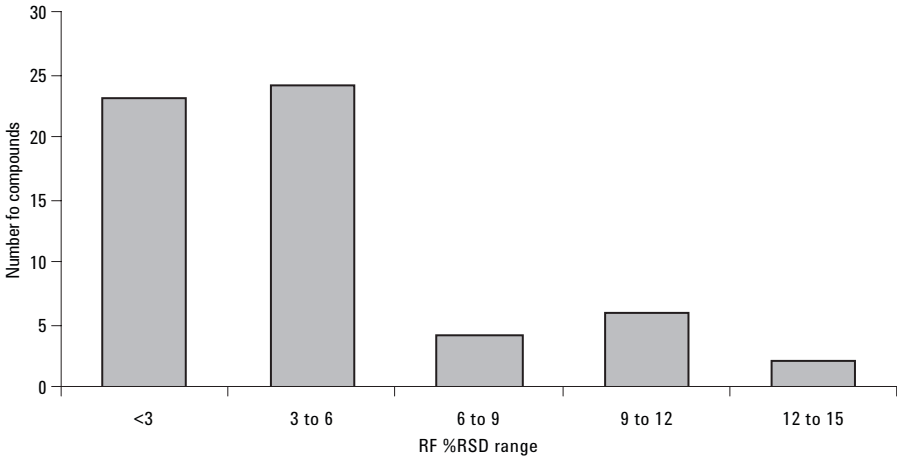

Figure 4. Distribution of the RF RSDs for the 59 calibrated peaks (m- and p-xylene were not resolved).

Response Stability

The longevity of any calibration depends upon having a consistent response for all compounds, even when running samples almost continuously over the course of several days, weeks, or even months. Some laboratories have observed a falloff in response over time that can jeopardize the calibration. Moreover, it has been observed that the recoveries for certain compounds may be dependent upon the presence or absence of other VOCs in the sample. A complete discussion of this problem and some simple solutions for it may be found in the "Optimization Techniques" section below.

In order to assess instrument stability over time, two types of samples were prepared. "System Blanks" contained only FBz, BFB, and 1,2-dichlorobenzene-d_4 (DCB-d_4) at 10 µg/L in water. The first compound was used as the ISTD while the latter two were chosen as surrogates. "Spiked" samples were the same as the system blanks but with the 60 target VOCs added at 20 µg/L each. These samples were analyzed alternately, typically for 22 runs, but sometimes many more runs over several days.

Figure 5 is a plot of the normalized recoveries for FBz, BFB, and DCB-d_4. It illustrates the two problems that can be observed when instrument parameters are not optimized. First, there is a gradual drop in response for all three compounds as illustrated by the sloping arrows. Superimposed upon this is a reduction in surrogate recovery in the absence of added VOCs. Because system blanks and spiked samples were alternated in the sequence, there was a "zigzag" appearance to the plot.

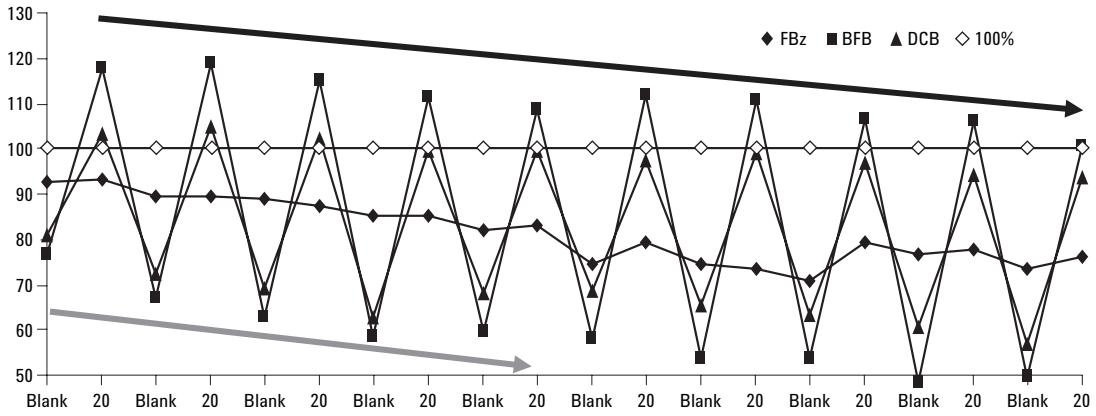

Figure 5. Normalized recoveries for FBz, BFB, and 1,2-DCB-d_4. System blanks (containing only FBz, BFB, and DCB-d_4 at 10 µg/L each) were analyzed alternately with system blanks spiked with an additional 60 VOCs at 20 µg/L each. Arrows show a gradual loss of response over the course of the sequence. The zigzag pattern arises because the recovery of BFB and DCB-d_4 is higher in the presence of other VOCs.

The problems illustrated in Figure 5 can be avoided rather easily by not overloading the MSD's electron multiplier (EM) and by ensuring that there are no active sites in the sample flow path. Figure 6 shows normalized recovery plots for BFB and DCB-d_4 that are typical when the instrument parameters are set correctly. Once again, system blanks and spiked samples were alternated, but this time there was no drop in response over time. Surrogate recovery was independent of sample spiking. Simple solutions for resolving these problems are discussed below.

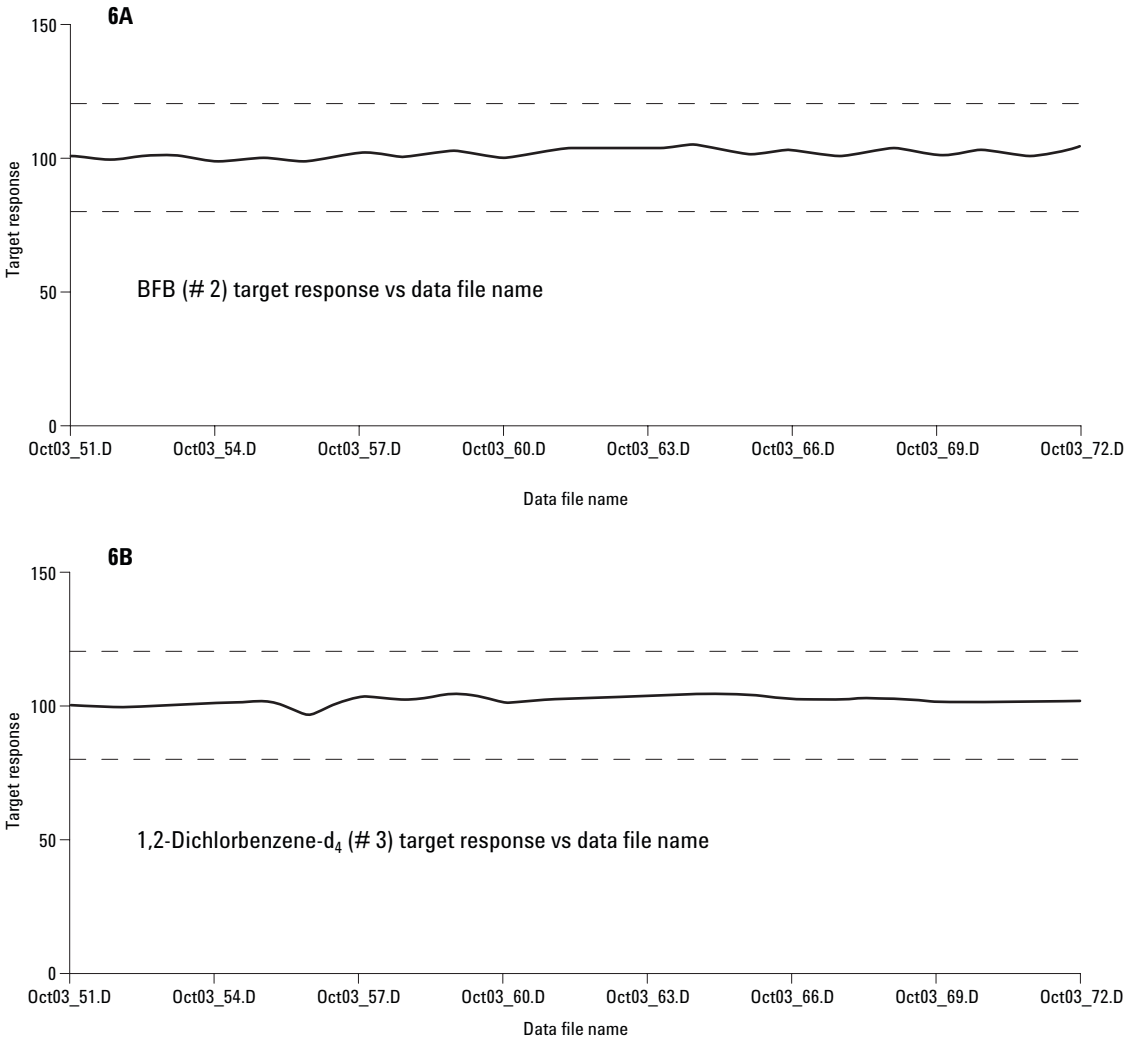

Figure 6. Normalized recovery for BFB (6A) and DCB-d_4 (6B) using the Agilent 6890N/5973 inert GC/MS coupled to the Velocity XPT P&T with optimized system parameters.

Optimization Techniques

MSD Tuning: Application Note 5988-4373EN [6] discusses three different ways to tune Agilent's 5973N MSD in order to meet BFB requirements. With the recent introduction of the 5973 inert MSD, these procedures still apply, though it is helpful to turn off the variable entrance lens setting when using the BFB autotune. The CLP Statement of Work specifications (Table 5) offers more latitude than the 8260B tuning requirements. Most importantly, ion 174 can be up to 120% of ion 95 (the reference ion). It is helpful to tune the MSD so as to produce a 174/95 ion ratio that is in the 90%–120% range because this improves the signal for bromoform (base peak = 173), which purges with poor efficiency. For this work, the "modified autotune" method was used and the 174/95 ratio was about 105%. It has been our experience that once the Agilent 5973 inert has been tuned to meet BFB requirements, the tune is stable for many weeks. It is impossible to say how long, because once tuned, it never failed to pass the BFB requirements.

MSD Parameter Optimization: When ISTD or surrogate responses fall off with repeated injections, overloading the Agilent 5973 MSD's high energy dynode (HED) EM may be the cause. The 5973 was designed to be significantly more sensitive than its predecessors and incorporates an HED in the EM. This reduces the noise and increases the signal, especially for ions of higher mass. However, this highly sensitive detector can be overloaded by continuous ion bombardment or by operating it at too high a voltage. The symptom is an unusually large loss of response over time.

Many GC/MS users erroneously believe that they can increase the sensitivity of their MSD by increasing the EM voltage. This can be done by raising the target value during tuning or by adding voltage to the tune value in the "MS SIM/Scan Parameters" window. However, in the electron impact mode, the noise increases at approximately the same rate as the signal. So, the true sensitivity (signal/noise) does not increase. The main consequence is to reduce the EM's lifetime. This can show up as a reduced response over time that might even be noticeable after several runs. (Note that these statements about signal/noise ratios do not necessarily apply to chemical ionization techniques.)

The solution to this "problem" is relatively simple. The easiest way is to reduce the EM voltage, which reduces the signal and noise, but not the signal/noise ratio. It may also be necessary to reduce the threshold value in the "Edit Scan Parameters" window in order to see the smaller ions. The default EM voltage values from an Autotune or BFB tune are usually correct, but these can be decreased somewhat if the above-mentioned symptoms occur.

It is easier to overload the EM in the selected ion monitoring (SIM) mode, because only a few ion fragments are monitored. During peak elution in the scan mode, there are "blank" spaces in all spectra where the signal is small or zero. With SIM, the signal is almost continuous and the ions monitored are usually the most abundant ones. Here again, the solution is relatively simple. One can reduce the EM voltage, decrease the SIM dwell time, and/or reduce the peak width by choosing the "High Resolution" option. The latter two values are set in the "Edit SIM Parameters" window. In any case, it is important to remember that both signal and noise are roughly proportional to the EM voltage and nothing is sacrificed by making small reductions in its value. Just remember to lower the threshold value or set it to 0 at the same time.

Reducing System Activity: When surrogate recoveries are higher in the presence of other analytes, as illustrated in Figure 5, active sites in the sample flow path are a likely cause. Surrogates can adsorb on these active sites, reducing their recovery. Surrogate recoveries improve when other analytes are present that compete for the active sites. To prevent such problems, one must use a highly inert P&T/GC/MS system and maintain its cleanliness by avoiding contamination from foaming samples. The Agilent 6890N/5973 inert GC/MS coupled to the Velocity XPT P&T showed no signs of sample adsorption. As seen in Figure 6, surrogate recoveries were highly stable with this system. If target or surrogate recoveries vary depending upon the presence of other analytes, it may be helpful to increase the temperature of the MSD source or upgrade an older 5973A or N with the new "Inert" source.

The P&T Method and Water Management: The VOC Teklink software used to control the new Teledyne Tekmar Velocity XPT concentrator and Aquatek 70 autosampler offers a "wizard" tool to help the user choose parameters for the method. Only minor modifications were made to the wizard-generated method. ISTDs were added manually to each sample so the "Fill I.S. Time" was set to 0.00 min. The bake time was increased to 3 minutes and the number of bake rinses was increased to three. The wizard chose all other parameters after the user provided information about the system configuration.

One of the primary concerns of P&T/GC/MS methods is the management of water that is inevitably purged along with the analytes. Since calibration, surrogate, and ISTD solutions are prepared in methanol, some of this solvent is also purged and retained by the trap. By starting the scan at 40 µ, methanol and water ions were not detected by the MSD. Nevertheless, transferring large amounts of water or methanol from the P&T to the GC/MS can result in poor reproducibility for those compounds that co-elute with them. Using the Velocity XPT with the Agilent 6890N/5973 inert system there were no problems that could be attributed to water. Because the P&T was configured with a Vocarb 3000 trap, the DryFlow trap was not required. Various dry purge times and flow rates were tried, but the only affect this had was to distort the peak shape of one or more early eluting peaks. Therefore, the dry purge option was not used. It is likely that some problems attributed to an excess of water actually result from overloading the MSD EM.

Standard preparation: The careful preparation of standards for calibration cannot be overemphasized. As with most laboratories, the initial dilutions were purchased as 2000 µg/mL/component concentrates, which were stored without problem in a refrigerator. Experience in this laboratory showed that best results were obtained when observing the following guidelines:

- Prepare secondary dilutions used for sample spiking from freshly opened standards.
- Transfer secondary dilutions to appropriately sized glass containers so that there is little or no headspace in the vial. Store small quantities in microvials.
- Mininert vial closures were tried for sample storage but were prone to leakage and their use was discontinued. In addition, they were not available for microvials.
- It works well to prepare calibration standards by spiking methanolic solutions into pure water through the septum of the VOA vial. It works equally well to prepare standards in 50- or 100-mL volumetric flasks and pour the aqueous solutions into VOA vials.
- If several VOA vials of the same solution are being prepared at one time, do not prepare the solution in a single large volumetric flask. There will be some VOC loss by pouring repeatedly from the flask. Instead, spike vials individually or use the apparatus described in Figure 1 for sample transfer.
- When preparing calibration standards, transfer the same amount of methanolic solution to each VOC sample. This requires preparing secondary dilutions in methanol for each calibration level instead of spiking different amounts of a single standard.

Leaks: Leaks anywhere in the system can result in poor precision, loss of sample, and calibration failure. Leaks in the carrier gas flow path can easily be detected by the MSD as a high background of oxygen and nitrogen. To correct leaks, tighten or replace the offending fittings after finding the leaks using established techniques. A more difficult problem to detect results from leaks in the fittings that connect the purge vessel to the P&T instrument. Even the smallest leaks during the purge cycle can result in the loss of VOCs and cause poor precision. Leaks that a helium leak detector might miss, can still cause VOC loss. If all the RFs for a given calibration level seem to be low by a similar amount, or if the RF RSDs are all very similar (but too large), then P&T leaks are the likely cause. Tighten or replace the fittings associated with the purge vessel.

Conclusions

EPA Method 8260B with P&T sample introduction is one of the most widely used water analysis methods. There are numerous P&T, GC, and MS variables to optimize in order to obtain long-lasting linear calibration curves and good analytical results. This application note summarizes much of Agilent's experience in optimizing all facets of this VOC method. Most analysts know how to prepare calibration and check samples, tune the MSD, and set instrument parameters; and they find this method to be very rugged with infrequent need for retuning and recalibration. The suggestions in this paper are designed to help in case problems do arise or when an analyst runs this method for the first time. Though the focus was on Method 8260B, these techniques apply to almost any P&T/GC/MS method.

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Printed in the USA
February 25, 2004
5989-0603EN

Agilent Technologies

Stir Bar Sorptive Extraction: A New Way to Extract Off-Flavor Compounds in the Aquatic Environment

Application

Food and Flavors

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Abstract

The objective of this study was to analyze organic off-flavors in water by gas chromatography/mass spectrometry (GC/MS) using Stir Bar Sorptive Extraction (SBSE). Six compounds were quantitatively determined using Selected Ion Monitoring (SIM): 2-methylisoborneol (MIB), geosmin, 2,4,6-trichloroanisole, 2,3,6-trichloroanisole, 2,3,4-trichloroanisole and 2,4,6-tribromoanisole. The Limit of Quantification (LOQ) was found to be from 0.1 ng/L to 0.2 ng/L for haloanisoles, 0.5 ng/L for geosmin and 1 ng/L for MIB. Relative standard deviation at the quantification limit ranges from 7% to 14.6%. Recovery was evaluated by spiking real water samples. It ranged from 80% to 120% depending on the compound. GC/MS detection in the scanning mode combined with olfactometry were used for qualitative analysis in order to characterize new odorous compounds. Using this technique, it was possible to extract and analyze more than 20 samples a day.

Introduction

Complaints received by water companies are most often due to bad taste and odors in drinking water. Furthermore, the presence of these unpleasant tasting but otherwise harmless compounds can be taken as unsafe water by the consumer. In most cases, complaints concern chlorine and earthy/musty smelling compounds. A better understanding of the chemical causes of taste and odors in drinking water supplies would help in the control of taste and odor problems.

For 30 years, it was commonly accepted that earthy/musty aromas in drinking water were associated with the presence of geosmin, MIB and/or haloanisoles [1, 2, 3]. MIB and geosmin have strong odors, which are detectable at extremely low thresholds. MIB has a woody or camphor odor, detectable at a threshold ranging from 5 to 10 ng/L, while geosmin has a characteristic earthy odor detectable in water at a threshold ranging from 1 to 10 ng/L [4, 5]. The presence of these compounds in water was previously associated with the presence of actinomycetes or their metabolic products [6, 7, 8] in raw water, as well as cyanobacteria and fungi [9, 10, 11]. Haloanisoles have a

musty odor at a low threshold. For instance, the threshold odor of 2,4,6-trichloroanisole ranges from 0.05 to 4 ng/L. Their formation is probably caused by microbiological methylation of halophenols during water treatment or during transport through the distribution system [12, 13, 14]. Halophenols are formed during chlorine disinfection of drinking water and some of them have been identified as natural halogenation products [15].

For a long time, the identification of these compounds in water has been a real analytical problem because they are odorous at very low concentrations. The main analytical method used to identify odorous compounds in water is Closed Loop Stripping Analysis (CLSA). With this method [16, 17], organic substances are released from the water sample in a hermetically sealed, closed circuit system, which uses air or inert gas at 40 °C to strip away the volatiles. These liberated substances are transferred to a very small amount of charcoal localized in the closed circuit. Finally, the organic substances are eluted from the charcoal with solvent and are analyzed by GC. "Purge and Trap" analysis is based on the same principles as CLSA, but it exhibits lower sensitivity and, therefore, is very useful for concentration levels above 100 ng/L. Nevertheless, these "stripping" techniques were not efficient enough for less volatile and/or more polar compounds. Some authors have used solid phase micro extraction (SPME) [18]. From a chromatographic point of view, GC linked with MS is the only detection method, which combines high powers of separation, identification, and quantitation.

Today, a novel extraction technique that is sensitive, simple, and fast is an alternative choice to conventional stripping methods. This SBSE technique is based on sorption instead of adsorption. The principle includes a magnetic stirring bar incorporated into a glass jacket coated with a 0.5-mm layer of polydimethylsiloxane (PDMS). Extraction is performed by placing a suitable sample amount in a vial, adding a stir bar, and stirring for 30 to 120 min. After extraction, the stir bar is introduced into a glass desorption tube and placed in a thermal desorption unit where it is desorbed at 200–300 °C. Compounds are detected using GC/MS.

The aim of the present study was to analyze six odorous organic compounds in water with the SBSE technique. These compounds (Table 1) must be quantified at the subnanogram/L level, under or close to their odor threshold.

Table 1. Analyzed Odorous Compounds

Name	Abbreviation	Taste	Odor threshold, ng/L	CAS number
2-methylisoborneol	MIB	Earthy	5–10	N/A
2,4,6-trichloroanisole	2,4,6-TCA	Musty	0.1–2	6130-75-2
2,3,6-trichloroanisole	2,3,6-TCA	Musty	0.1–2	50375-10-5
Geosmin	Geosmin	Camphor	1–10	19700-21-1
2,3,4-trichloroanisole	2,3,4-TCA	Musty	0.2–2	54135-80-7
2,4,6-tribromoanisole	2,4,6-TBA	Musty	0.15–10	607-99-8

Principles of SBSE

The analysis of odorous organic compounds in aqueous environmental samples must be performed after extraction and enrichment of the solutes from the matrix. Some 10 years ago, a new method was developed called SPME. With this extraction based on sorption, a relatively thin layer of PDMS (7–100 µm) coated on the outside of a needle device was used as the extraction medium. Sorptive enrichment offers several advantages over adsorption processes. These advantages include:

- Predictable sorption thanks to calculated or experimental $K_{O/W}$ [19]
- Absence of displacement effect (no breakthrough volume)
- Faster and milder desorption

In contrast to stripping techniques, SPME and SBSE are equilibrium techniques by nature, based on the partitioning of the solutes between the PDMS phase and the aqueous (or gas) matrix. In fact, the principle of these techniques is the same as liquid-liquid extraction (LLE), but with a very low quantity of solvent (0.5 µL of PDMS for SPME and 24 to 100 µL of PDMS for SBSE).

The theory of SBSE is straightforward and similar to SPME. With the approximation that the partitioning coefficient between PDMS and water ($K_{PDMS/W}$) is proportional [19] to the octanol-water partitioning coefficient ($K_{O/W}$), it can be shown that equilibrium is based on Equation 1. Recovery (R) is based on Equation 2 where m_{PDMS} is the quantity absorbed in the PDMS phase, m_W is the quantity of non-extracted analyte, β is the ratio of the volume of water/the volume of PDMS, and m_0 is the initial quantity.

$$1.\quad K_{o/w} \approx K_{PDMS/W} = \frac{C_{PDMS}}{C_W} = \frac{m_{PDMS}}{m_W} \times \frac{m_{PDMS}}{m_W} = \frac{m_{PDMS}}{m_W} \times \beta$$

$$2.\quad R = \frac{m_{PDMS}}{m_0} = \frac{\left(\frac{K_{O/W}}{\beta}\right)}{1 + \left(\frac{K_{O/W}}{\beta}\right)}$$

The only parameter governing the recovery of an analyte from the sample is the ratio of distribution coefficient and the phase ratio between the PDMS coated on the stir bar and the water sample.

Figure 1 illustrates the extraction recovery of a compound as a function of $K_{O/W}/\beta$ ratio. At a $K_{O/W}/\beta$=1, the recovery is 50%. At low $K_{O/W}/\beta$ values, the recovery is closely proportional to $K_{O/W}/\beta$ and extraction is minimal.

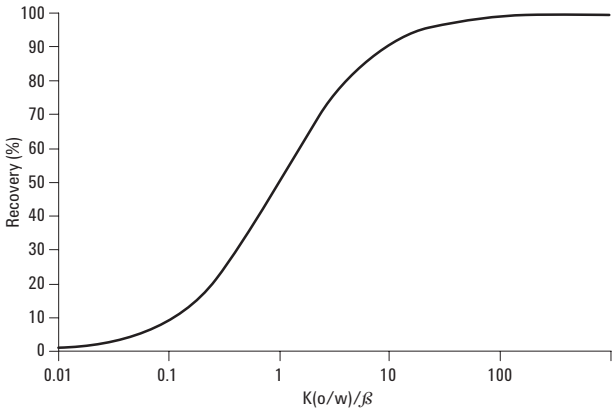

Figure 1. Recovery as a function of octanol-water partitioning constant and phase ratio.

In SPME, the maximum volume of PDMS coated on the fiber is 0.5 µL. For a typical sample volume of 10 mL, the phase ratio equals 2×10^4. This implies that quantitative extraction is only obtained for compounds with a $K_{O/W}$ in excess of 10^5. Only a very limited number of components exhibit such high $K_{O/W}$ values and, moreover, it was recently shown [20] that this type of apolar solute strongly adsorbs onto the stir bar and glass vial, as used in SPME. In SBSE, on the other hand, the situation is more favorable. A stir bar coated with 100 µL of PDMS can easily be used to extract 10 mL of water leading to a β factor of 100, which implies that solutes with $K_{O/W}$ in excess of 500 are quantitatively extracted into the PDMS coated stir bar. This not only renders quantification straightforward but also ensures a significant sensitivity for those compounds with $K_{O/W}$ below 10^5.

In Figure 2, the theoretical extraction recovery of analytes from a 10-mL water sample is shown for SPME and SBSE. It is clear that quantitative extraction is obtained at much lower $K_{O/W}$ in SBSE compared to SPME. This is due solely to the much lower phase ratio in SBSE. In case of incomplete extraction with SBSE, calibration is still possible using water samples with known concentrations of the target solutes.

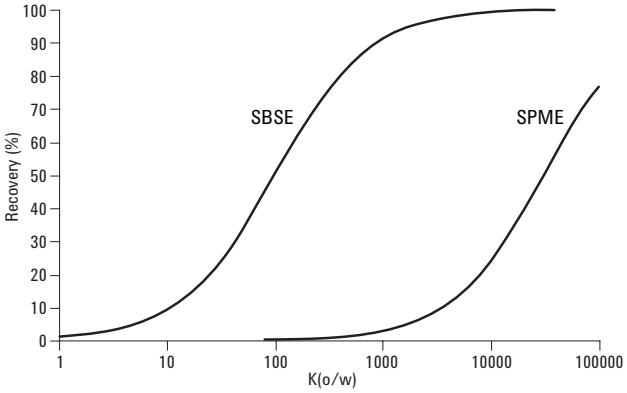

Figure 2 . Theoretical recovery as a function of octanol-water partitioning constant and typical phase ratio for SBSE and SPME (that is, the volume of PDMS on the SPME fiber = 0.5 µL, the volume of PDMS on the SBSE stir bar = 100 µL, and the volume of extracted water = 10.0 mL).

So far, the discussion has been limited to the equilibrium conditions of SBSE. However, considering the thickness of the coating (0.5 or 1 mm), the speed of extraction (required equilibration time) is also an important factor to consider. Due to the thickness of the coating, it is assumed that all resistance to mass transfer is in the coating and that the sample is perfectly stirred. For this situation it is possible to apply Equation 3 [21]

$$3.\quad t_{95\%} = \frac{d^2_{PDMS}}{2D_{PDMS}}$$

where $t_{95\%}$ is the time required to reach 95% extraction, d_{PDMS} is the thickness of the PDMS layer used (in meter), and D_{PDMS} is the diffusion coefficient of the analyte under investigation in PDMS, in m^2/s. For instance, for benzene (D_{PDMS}=2.5*10^{-10} m^2/s) the equilibration time is 30 minutes.

Experimental

Equipment

The gas chromatograph used was an Agilent 6890 - Agilent 5973 MSD (Agilent Technologies, Palo Alto, CA, USA)-olfactometric detector combination (GERSTEL® GmbH, Mülheim a/d Rhur, Germany). This chromatograph was equipped with a thermal desorption unit (TDSA) and a PTV inlet (CIS-4) from GERSTEL GmbH, Mülheim a/d Rhur, Germany.

Samples were extracted with 20-mm long stir bars (also called GERSTEL-Twister®) having a 0.5-mm layer of PDMS. The stir bar was thermally desorbed in the splitless mode using the following desorption temperature program: 30 °C (0.8 min), 60 °C/min to 280 °C (5 min). The desorbed solutes were cryofocused in the CIS-4 at -100 °C. After the stir bar desorption, the PTV inlet was programmed to 300 °C at 10 °C/s and held for 2 min. Injection was done in solvent vent mode. The compounds were separated on a 30 m × 0.25 mm id × 0.25 µm HP5-MS capillary column using helium carrier gas at 1.5 mL/min (constant flow). The oven was programmed from 50 °C (2 min) to 200 °C at 10 °C/min then to 300 °C at 25 °C/min (2 min). Detection was achieved in SIM mode for quantitative analysis and in scan mode for qualitative analysis. The olfactometer transfer line was heated at 250 °C. One-third of the effluent was directed to the mass spectrometer and two-thirds to the olfactometer.

Chemical Standards and Reagents

- Methanol (pesticide grade) obtained from Merck (Darmstad, Germany)
- Spring water to prepare blanks and standards
- The standard compounds 2-methylisoborneol; 2,4,6-trichloroanisole; 2,3,6-trichloroanisole; 2,3,4-trichloroanisole; 2,4,6-tribromoanisole; geosmin; and 2,4,6-trichloroanisole-d_5 obtained from Promochem (France).
- A stock solution containing MIB, geosmin, and the haloanisoles at 1 µg/L was prepared in spring water. Storage conditions for this stock solution: 4 °C for 1 month.
- An internal standard solution of 2,4,6-TCA-d_5 prepared in spring water at 20 µg/L. Storage conditions for this solution: 4 °C for 1 month.

Extraction Procedure

Extractions were performed in duplicate by placing a Twister (20-mm long, 0.5 mm of PDMS) into a 125-mL vial with 100 mL of the water sample and 5 mL of methanol. Each vial was spiked with 40 µL of the 2,4,6-TCA-d_5 internal standard solution. After stirring both samples for 2 hours at room temperature, the Twisters were removed from the duplicate samples and dried with a clean wipe. In order to increase sensitivity, both Twisters were introduced into a single glass desorption tube and desorbed using the conditions noted above.

Results and Discussion

Tuning the Mass Selective Detector (MSD)

To enhance sensitivity even further, the MSD was tuned manually in order to increase transmission of desired ions. Perfluoro-5,8-dimethyl-3,6,9-

trioxadodecane (PFDTD) was used as the calibrant. Conventional Autotunes are performed for optimum monitoring of the 69, 219, and 502 PFTBA ion ratios. The 219/69 and 502/69 ratios are usually about 60% and 3%, respectively, although this can vary considerably. Since the target compounds have masses ranging from 112 to 344, manual tuning was used to adjust the 219/69 ratio to 110% and the 414/69 ratio to 10%. The 414 ion was used instead of 502 because it is closer in mass to the target ions of the analytes. These ratios could be obtained using one of two procedures. The first approach was to ramp the repeller for ions 69, 219, and 414 and to choose the optimum response for 219. The second way was to perform a Target Tune by specifying the desired abundances and ion ratios for selected ions in the Agilent ChemStation.

This manual tune was used only for quantitative applications because structural information was not required. When using this manual tune, the Probability Based Matching System gave no satisfactory matching between an unknown and a reference spectrum from the NIST or Wiley libraries.

Mass Spectra of MIB, Geosmin and Haloanisoles

Figure 3 shows the experimental mass spectra for the target compounds listed in Table 1. For monitoring ions in the SIM mode, 95, 108, 110 were chosen for MIB, 112 and 125 for geosmin, 210 and 212 for the three chloroanisoles, 346 and 344 for the 2,4,6-tribromoanisole. The internal standard, 2,4,6-trichloroanisole-d_5, was monitored at *m/z* 217.

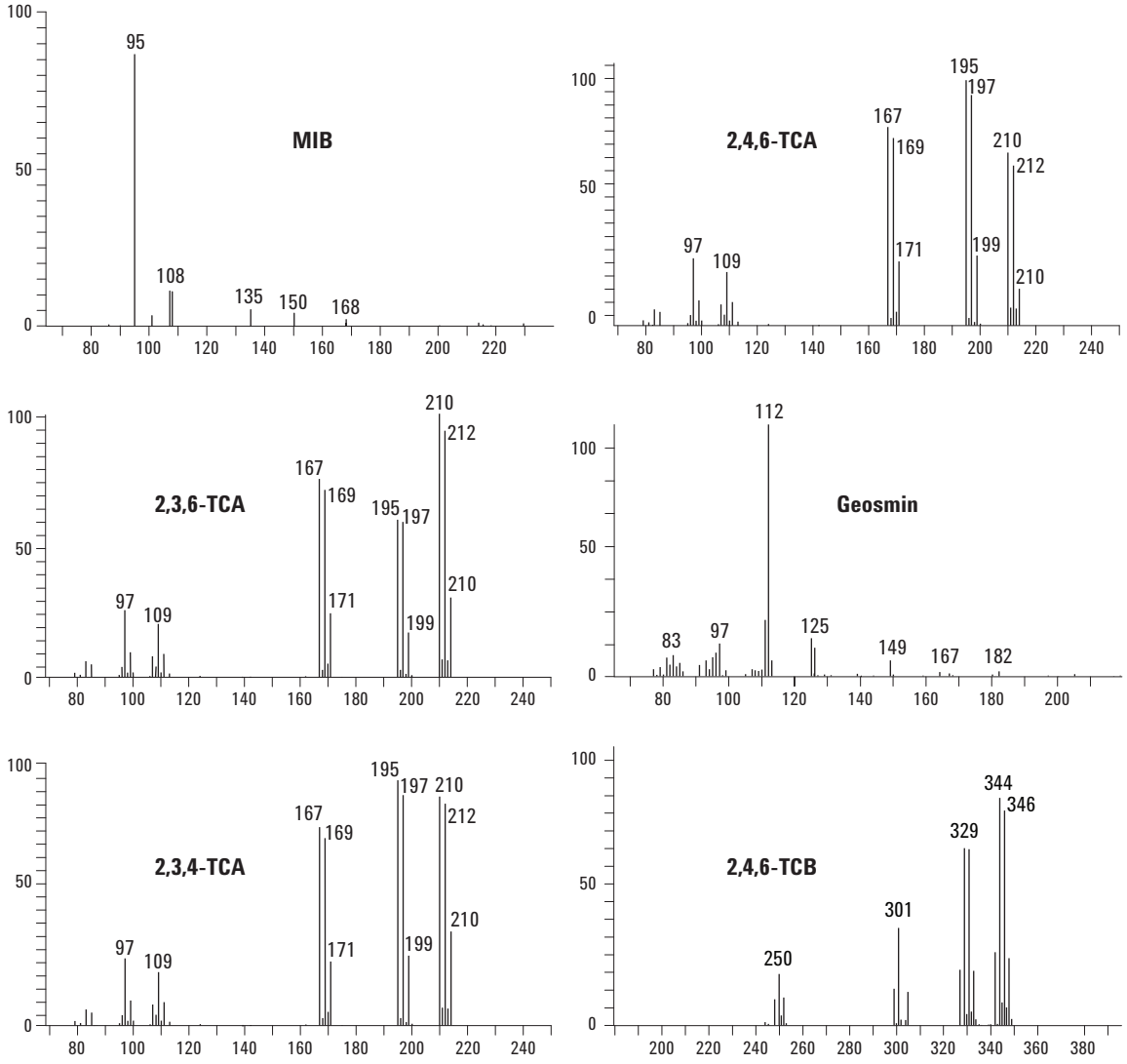

Figure 3. Experimental mass spectra of target compounds.

Figure 4 shows a SIM chromatogram of spring water spiked with 2 ng/L of each target compound.

Influence of Extraction Time

This experiment measured the sorption rates of compounds into PDMS. Spring water spiked with 2 ng/L of each compound was analyzed after extraction times ranging from 15 min to 300 min. Figure 5 shows the relationship between the extraction time and the response obtained for target compounds.

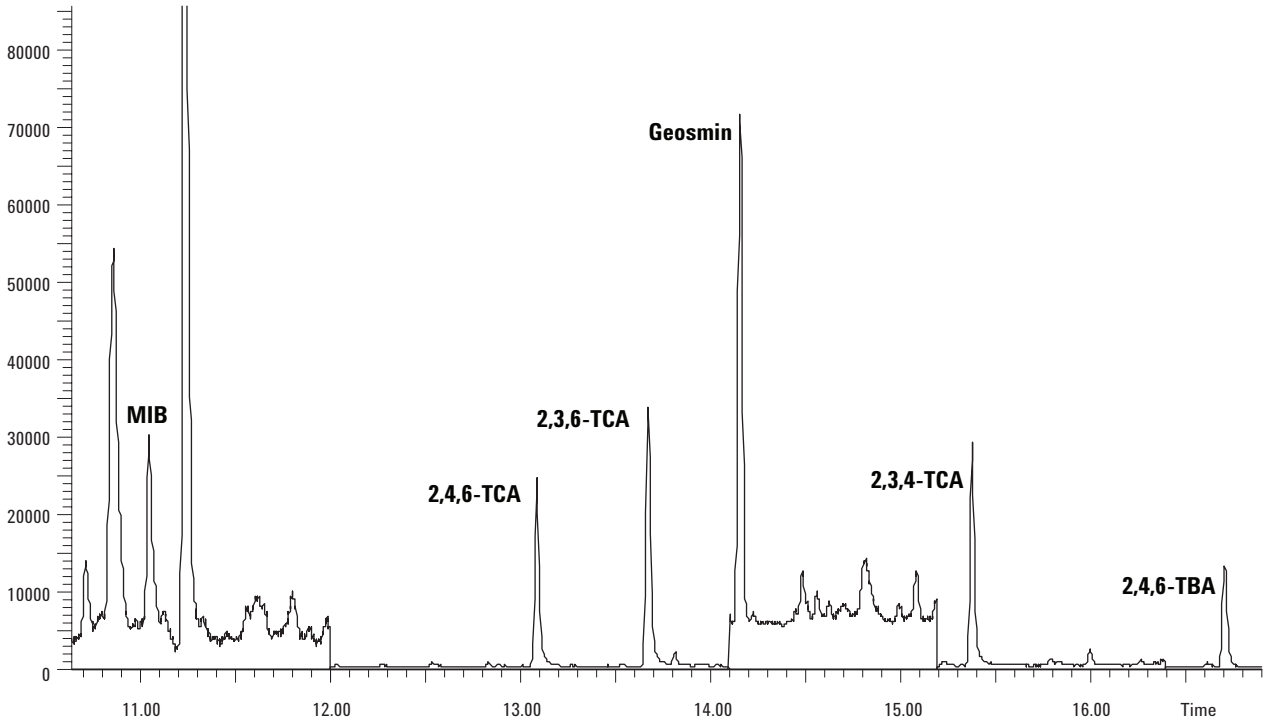

Figure 4. SIM chromatogram of target compounds.

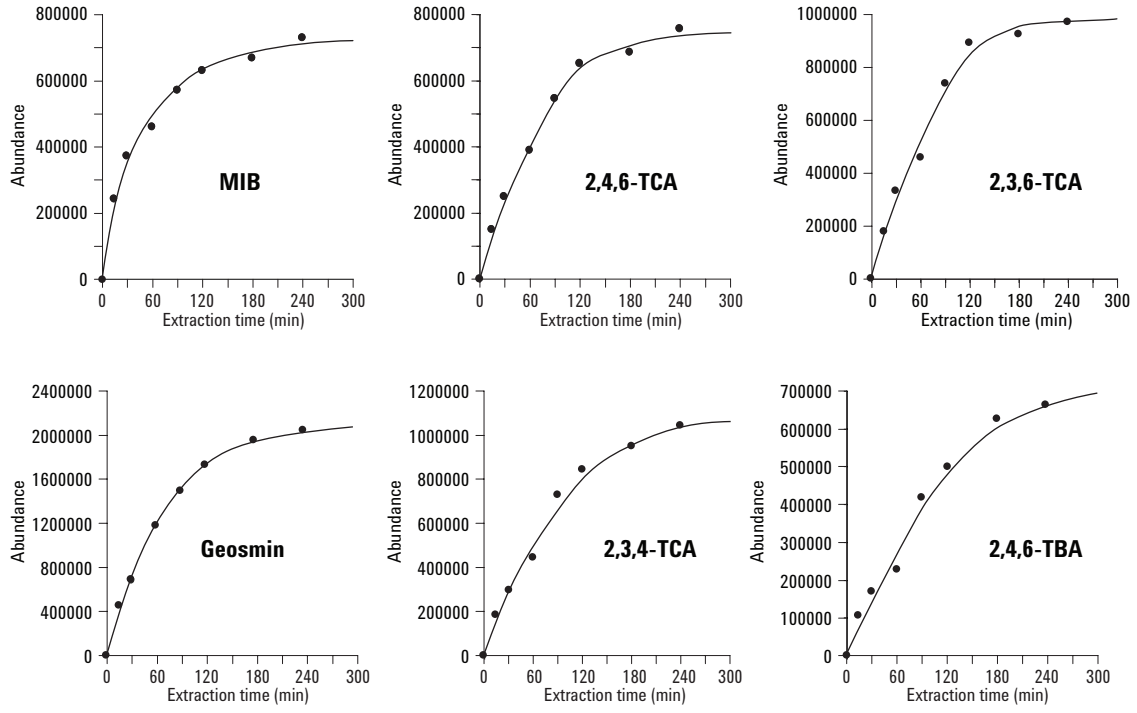

Figure 5. Influence of extraction time upon quantity extracted on PDMS.

For all compounds, the sorption rate is fast for the first 120 minutes and then slows without reaching a plateau. For routine analysis with high sample throughput, an extraction time of 120 minutes was empirically chosen.

Influence of Sample Volume

According to Equation 2, maximum recovery can be estimated with the octanol/water distribution coefficient ($K_{O/W}$). Log $K_{O/W}$ values for the target compounds were experimentally determinated and calculated using KnowWin software [19].

Table 2. Octanol/Water Distribution Coefficients ($K_{O/W}$) of Investigated Compounds

Name	Experimental log $K_{O/W}$	Calculated log $K_{O/W}$
2-methylisoborneol	3.31	2.85
2,4,6-trichloroanisole	3.85	4.01
2,3,6-trichloroanisole	3.64	4.01
Geosmin	n/a	3.57
2,3,4-trichloroanisole	3.74	4.01
2,4,6-tribromoanisole	4.48	4.75

For this experiment, different sample volumes of spring water from 10 to 200 mL were spiked with 1 ng of each compound. Extraction was done for 2 hours with a 2-cm long Twister (47 µL PDMS). Figure 6 shows experimental recoveries (A) compared to theoretical recoveries estimated using calculated $K_{O/W}$ (B) and experimental $K_{O/W}$ (C) values.

Experimental results were in accordance with theory (the more sample volume increases, the more the recovery decreases), but were inferior to expected values. This experiment proved that equilibrium was not reached after 2 hours of stirring. The difference between experimental and expected values increased when the sample volume increased and it was dependant on the compound.

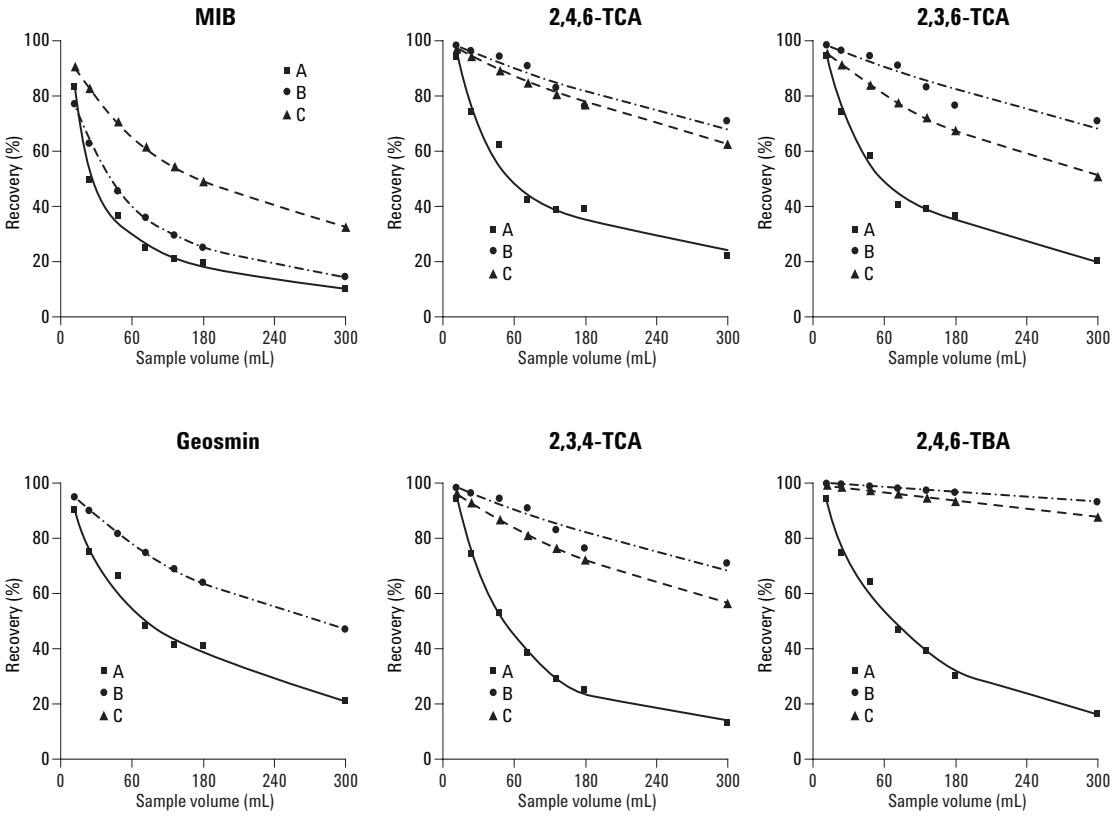

Figure 6. Influence of sample volume on recovery. A: Experimental results, B: Theoretical results (calculated $K_{O/W}$), C: Theoretical results (experimental $K_{O/W}$).

However, the enrichment on the PDMS media increases with the sample volume as shown in Figure 7. For most of the compounds, the extracted amount increases up to 100 mL of the sample and a volume of 200 mL does not lead to a significant gain in response. In order to achieve concentrations close to the odor threshold, it was necessary to use two 100-mL aliquots of each sample and two Twisters, which were desorbed together.

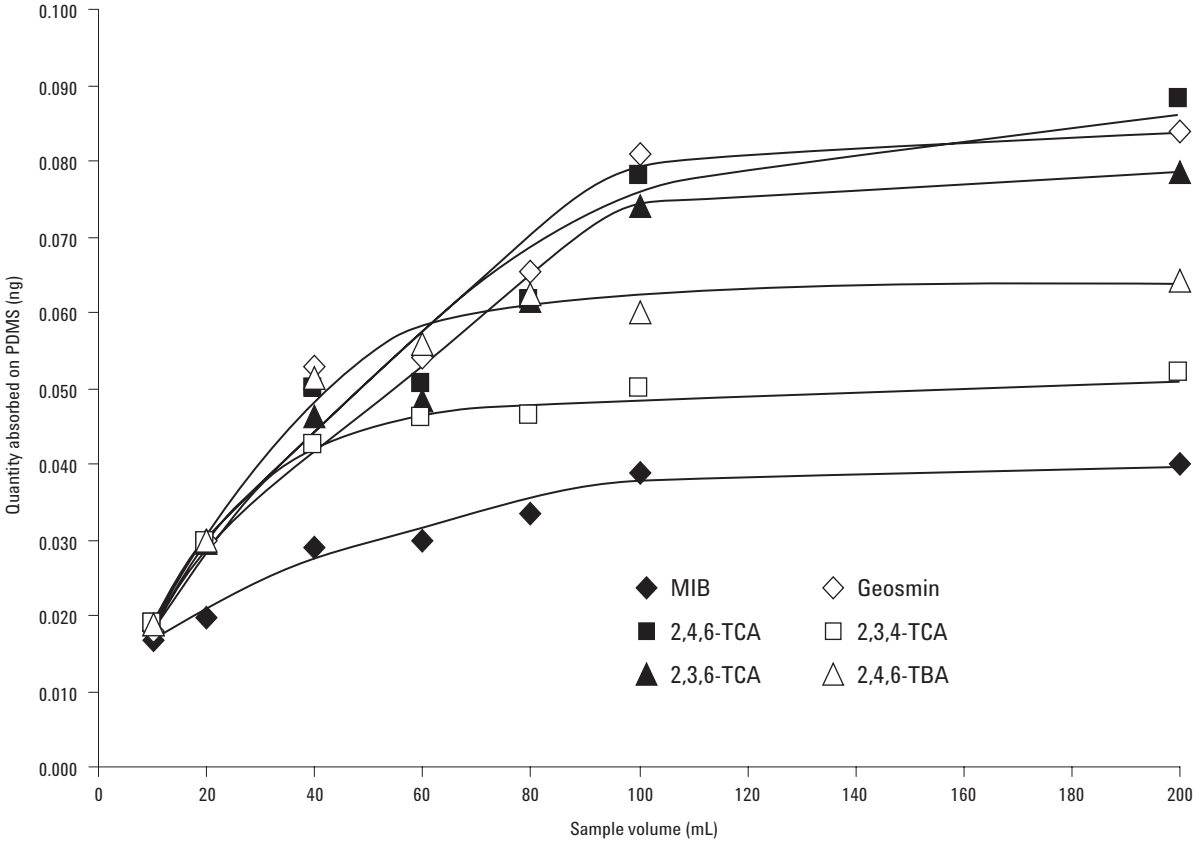

Figure 7. Influence of sample volume on quantity extracted.

Influence of Storage After Extraction

Extraction of a spring water sample spiked with 2 ng/L of each compound was replicated six times; each 100-mL sample was extracted by one Twister for 2 hours. One Twister was analyzed immediately and the others were stored at 4 °C in closed vials for later analysis. Figure 8 shows the influence of storage time on the response for all compounds.

These results show that no compound loss occurs during 1 week of storage and imply that:

- It is possible to store the Twister after extraction instead of storing water samples when the chromatographic analysis cannot be done immediately.
- Instead of sending bad tasting or odorous water samples to the laboratory, it would be possible to extract off-flavor compounds directly at the consumer's home.

Method Validation

This method was validated according to the AFNOR regulation XP T 90-210. This validation determines the following:

- The scope of linearity: linearity was studied over seven concentration levels, from 0.1 to 10 ng/L, replicated five times. Calibration was done in internal standard mode with 2,4,6-TCA-d_5. Linearity is achieved when the correlation coefficient (R) is better than 0.999.
- The LOQ is validated when the relative standard deviation (RSD) of 10 replicate samples, spiked with supposed LOQ, is under 20%.
- The repeatability is expressed as %RSD and is calculated on the basis of three replicates of eight different water samples. It must be under 20%.
- The trueness is expressed as the percent recovery of spiked real water samples and must be between 80% and 120%.
- The reproducibility is expressed as a %RSD of a check calibration standard (2 ng/L). It must be under 20%.

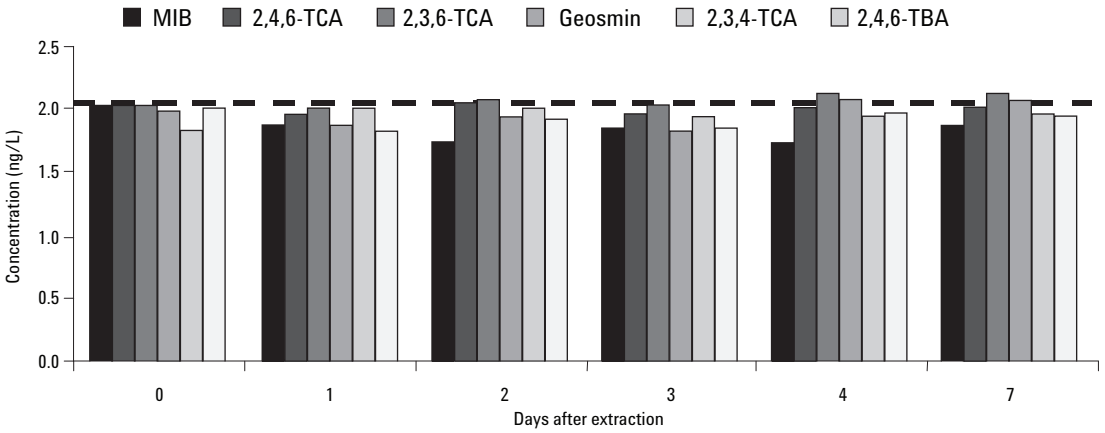

Figure 8. Influence of Twister storage time after extraction.

The results of this validation are summarized in Table 3 and in the calibration curves shown in Figure 9.

Table 3. Validation Results for Target Compounds

	R	LOQ, ng/L	Repeatability %	Trueness %	Reproducibility %
MIB	0.9987	1	4–10	89–110	13
2,4,6-TCA	0.9998	0.1	1–5	97–110	4
2,3,6-TCA	0.9998	0.1	4–11	97–117	5
Geosmin	0.9991	0.5	2–10	83–101	9
2,3,4-TCA	0.9998	0.2	7–15	87–110	13
2,4,6-TBA	1.0000	0.2	2–9	91–104	15

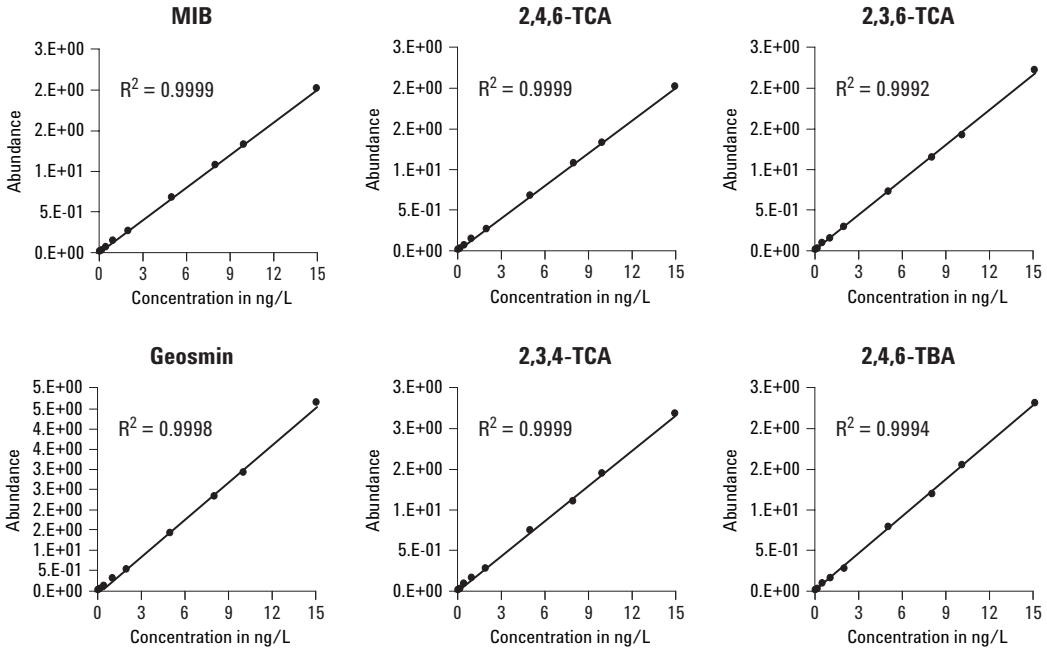

Figure 9. Calibration curves for investigated compounds.

The validation criteria were achieved for all target compounds.

Application to Real Water Samples

Different water samples were analyzed following complaints about taste and odor problems.

Case 1

Two samples (A and B) were collected at the consumer's home. Sample A gave a very pronounced musty odor and sample B gave a soft musty odor and a pronounced metallic odor. Samples A and B were treated by SBSE and analyzed in SIM mode in order to detect MIB, geosmin, and the haloanisoles. Quantitative results and chromatograms for each sample appear in Table 4 and in Figure 10.

Table 4. Concentration of Target Compounds in Sample A and B

	Sample A [C] (ng/L)	Sample B [C] (ng/L)
2-methylisoborneol	<1	<1
2,4,6-trichloroanisole	**8.9**	**0.2**
2,3,6-trichloroanisole	<0.1	<0.1
Geosmin	**5.2**	<0.5
2,3,4-trichloroanisole	<0.2	<0.2
2,4,6-tribromoanisole	**0.4**	**1.3**

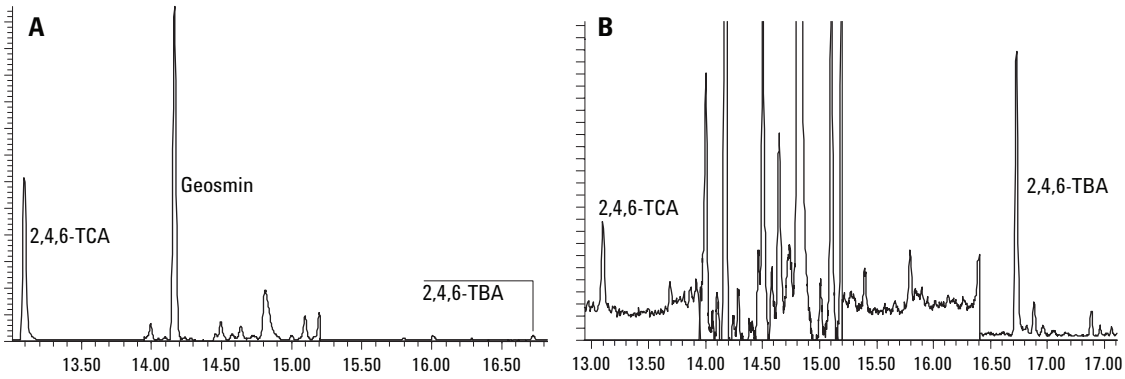

Figure 10. SIM chromatograms for samples A and B.

The concentration levels found in both samples can certainly explain the musty odor. In order to identify the other odorous compounds, the water samples were treated another time by SBSE without internal standard, and the GC/MS was run in the scan mode.

For sample A, the olfactometric detection showed a pronounced musty odor at the retention times of 2,4,6-TCA and geosmin, but also a medicinal one at 8 minutes and a solvent-like one at 14 minutes. For sample B, the olfactometric detection gave a mild musty odor at the retention time of 2,4,6-TBA and also a medicinal one around 8 minutes.

Interpretation of isotope ratios in the spectra for sample A showed two halogenated compounds - a brominated one (8.4 min) and a chlorinated one (13.9 min). The medicinal odor was associated with dibromoiodomethane, which is a chlorination byproduct. The solvent odor was associated with tetrachlorobenzene as shown in Figure 11. For sample B, dibromoiodomethane was also detected.

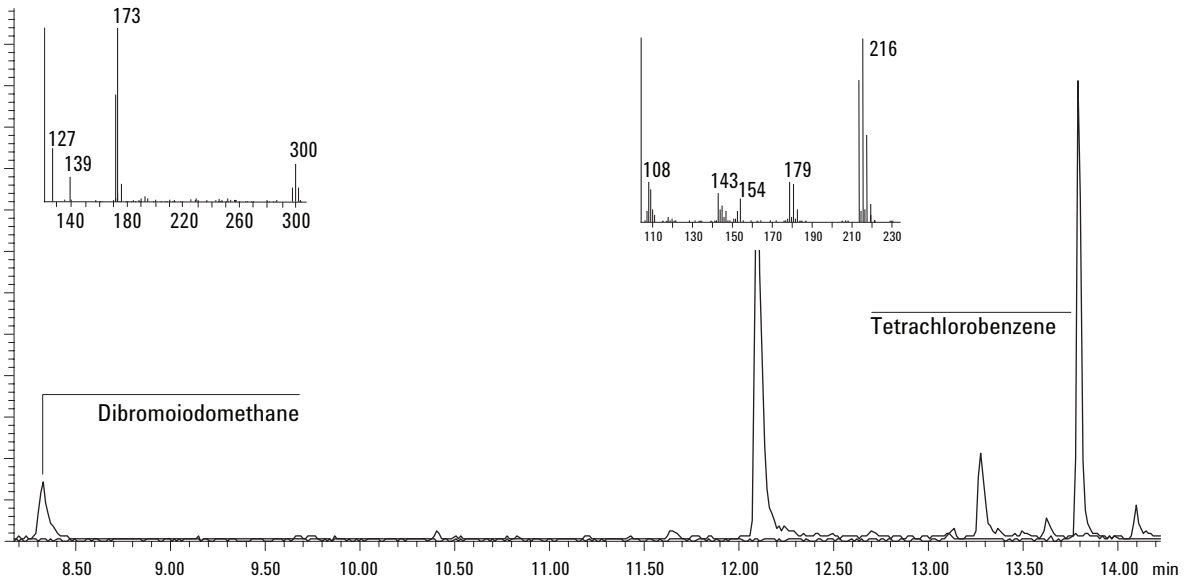

Figure 11. Sample A Scan chromatogram.

Case 2

In this case, an off-flavor episode occurred in a tank located near Paris. Degradation of the water's organoleptic quality was observed soon after some cracks appeared on the tank's coating and important living organisms were found on the interrior surface. Following complaints, several flavor analyses were performed on water originating from the tank. Results indicated that the chlorinous taste of the treated water was masked by an intense musty taste (Threshold Test Number: 5).

Drinking water stored in this tank is produced from ground water which undergoes a two-step treatment process: the water first undergoes aeration and sand filtration for iron removal and then the water is chlorinated just prior to entering the tank. The tank's coating, which must provide an impermeable seal to the water during storage, is a synthetic coating prepared by mixing a gray elastic cement and a white synthetic resin in aqueous solution. The theoretical mechanical and physical properties of this coating ensure high elasticity and no release of organic compounds. Filtered and chlorinated waters were treated by SBSE for quantitative analysis in order to search for the target odorous compounds.

Quantitative results and chromatograms of each sample appear in the Table 5 and in Figure 12.

Table 5. Concentration of Target Compounds in Filtered and Chlorinated Waters

	Filtered water [C] (ng/L)	Chlorinated water [C] (ng/L)
2-methylisoborneol	<1	<1
2,4,6-trichloroanisole	<0.1	<0.1
2,3,6-trichloroanisole	<0.1	<0.1
Geosmin	<0.5	<0.5
2,3,4-trichloroanisole	<0.2	<0.2
2,4,6-tribromoanisole	< 0.2	**5.6**

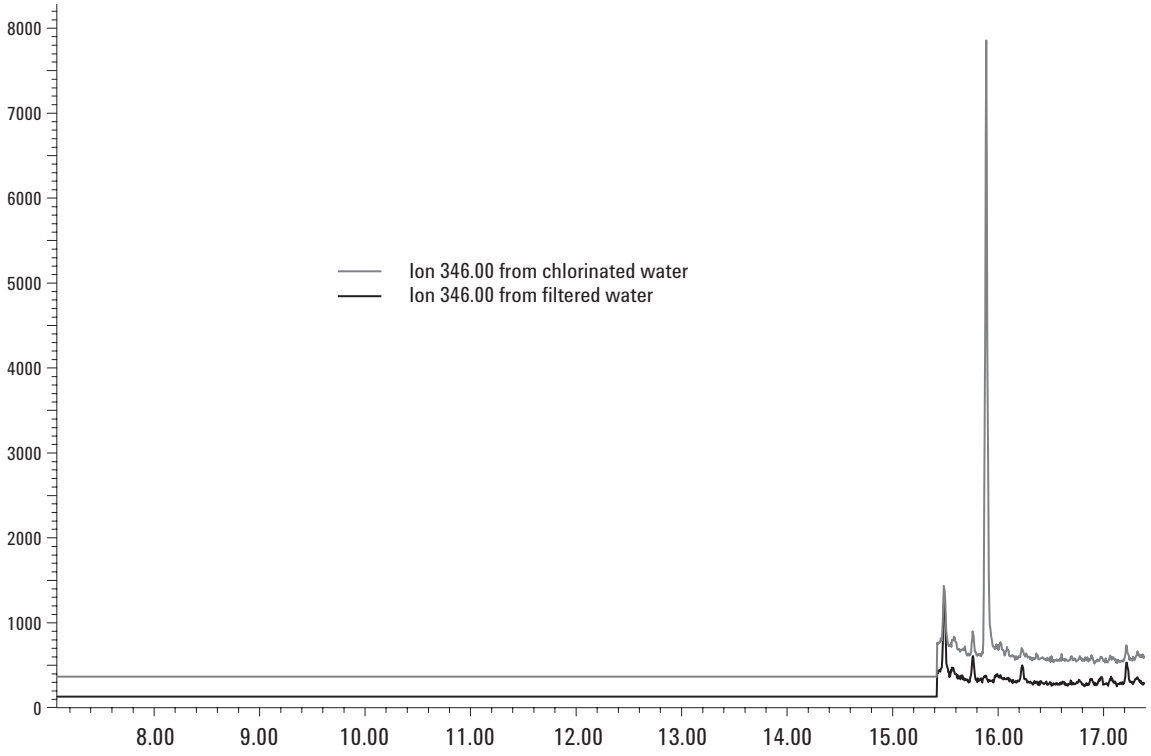

Figure 12. EIC (*m/z:* 346) of chlorinated and filtered water.

The only compound found among the six targets was 2,4,6-tribromoanisole at a concentration of 5.6 ng/L. The presence of 2,4,6-TBA can easily explain the significant musty taste imparted to the water. None of the target compounds was found in the filtered water.

GC with olfactometric detection of filtered water did not exhibit any of the characteristic odors. However, for chlorinated water, it gave a significant musty odor at the retention time of 2,4,6-TBA in addition to different phenolic odors at around 8, 14, and 17 minutes. In order to make phenolic compounds more amenable to GC, they were derivatized with 1 g of K_2CO_3 and 500 µL of acetic anhydride for 100 mL of water sample. Detection was achieved in scan mode for qualitative analysis. Results obtained for both sniffing and MS detection appear in Table 6 and in the scan chromatogram in Figure 13.

Table 6. Odors Generated During the Chromatographic Run Time for Sample B

Tr (min)	Odor	Intensity	Qualification (acetate derivative)
8.5	Phenolic	++++	Phenol
13.7	Phenolic	++	2,4,6-trichlorophenol
15.9	Musty		2,4,6-tribromoanisole
16.8	Phenolic	+++	2,4,6-tribromophenol

According to these results, the hypothesis was that the tank's coating released phenol, which was halogenated to 2,4,6-TCP and 2,4,6-TBP because of the residual chlorine. 2,4,6-TBA was then synthesized by living organisms present at the surface of the coating. The authors cannot yet explain why only 2,4,6-TBA was formed by living organisms despite the presence of both 2,4,6-TCP and 2,4,6-TBP.

Case 3

This case consisted in studying deterioration in organoleptic quality of water along the network distribution system. The complaints came only from consumers who were located far from the treatment plant. Two samples were taken - the first one at the outlet of the treatment plant (sample A) and the second one at the consumer's home at the end of the network (sample B). Sample A gave only a chlorine odor whereas sample B gave musty, swampy, earthy odors (Threshold Test Number: >10).

The two samples were treated by SBSE in order to monitor MIB, geosmin, and haloanisols. The results showed that sample A was free of these compounds. In sample B, 2,4,6-TCA and 2,3,4-TCA were found at 0.1 ng/L and 0.2 ng/L, respectively. However, these concentrations cannot explain the significant taste and odor impairment. Fresh water samples were treated another time by SBSE without internal standard. These were analyzed by TDS/GC/MS in the scan mode and by olfactometry.

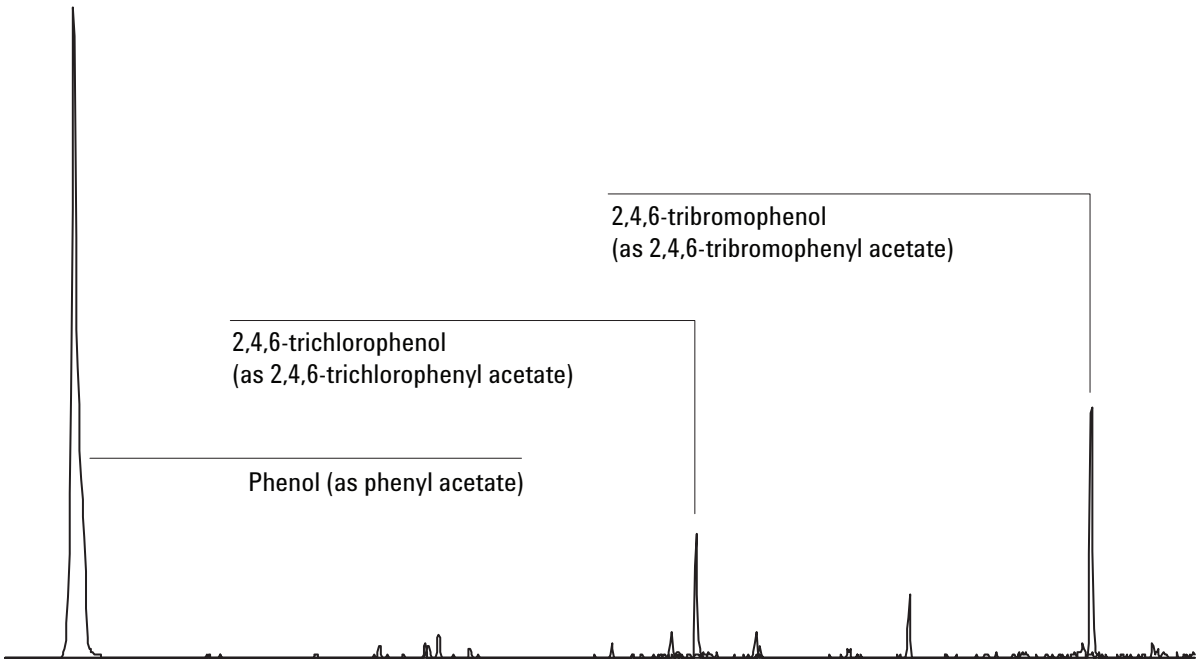

Figure 13. TIC of water sample after insitu-derivatization.

Olfactometry allowed the detection of various odors along the chromatographic run time for sample B, whereas nothing was smelled in sample A. The results for sample B for both sniffing and MS detection are listed in Table 7.

Table 7. Odors Generated During the Chromatographic Run Time for Sample B

Tr (min)	Odor	Qualification
7	Sweaty	Phenylacetaldehyde
7.8	Swampy	Dimethyltrisulfide
10.7	Citrus	Decanal
12	Flower	Undecanal
12.8	Sweaty	Not qualified
12.9 to 15.2	Musty	Alkylbromobenzene isomers
16.07	Rancid	Isopropyldodecanoate
16.8 to 17.4	Tar	Diisopropylnaphthalene
20.15	Tar	Dodecahydrophenanthrene

Seven different odors were detected by sniffing detection and some of them were in good agreement with the flavor profile analysis, as for instance, the swampy smell associated with dimethyl trisulfide. A musty odor was smelled from 13 to 15 minutes and was matched to different alkylbromobenzene isomers, of which the major component was 2-methyl-4-isopropylbromobenzene. Rancid and tar odors corresponded with isopropyldodecanoate and dodecahydrophenanthrene, respectivly. Pleasant odors like sweet and fruity (aldehyde compounds) were not detected by tasters.

Figure 14 shows the comparison of total ion chromatograms (TIC) of each compound that could be smelled in samples A and B.

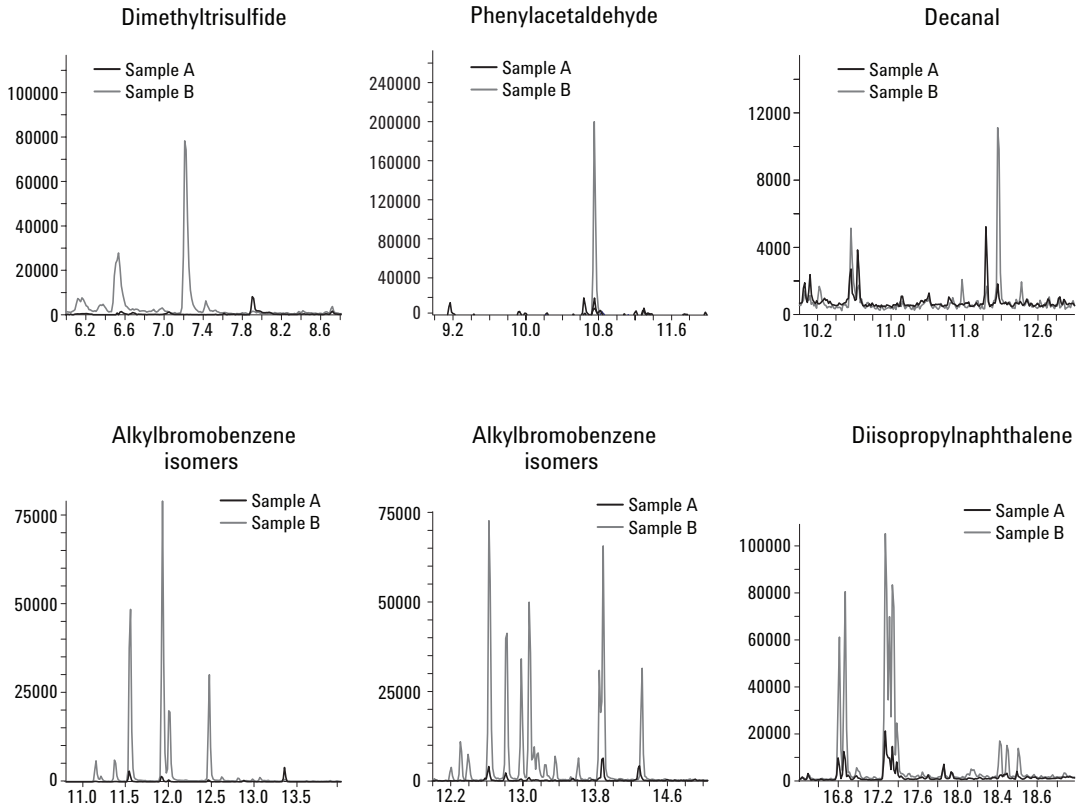

Figure 14. EIC of samples A and B for dimethyltrisulfide, phenylacetaldehyde, decanal, alkylbromobenzene isomers, and diisopropylnaphthalene.

Conclusions

- Most often, taste and odor problems in drinking water are due to very low traces of compounds present in a complex mixture. That is why GC/MS is the best separation and detection choice to quantify odorous compounds.
- A rapid SBSE-TD-GC/MS-Olfactometry method for the determination of MIB, geosmin, and haloanisol compounds in water samples was developed. The combination of TD-GC/MS and the SBSE made it possible to quantify all of the odorous components at levels close to or under their odor threshold limit.
- The influence of extraction time, sample volume, and storage time were studied in order to optimize the method's sensitivity. The final method was validated according to the AFNOR regulation. Linearity was checked with the correlation coefficient (R) ranging from 0.9987 to 1.0000. The repeatability and reproducibility values were under 15%. Recoveries were all between 87% and 117%, depending upon the compound.
- Storage time for Twisters is for at least 7 days after extraction without loss of the extracted compounds.
- When applied to real odorous water samples, SBSE showed a good correlation between flavor profile analysis, MS analysis, and olfactometric detection. In addition to the target compounds, it was possible to identify unknown odorous compounds at very low levels far more rapidly than possible using conventional techniques.

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Printed in the USA
March 31, 2003
5988-8900EN

Agilent Technologies

Analysis of Low Concentration Oxygenates in Environmental Water Samples Using Purge and Trap Concentration and Gas Chromatography/Mass Spectrometry

Environmental

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Abstract

The frequent use of oxygenate additives in gasoline to produce clean burning fuels has led to widespread and well documented contamination of ground water and drinking water supplies. The phasing out of methyl tert-butyl ether (MTBE) as the oxygenate of choice has led to an increased interest in testing for other common additives. These other additives may be ethers, other than MTBE, but also methanol or ethanol may be considered. Traditional techniques used for the analysis of volatile organic compounds in drinking and ground waters frequently employ the use of a purge and trap concentrator interfaced with a gas chromatograph. Detectors being used range from photoionization detectors (PID) and electrolytic conductivity detectors (ELCD) to mass selective detectors (MSD). Mass spectrometry is becoming the detection mode of choice for these additives, as it provides an additional level of confirmatory confidence in the presence of many potential matrix interferences. However, the challenge of extracting extremely polar analytes from an aqueous matrix requires modification and optimization of the purge and trap concentrator from its typical settings. As laboratories are seeking to determine these polar additives in the low part-per-billion (ppb) range, it is important that all aspects of the system be optimized. This application note will discuss system settings necessary for achieving low level quantitation of additives such as methanol and ethanol.

Experimental

This work was performed using a 6890 Plus gas chromatograph equipped with a 5973 mass spectrometer (Agilent Technologies, Inc. Wilmington DE). The purge and trap (P&T) used in the study was a model 3100 obtained from Tekmar/Dohrman (Cincinnati, OH). The J&W Scientific brand capillary column used, DB-VRX, was obtained from Agilent Technologies Inc. (Folsom, CA).

All standards used were prepared in-house from neat materials. Standard solutions were prepared in purified water.

Discussion

More and more frequently environmental laboratories are being asked to analyze for oxygenated analytes in drinking, ground and wastewater samples using pre-existing P&T GC/MS technology. Analytes such as acetone, ethyl ether, methyl-*tert*-butyl ether (MTBE), *tert*-butanol (TBA) and 2-butanone (MEK) are common, but now labs are beginning to receive an increasing number of requests for methanol and ethanol. As some laboratories are reporting very low method detection limits for these polar analytes, it is becoming apparent to others that not matching these low levels may eventually result in a loss of business. This work was performed for two primary reasons:

- To optimize P&T system conditions in order to achieve the best sensitivity possible for oxygenates in water
- To ascertain whether or not the low detection levels being reported by laboratories are realistic and achievable

The basic study design was as follows: Multiple replicates of an oxygenate standard (Table 1) were run using typical P&T conditions to establish a base response and to assure that reproducible results were being obtained. The %RSD for the multiple runs averaged 0.6%–0.8%. Once the base response was established analysts made modifications to the method parameters and charted any response changes for the same standard solution. Once the most significant modifications were defined they were combined to provide the best possible response enhancement. At this point a calibration curve was performed to establish that this technique was truly valid for quantitative work spanning a wide range of concentrations.

The oxygenate standard shown in Table 1 was prepared from neat materials in purified water and was used for all of the response enhancement work. Analytes are in solution at concentrations ranging from 5 to 500 µg/L. These concentrations were derived through experimentation using typical labratory P&T condtions so that all of the peaks of interest were on the same scale at the beginning of the study.

Table 1. Maximizing Oxygenate Response Analyte Concentrations in Purged Standard

Compound	Concentration (µg/L)
Methanol	500
Ethanol	500
Acetone	50
Ethyl ether	5
tert-Butanol (TBA)	50
Methyl-*tert*-butyl ether (MTBE)	5
Methyl ethyl ketone (MEK)	50

All stock solution prepared in purified water

Figure 1 shows the chromatogram obtained for the oxygenates standard using typical P&T concentrator conditions. Note that the abundance counts on the Y axis range up to approximately 35,000. This is an extracted ion chromatogram for *m/z* values of 31, 43, 73, and 59.

The primary variables considered in this work were:

- Purge gas flow
- Sample volume
- Sample temperature
- Matrix modification

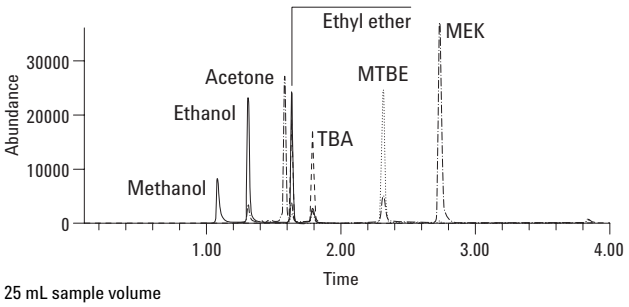


Figure 1. Oxygenates standard run using typical P&T conditions and DB-VRX capillary column.

Purge Gas Flow

The purge-gas flow was not adjusted or modified from typical settings as in most cases the laboratory will use the same P&T/GC/MS system for this analysis as well as for their standard 8260B and 524.2 work. Purge flow in most, if not all, P&T systems is a manual adjustment. Purge flow has a definite impact upon analyte recovery and if it is not kept constant calibration curves may become invalid and need to be rerun. If purge flow were manually increased for the oxygenate work and manually adjusted back down to return to 8260B or 524.2 work, it may jeopardize the current calibration curve. In addition, excessive purge flow can lead to trap breakthrough for some of the more volatile analytes contained in such methods. It was deemed more important that laboratories be able to easily adopt the changes suggested here without causing any loss in productivity for other methods of interest. As such, a purge flow of 40 mL/min for 11 minutes was maintained.

Sample Volume

It was shown in O.I. Analytical application note number 13271198 that utilization of a 25 mL sample volume vs. the typical 5 mL results in better sensitivity and improved calibration reproducibility. In its simplest form, five times the sample means five times the nanogram amount in solution. This does not mean five times the response will be achieved for all analytes, but for most a significant increase in response will be noted. All subsequent work was performed using a 25 mL sample size.

Sample Temperature

Multiple runs were performed with the sample temperature at ambient, 45 °C, 55 °C, 65 °C, 75 °C and 85 °C. Figure 2 shows the response enhancement with temperature increase for each analyte in the oxygenate standard. The responses graphed are all relative to the sample purged at ambient temperature, which is considered 100% recovery. There is a consistent response increase with temperature up to 75 °C but at 85 °C a dramatic increase is noted. As the response increase was so significant at 85 °C, this temperature was considered optimum. The sample preheat (heating prior to purge) was set for 1 minute for all temperatures. In reality, after 1 minute the purge temperature had only reached approximately 55 °C. After 2 minutes the temperature was around 65 °C, 3 minutes was 75 °C and not until the 4-minute mark did the vessel actually reach 85 °C. While the temperature was not at the set-point when purging began, it was deemed as acceptable as the heating rate of the sample was very consistent and even in the worst case, with an 85 °C set-point, the sample was purged for 8 minutes at full temperature. Increasing the preheat time to 3 and 4 minutes did not result in any significant response improvement, but definitely increased the overall purge and trap cycle time, thus reducing sample throughput. One minute of preheat gave excellent response with a minimal cycle time.

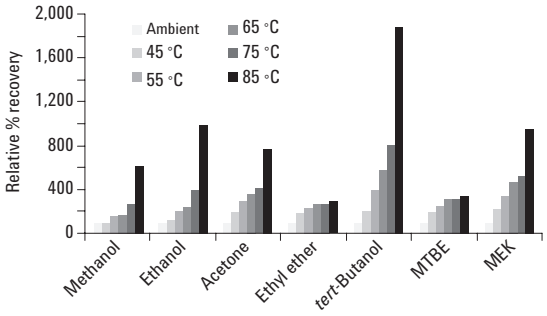


Figure 2. Analyte response vs. sample temperature.

When heating the sample to this degree, the amount of moisture transferred to the sorbent trap is significant. One benefit of the Vocarb™ 3000 trap used in this work is that it is dry-purgeable. Whether or not the water is actually purged from the trap during this step or simply purged completely into an appropriate sorbent was not explored, but a benefit in chromatographic performance is evident (Figure 3).

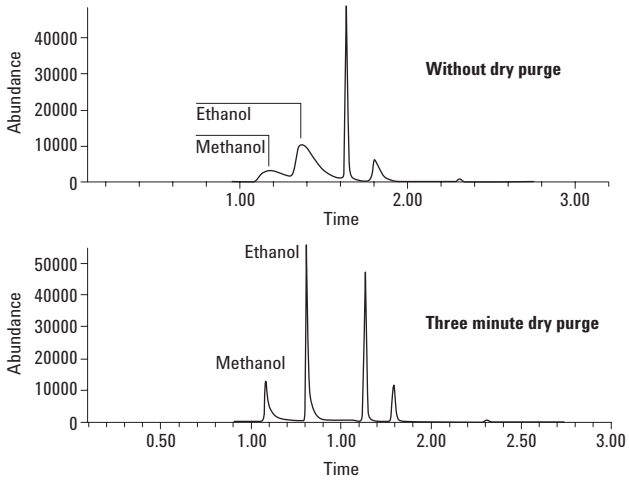


Figure 3. Modifying P&T conditions–secondary effects of increased sample temperature.

Matrix Modification

It is well understood that increasing the ion content of an aqueous solution can lead to improved recovery of non-ionic organic species from that solution. The process of increasing the ion content is generally termed 'Salting'. The primary considerations when salting a solution are what type of salt and how much salt to add. Table 2 refers to the benefits and drawbacks of several common salts. Considering the obvious drawbacks of sodium carbonate and potassium phosphate, this study focused on the use of sodium chloride and sodium sulfate. Figures 4 and 5 show the improved response achieved with different mass additions of both sodium chloride and sodium sulfate, respectively. Figure 6 gives a direct comparison of 'no salt' relative to optimum amounts of sodium chloride and sodium sulfate. It is obvious that sodium sulfate gives superior performance and, as it does not have the same corrosive characteristics as sodium chloride, it was chosen for all further work.

Table 2. The Benefits and Drawbacks of Several Common Salts

Compound	Results
Sodium chloride (NaCl)	Highly soluble in water (~8 g/25 mL), readily available, chlorine ion very reactive
Sodium sulfate (Na_2SO_4)	Highly soluble in water (~6 g/25 mL), neutral pH in solution, 2 sodium ions per molecule of salt
Sodium carbonate (Na_2CO_3)	Highly alkaline in solution
Potassium phosphate dibasic (K_2HPO_4)	Extremely soluble in water (~37 g/25 mL), difficult to work with

Figure 4 shows the effect of sodium chloride salt quantity on response relative to the 'no salt' standard P&T condtions. Above 6 g of NaCl the salt was not dissolving completely into solution and so addition was stopped here.

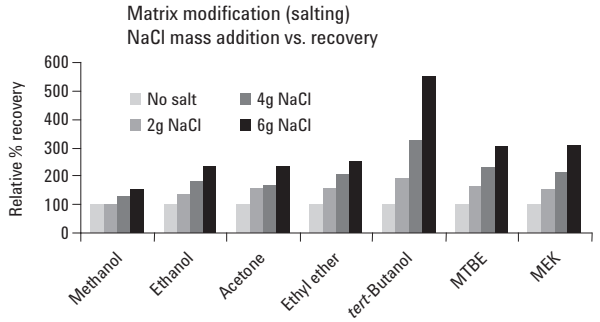


Figure 4. The effect of sodium chloride salt quantity on response relative to the 'no salt' standard P&T condtions.

Figure 5 shows the effect of sodium sulfate salt amount on response relative to the 'no salt' standard P&T conditions. There is a distinct rise in response at 6 g as we approach full saturation of solution.

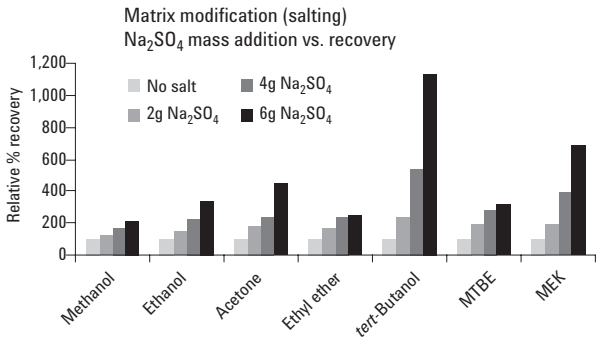


Figure 5. The effect of sodium sulfate salt amount on response relative to the 'no salt' standard P&T conditions.

In Figure 6, this combined bar-graph shows directly the response differences experienced with the two different types of salt (both at 6 g) relative to the 'no salt' standard P&T conditions. It is clear that there is a definite response advantage to using sodium sulfate vs. sodium chloride.

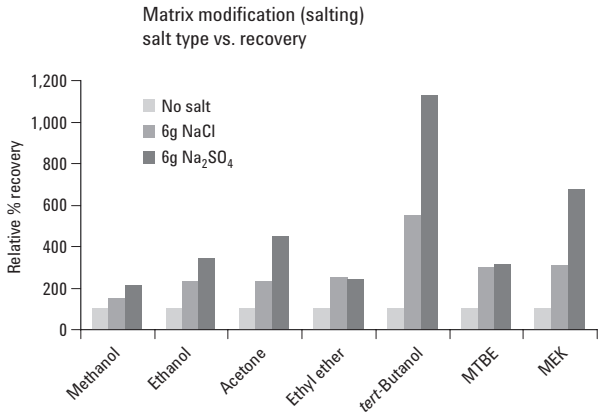


Figure 6. The response differences experienced with the two different types of salt relative to the 'no salt' standard P&T conditions.

Combining Parameters

Figure 7 shows the effect of temperature and salt addition alone, relative to standard conditions, but also shows how much more impact these modifications made once they were combined. For instance, *tert*-Butanol response was increased roughly 20 times using an 85 °C purge temperature and roughly 10 times using the addition of 6 g of sodium sulfate, but when these two modifications were combined it resulted in an overall response increase of over 75 times relative to standard P&T conditions.

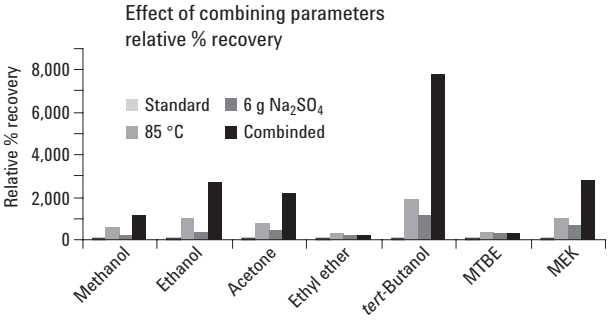


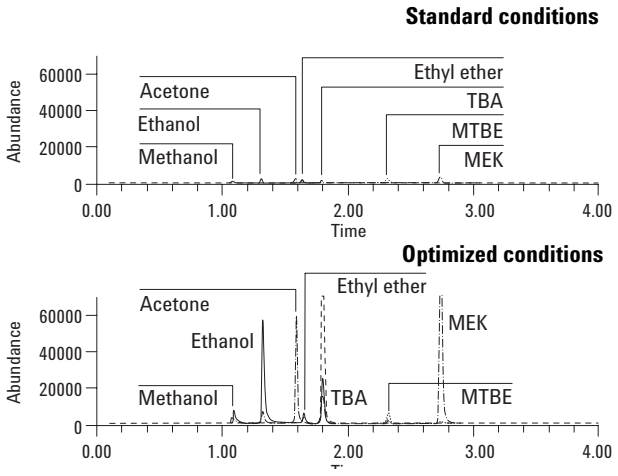
Figure 7. Effect of combining optimized purge and trap parameters.

Table 3 shows the final optimized run conditions for this analysis. Figure 8 is a direct visual comparison of standard vs. optimized P&T conditions. Again, methanol and ethanol are spiked into solution at 500 ppb. Note the much smaller response for ethyl ether and MTBE in the optimized chromatogram. Recall that they are in solution at 5 ppb vs. 50 ppb for acetone, TBA and MEK and 500 ppb for methanol and ethanol.

Table 3. Optimized Run Conditions

Column:	**DB-VRX**
P/N:	**121-1524**
Length:	20 m
Diameter:	0.18 mm
Film thickness:	1.0 µm film
Carrier:	Helium at 45 cm/sec (1.0 mL/min)
Oven:	45 °C for 3.5 minutes
	45–150 °C at 15 °C/min
Injector:	Tekmar 3100 Purge and Trap
Trap:	Vocarb 3000
Sample volume:	25 mL
Sample temp:	85 °C (1 minute preheat)
Purge:	11 Minutes
Dry purge:	3 Minutes
Desorb preheat:	245 °C
Desorb:	1 Minute at 250 °C
Bake:	10 Minutes at 260 °C
Line and valve temp:	125 °C
Interface:	Split injector at 200 °C,
	60:1 Split ratio
Gas saver:	150 mL/min at 1 minute
Agilent 5973 MSD	
Scan range:	29-260 amu
Scan rate:	3.17 scans/sec
Quad temperature:	150 °C
Source temperature:	230 °C
Transfer line temp:	250 °C
Matrix modification:	6 g Sodium sulfate

Figure 8 shows a chromatographic comparison between typical and optimized P&T conditions. The 'Y' scales are normalized for comparative purposes.



Abundance normalized for comparative reasons

Figure 8. A chromatographic comparison between typical and optimized P&T conditions.

Performing a Calibration Curve

Eight points were used with the realistic expectation that not all analytes were going to be linear from 0.5 ppb up to 200 ppb. It was expected that the methanol and ethanol may not achieve single digit ppb levels. As expected, methanol was not able to be calibrated down to 0.5 ppb or even 5 ppb. At these lower concentrations the signal-to-noise ratio was simply too low to be reliable. At 10 ppb it was approaching a more reasonable 10:1 ratio. Figure 9 shows the calibration curve for methanol. Using linear regression per EPA methodology and a calibration range of 10–200 ppb the R-squared value was 0.9987, which is well above the EPA required 0.990 needed for valid quantitative use. Ethanol was calibrated from 0.5–200 ppb with an R-squared value of 0.998 (Figure 10). Both methanol and ethanol calibration ranges could likely be extended to well above 200 ppb.

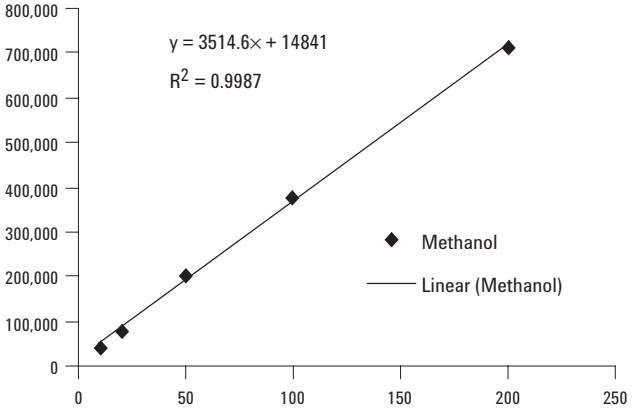


Figure 9. Calibration curve for methanol with a calibration range of 10–200 ppb

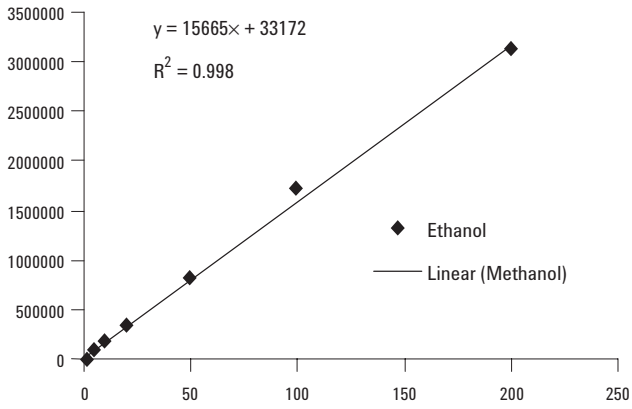


Figure 10. Calibration curve for ethanol with a calibration range of 0.5–200 ppb.

For all components, except methanol, a calibration range of 0.5–200 ppb was achieved with R-squared values ranging from 0.990–0.998 (Table 4), all at or above the Environmental Protective Agency (EPA) requirements for valid quantitation. Methanol as stated earlier was calibrated over a range of 10–200 ppb with an R-squared value of 0.9987.

Table 4. Calibration Curve Summary Using Optimized Analysis Conditions

Compound	Calibration range (ppb)	R^2 Value
Methanol	10–200	0.999
Ethanol	0.5–200	0.998
Acetone	0.5–200	0.993
Ethyl ether	0.5–200	0.994
TBA	0.5–200	0.990
MTBE	0.5–200	0.995
MEK	0.5–200	0.994

USEPA requires R^2 value of 0.990 or greater for quantitative use

Additional Considerations

Automated sampler systems, that accept full VOA vials to reduce sample handling, may require some special approaches to facilitate salt addition. It may be necessary to contact the manufacturer of the autosampler to find out the feasibility of salt addition.

Many laboratories attempt to run the low level oxygenates in conjunction with their 8260 or 524.2 methods. The conditions presented in this work likely will not work well with these standardized EPA methods, but this has not yet been confirmed. If it is desired to run the oxygenates together with the full VOC list these analysis conditions may need to be pared back somewhat, though this will reduce the sensitivity for methanol and ethanol. For example, heating the sample to 65 °C with no salt addition may work for full VOCs and will likely allow for calibration down to around 100 ppb for methanol and ethanol. If the selected ion monitoring (SIM) mode of the MSD is used, per EPA method 8260B section 7.5.12, increased sensitivity will be gained. This may allow for less aggressive conditions than those used in Table 3 while still achieving low ppb quantitation levels.

Salt should be baked to remove moisture and any possible contaminants.

Dry blanks should be run often and line/valve temperatures kept hot (125–150 °C) to reduce water build-up and carryover problems. If a single purge vessel is used for all samples it should be rinsed and/or baked thoroughly after every run.

Conclusion

With optimized P&T conditions it is possible to detect and accurately quantitate low ppb levels of oxygenated contaminants in aqueous sample matrices. This application note provides some of the tools needed if this type of work is to be considered.

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Printed in the USA
March 12, 2003
5988-8993EN

Application 707

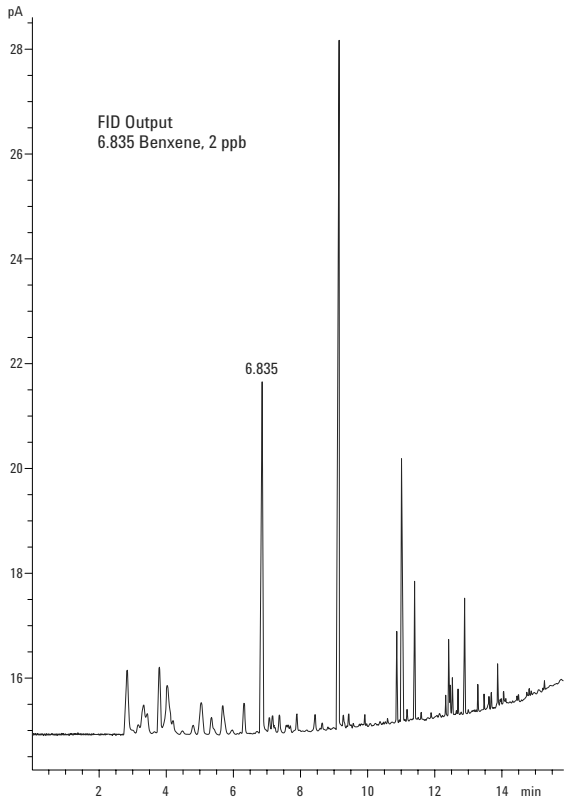
Gas Phase Sample Extraction System

Analysis of Trace Organic Components in Water

Technical Overview

Application Highlights

- Bench-top or wall-mounted system
- The extraction is performed by flowing the water sample past a thin, solid, polymer barrier.
- Various detector choices allow a wide range of selectivity and sensitivity.
- Concentration ranges down to ppt levels



Optional Configurations

- Trace hydrocarbon impurities in cooling tower/exchanger water
- Trace hydrocarbon impurities in high purity drinking water
- TOGA Analysis utilizing capillary column technology
- Contaminants in discharge water

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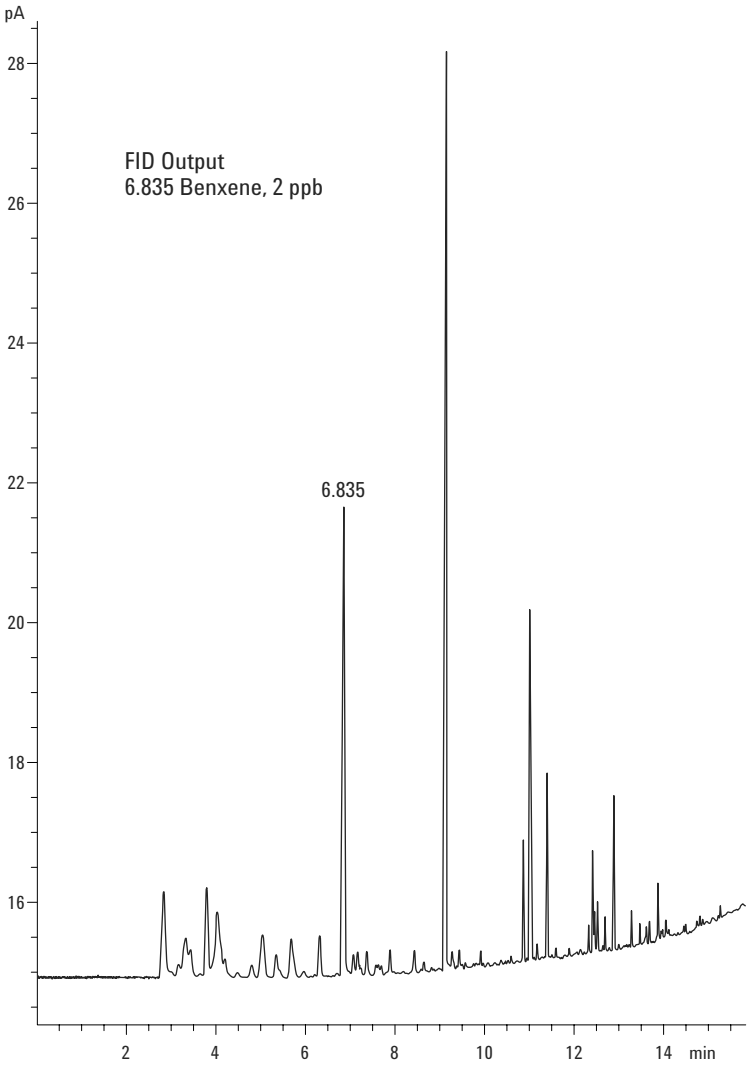

FID output from the Agilent Gas Phase Sample Extraction System.

Printed in the USA
December 6, 2002
5988-7673EN

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WASSON·ECE INSTRUMENTATION

BFB Tuning for Environmental Analysis: Three Ways to Succeed

Application

Environmental

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Abstract

The United States Environmental Protection Agency methods 524.2, 8260B, and Contract Laboratory Program Statement of Work employ purge and trap concentration of volatile compounds in water samples with analysis by gas chromatography/mass spectrometry. Each method requires the mass spectrometer to meet specific tuning criteria before proceeding to actual samples. This paper summarizes these tuning criteria, and shows three different ways that the Agilent Technologies 6890/5973 gas chromatograph/mass selective detector system can be tuned to meet them. A very simple and robust procedure is described in the Modified Autotune section. A quick reference guide for this procedure is given at the end of the paper under Modified Autotune Summary.

Introduction

If you are already familiar with 4-bromofluorobenzene (BFB) tuning and evaluation procedures, you may want to go directly to the section titled "Modified Autotune Summary" found at the end of this paper. It offers an alternative approach for tuning Agilent 6890/5973 GC/MSD systems that is routinely successful in this laboratory.

The United States Environmental Protection Agency (USEPA) has developed several methods for the analysis of volatile organic compounds (VOCs) in water samples. The three most widely used procedures all employ purge and trap (P&T) sample introduction followed by capillary column gas chromatography with mass spectral detection (P&T/GC/MS). USEPA Method 524.2 revision 4[1] is used for drinking water analysis while Method 8260B revision 2[2] is used for wastewater. The USEPA Contract Laboratory Program Statement of Work (CLP-SOW)[3] uses a similar P&T/GC/MS method for the analysis of hazardous waste.

There are many similarities among these three USEPA volatiles methods. One common requirement is that the GC/MS system must be tuned in such a way that 4-bromofluorobenzene (BFB) meets specific ion abundance criteria. This requirement helps to ensure that data are comparable between instruments of different design and

among various laboratories. This paper summarizes USEPA method 524.2, 8260B, and CLP tuning criteria, and shows three different ways that the Agilent Technologies 6890/5973 GC/MSD system can be tuned to meet them.

Experimental

A standard containing fluorobenzene, 1,2-dichlorobenzene-d_4, and 4-bromofluorobenzene at 2.0 mg/mL was purchased from AccuStandard (New Haven, CT). A portion of this solution was diluted in methanol (B&J HPLC and pesticide grade) to a concentration of 50 ng/µL.

Standards for tune evaluation were injected by syringe or P&T into several different Agilent Technologies 6890/5973 GC/MS systems. When making syringe injections into the split/splitless inlet, a liner with a 900-µL volume was used and no more than 1.0 µL was injected to avoid over-expansion in the inlet.

Results and Discussion

Tuning Criteria

Table 1 lists the tuning criteria for USEPA methods 524.2, 8260B, and CLP-SOW. All three methods base their tuning criteria on the ion responses of BFB. All ion responses are reported relative to *m/z* 95, which is assumed to be the base peak even though ions 174 and 176 may be larger in the CLP-SOW method.

While many of the requirements in Table 1 are the same for all three methods, some important differences are worth noting. Method 8260B actually allows the analyst to use the tuning criteria specified in either of the other two methods. More importantly, it allows one to use "manufacturers tuning (sic) instructions" so long as it does not hurt method performance. However, many laboratories still follow the BFB tuning requirements specified in method 8260B or choose to substitute CLP-SOW tuning criteria.

Methods 524.2 and 8260 require that *m/z* 95 be the base peak in the BFB spectrum, which caps the *m/z* 174 relative abundance at 100% (relative to *m/z* 95). The CLP-SOW requirements allow *m/z* 174 to be up to 120% of *m/z* 95. Tuning procedures that reduce the response of *m/z* 174 too much may lead to lower overall sensitivity, especially for bromoform which has a quant ion of *m/z* 173. Conversely, maximizing this ratio, within the requirements of the method, can enhance overall sensitivity.

Automated BFB Tuning

The Agilent 5973 MSD uses perfluorotributylamine (PFTBA) for electron impact tuning because it exhibits good stability, the right volatility, and a wide range of fragment masses. However, USEPA volatiles methods evaluate the tune using BFB which produces an entirely different spectrum.

Table 1. Criteria for BFB Tuning for Three Capillary GC/MS Volatiles Methods

	Relative Abundance Criteria		
Mass (*m/z*)	**Method 524.2**	**Method 8260B[a]**	**CLP-SOW**
50	15 to 40% of 95	Same as 524.2	8 to 40% of 95
75	30 to 80% of 95	30 to 60% of 95	30 to 66 % of 95
95	Base Peak, 100%	Same as 524.2	Same as 524.2
96	5 to 9% of 95	Same as 524.2	Same as 524.2
173	<2% of 174	Same as 524.2	Same as 524.2
174	>50% of 95	Same as 524.2	50 to 120% of 95
175	5 to 9% of 174	Same as 524.2	4 to 9% of 174
176	>95 to <101% of 174	Same as 524.2	93 to 101% of 174
177	5 to 9% of 176	Same as 524.2	Same as 524.2

[a]Alternative tuning criteria may be used (for example, CLP or Method 524.2) including manufacturer's instructions provided that method performance is not adversely affected.

Therefore, automated (or manual) tuning procedures must adjust PFTBA ion responses in order to get the desired BFB response ratios. Agilent G1701CA EnviroQuant ChemStation software automates BFB tuning so that the instrument typically passes the more restrictive USEPA Method 524.2 and 8260B requirements listed in Table 1. After tuning, the analyst must inject a BFB standard by syringe or P&T to verify that the tune passes the requirements for the method in use.

Automated BFB tuning adjusts MSD source parameters so that PFTBA ion abundances meet predetermined "targets." The default PFTBA target values are set so that a subsequent BFB injection should meet the requirements for all three methods. Table 2 shows a portion of a BFB tune report that includes the target responses (as a percentage of *m/z* 69) for *m/z* 50, 69, 131, 219, 414, and 502. The actual abundances achieved by the tune are shown on the last line. When these targets are met, the Agilent 5973 MSD normally passes any of the tuning criteria listed in Table 1.

Table 2. A Portion of a Typical BFB Tune Report

Target Mass:	50	69	131	219	414	502
Target Abund (%):	1.0	100.0	45.0	55.0	2.4	2.0
Actual Tune Abund (%):	1.2	100.0	48.1	59.3	2.7	2.3

Figure 1 shows an average spectrum obtained for a 1-µL manual injection of BFB (50 ng/µL split 50:1) using the tune shown in Table 2. Agilent G1701CA EnviroQuant ChemStation Environmental Data Analysis software can evaluate the spectrum automatically and generate a report that is archived with the data file. Because BFB tuning criteria are not uniform among USEPA methods, the analyst must first specify the allowable ranges using the form shown in Figure 2. The form is accessed in Environmental Data Analysis by selecting Tuner/Edit BFB Criteria on the dropdown menu.

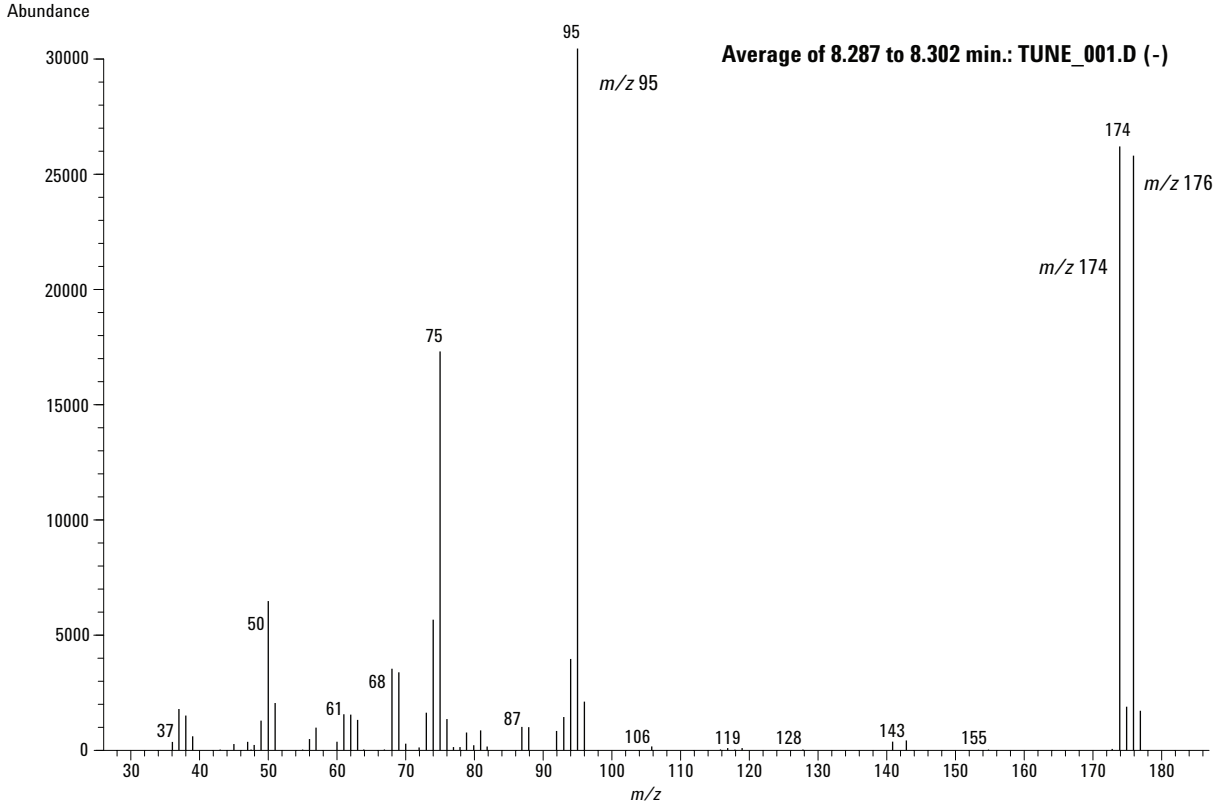

Figure 1. Average spectrum of BFB after performing a standard BFB automated target tune. One µL of a methanol solution containing 50 ng/µL of BFB was injected by hand.

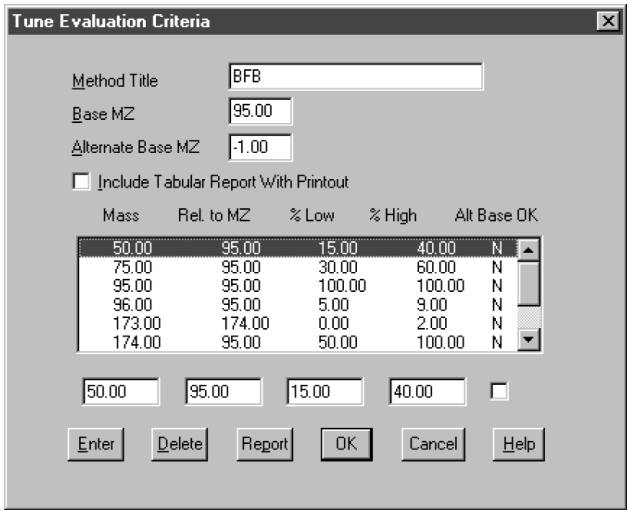

Figure 2. The Agilent G1701CA EnviroQuant ChemStation screen for entering BFB tune criteria. The user can modify the parameters to meet the requirements of the method in use. These values are used by the ChemStation for automated tune evaluation.

Having entered abundance criteria for the method in use, one can automatically assess the suitability of the tune using the EnviroQuant software (Figure 3). One can choose to "Evaluate BFB to Screen/Printer" in which case it will evaluate the current spectrum. This can be a single spectrum or an average. Alternatively, by choosing "Autofind BFB to Screen/Printer," the software automatically finds BFB in the chromatogram, averages the top three spectra and subtracts a baseline spectrum. In either case, a report such as the one in Figure 4 is generated. The most recent report is archived in the datafile.d directory in a file called tuneeval.txt.

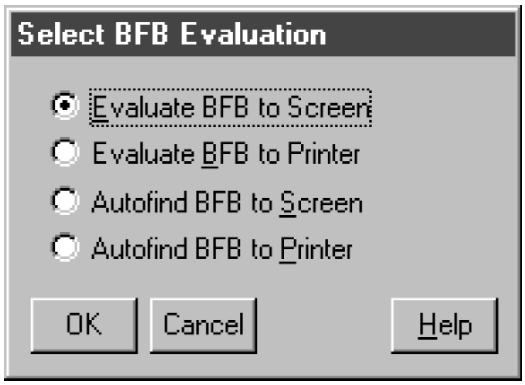

Figure 3. Choices for automated BFB tune evaluation by the EnviroQuant software. The "Evaluate BFB..." choices use the spectrum (single or averaged) in Data Analysis window 1 for evaluation. The "Autofind..." choices automatically find the BFB peak, average the top three BFB spectra and subtract a baseline spectrum prior to evaluation.

```
                                   BFB

Data File : C:\HPCHEM\1\DATA\Sep24_01\TUNE_001.D          Vial: 1
Acq On    : 24 Sep 2001   2:25 pm                     Operator:
Sample    : BFB for tuning 1 uL                       Inst: GC/MS Ins
Misc      :                                           Multiplr: 1.00
MS Integration Params: rteint.p

Method    : C:\HPCHEM\1\methods\envdef.m (RTE Integrator)
Title     :

AutoFind: Scans 1567, 1568, 1569; Background Corrected with Scan 1559

| Target | Rel. to | Lower  | Upper  |  Rel.  |    Raw    |   Result  |
|  Mass  |  Mass   | Limit% | Limit% |  Abn%  |    Abn    | Pass/Fail |
-------------------------------------------------------------------------
|   50   |    95   |    15  |    40  |  21.6  |     6769  |   PASS    |
|   75   |    95   |    30  |    60  |  56.5  |    17708  |   PASS    |
|   95   |    95   |   100  |   100  | 100.0  |    31317  |   PASS    |
|   96   |    95   |     5  |     9  |   7.1  |     2209  |   PASS    |
|  173   |   174   |  0.00  |     2  |   0.0  |        0  |   PASS    |
|  174   |    95   |    50  |   100  |  82.8  |    25933  |   PASS    |
|  175   |   174   |     5  |     9  |   7.4  |     1910  |   PASS    |
|  176   |   174   |    95  |   101  |  99.3  |    25747  |   PASS    |
|  177   |   176   |     5  |     9  |   6.6  |     1702  |   PASS    |
-------------------------------------------------------------------------

TUNE_001.D  envdef.m      Mon Sep 24 16:00:27 2001   MSVOC04
```

Figure 4. The Agilent EnviroQuant ChemStation BFB Tune Evaluation Report for the spectrum shown in Figure 1.

In this case the automated BFB tuning procedure produced a tune that passes Method 524.2 and 8260B criteria with a 174/95 ratio of 82.8%. This ratio is limited to 100% by these USEPA methods, which specify that *m/z* 95 must be the base peak. To meet these strict guidelines, one has to "de-tune" the Agilent 5973 MSD which results in somewhat lower instrument sensitivity. Laboratories may want to increase the 174/95 ratio so it more closely approaches the 100% limit of Methods 524.2 and 8260B or so that it approaches the 120% limit specified in the CLP-SOW method. Most laboratories that perform Method 8260B tune their instruments to meet the CLP-SOW requirements because the method allows laboratories to use these tune criteria and the MSD performance is closer to optimum.

In addition to the automated BFB tune, there are two procedures that can be used to improve instrument sensitivity, to meet the more liberal CLP-SOW requirements, or to create a passing tune should the standard BFB autotune fail. In this laboratory, the "Modified Autotune" procedure was found to produce tunes that routinely passed BFB criteria for any of the three methods. As shown below, changing the BFB tuning targets can also produce a passing BFB tune while enhancing the signal for bromoform.

Target Tuning

Automated BFB tuning adjusts MSD source parameters to achieve the target responses required for the method in use. This is essentially a "target tune" procedure where the initial target abundances provided by the software are designed to

meet the more restrictive 524.2 and 8260B requirements. When needed, it is easy to change the target PFTBA relative abundance criteria to produce the desired affect on the BFB ions. This is done by selecting View/Manual Tune/Set Tune Targets.

For example, consider the spectrum in Figure 1 which passed all of the tuning criteria, but which had a lower than optimum *m/z* 174 response. Experience in this laboratory has shown that increasing the relative abundance of *m/z* 174 will increase the overall sensitivity of the instrument, in particular for the bromoform response at *m/z* 173. As shown in Figure 5, the target abundances for ions 131 and 219 were each increased to 70% from their default values of 45% and 55% respectively. These choices were saved to the BFB.U tune file and a new BFB Target Tune was run. Figure 6 shows the new BFB spectrum (average of three spectra across the apex with baseline subtraction) which passes CLP-SOW criteria (Table 1) and is, therefore, satisfactory for either CLP or 8260B volatiles methods.

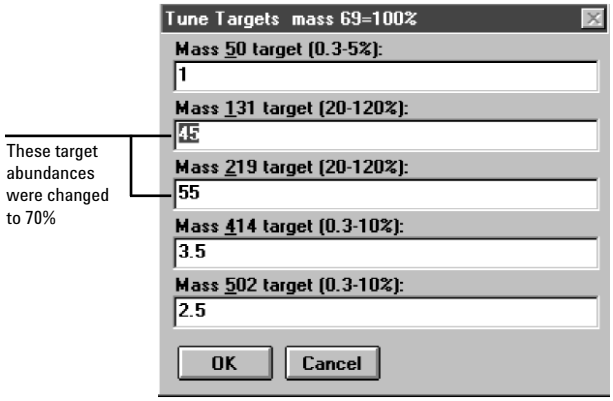

Figure 5. PFTBA target abundance values (relative to *m/z* 69) used for "target" tuning. When these abundances are saved to the BFB.U tune file, they are used by the BFB target tune algorithm.

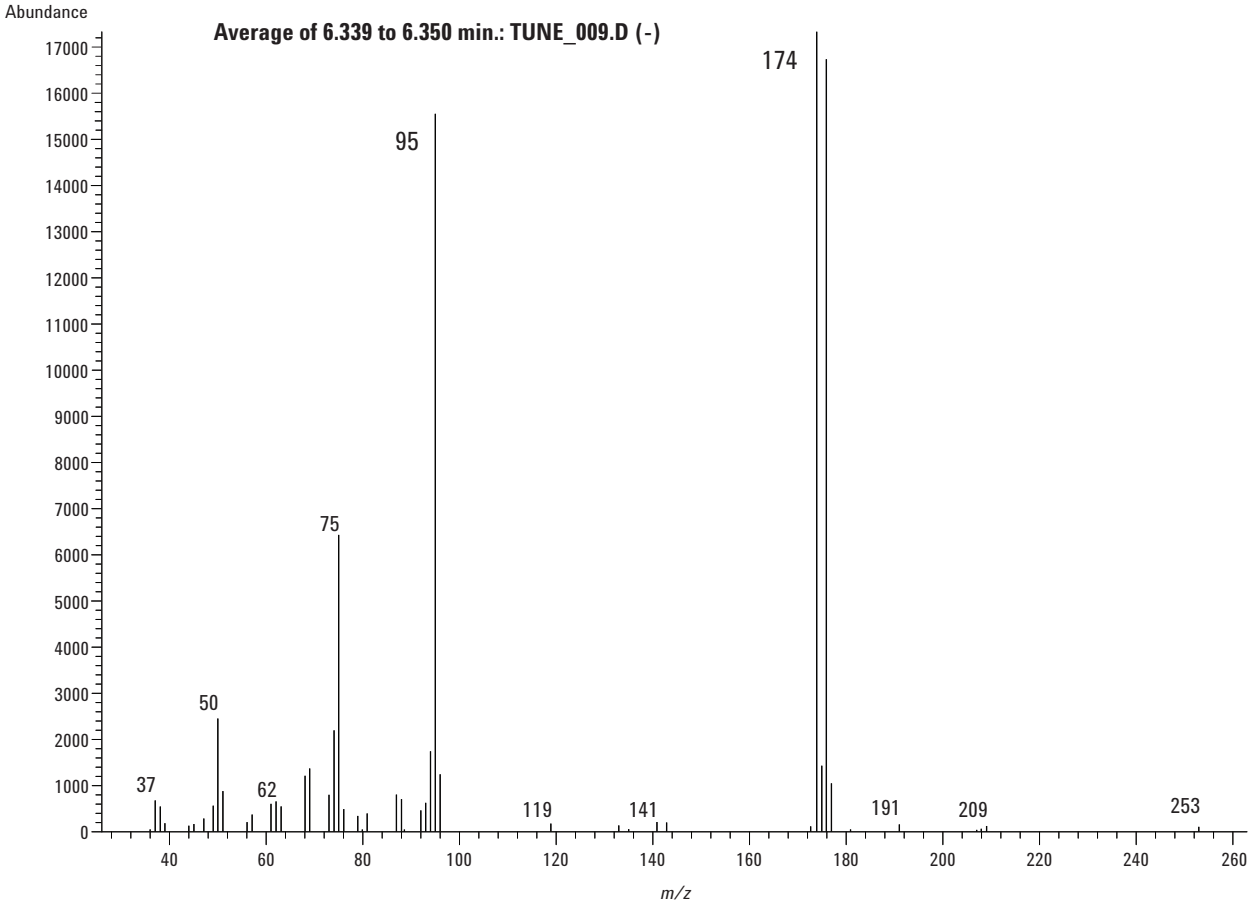

Figure 6. Average BFB spectrum obtained by changing the tune targets for *m/z* 131 and 219 to 70% (relative to *m/z* 69). This spectrum passes CLP-SOW tuning criteria.

Modified Autotune

With the convenience of automated tuning procedures available in the Agilent ChemStation software, most analysts have gladly given up the idea of manually tuning their 5973 MSDs. A combination of automated tuning with a slight manual modification has given excellent BFB results in this laboratory. The total process is easy and usually takes just a few extra minutes after the autotune is complete. The steps are described below and are summarized in a "quick reference" format in the next section.

1. From the Manual Tune portion of the software, perform an Autotune (select Tune/Autotune). This algorithm tunes the Agilent 5973 MSD for maximum sensitivity over the entire mass range and is widely used by methods that do not specify other tune criteria. This autotune emphasizes overall sensitivity by improving abundances for higher mass ions (for example, 502). As a result, the Autotune procedure typically gives an abundance for *m/z* 50 that is too low to meet 524.2 and 8260 criteria and an abundance of *m/z* 174 that may be too high, even for CLP-SOW tuning.
2. After completing the Autotune procedure, choose Edit MS Params (under the AdjParam menu item) which will display the screen shown in Figure 7.

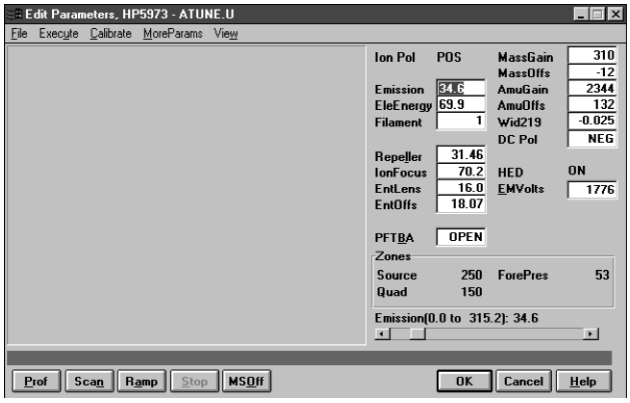

Figure 7. The Edit Parameters screen found by selecting AdjParam/Edit MS Params in the main Manual Tune window.

3. Two changes are required in the default values used for adjusting parameters in this view. First, under the MoreParams menu, choose Ramp Params and change the "Stop" value for the ion focus to 140 as shown in Figure 8. Close this window and choose AcqParams under the MoreParams window and change Mass 3 from 502 to ion 50 as shown in Figure 9. Close this window and return to the main Edit Parameters screen (Figure 7).

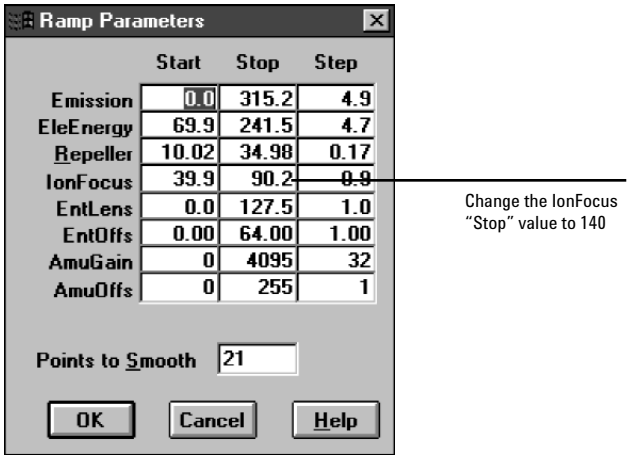

Figure 8. This window allows the user to set ranges for the various tuning parameters. The default ion focus "Stop" setpoint of 90 was set to 140.

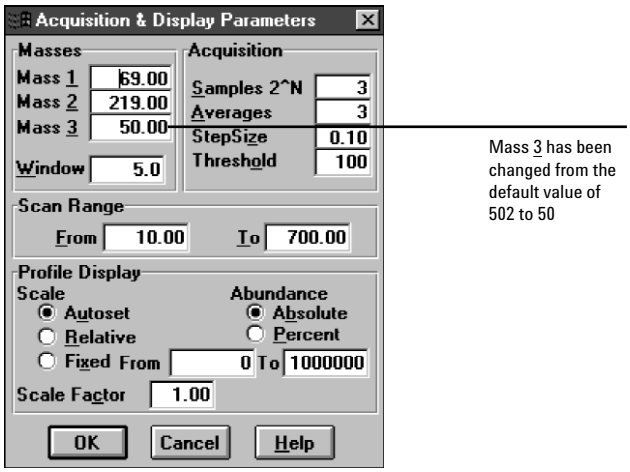

Figure 9. Acquisition and Display Parameters window. *M/z* values of 69, 219, and 50 have been chosen so that these responses can be ramped and their relative abundances displayed.

4. Highlight the IonFocus window with the cursor and then select Ramp. This gradually ramps the ion focus voltage over the specified range while monitoring the response of ions 69, 219, and 50. After about a minute, a plot of these ion responses vs. the ion focus voltage appears in the window (Figure 10).

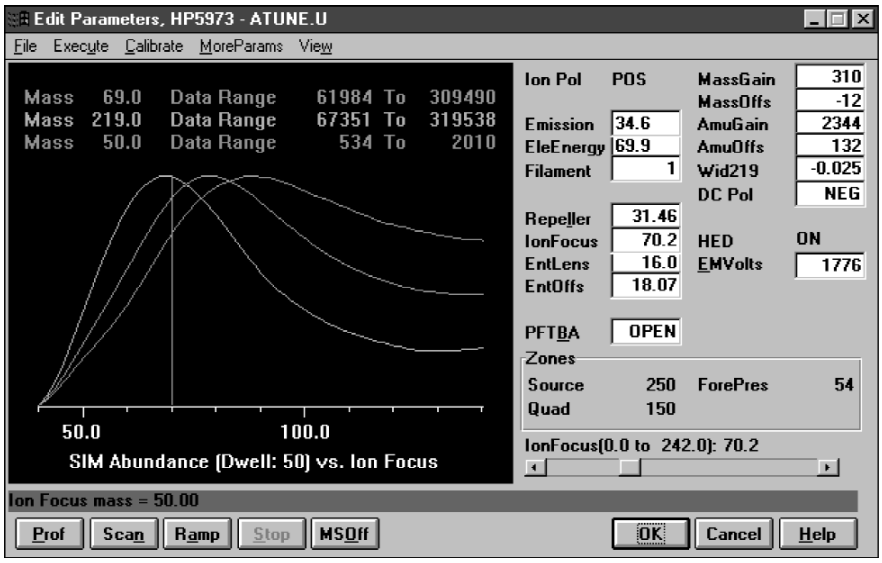

Figure 10. Abundances for ions 69, 219, and 50 while ramping the Ion Focus from 40 to 140.

5. Under the View dropdown menu item, choose Expand. This view shows the current Ion Focus setting, the abundance of *m/z* 69 and the relative abundances of ions 219 and 50 (Figure 11). From the plot, it is easy to see that an increase in the Ion Focus value should increase the 50:69 ratio while reducing the 219:69 ratio. These are exactly the changes that should enable the MSD to pass BFB tuning criteria.

   Note that the ion focus ramping procedure can also be performed from the main Manual Tune screen by choosing Ramp/Ramp Ion Focus on the dropdown menu.

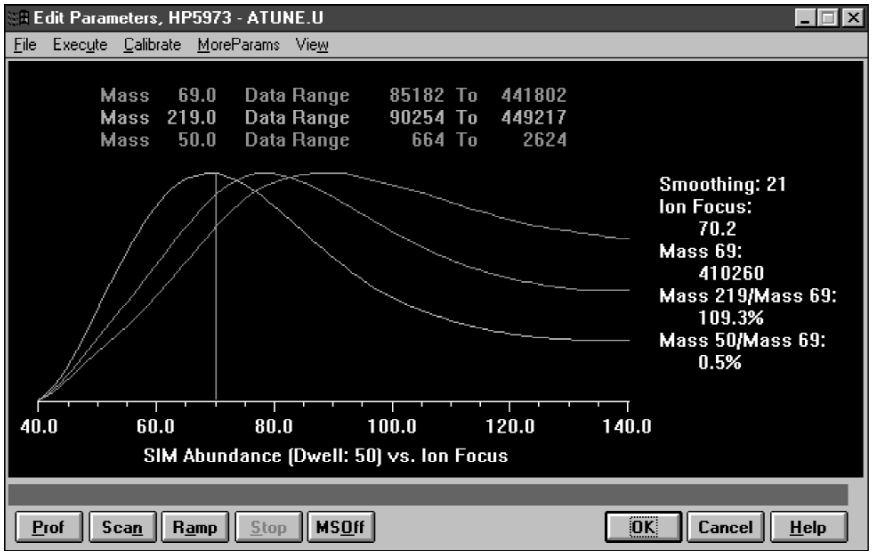

Figure 11. An expanded view of the SIM-Abundance-vs-Ion Focus plot obtained by selecting View/Expand. This view allows one to drag the vertical line to different setpoints while observing changes in the ion relative abundances.

6. The vertical line indicates the current ion focus setpoint. Use the cursor to drag this setpoint line to the right while observing the change in the 219:69 and 50:69 ratios. Agilent laboratories have had good success by setting the Ion Focus to values between 100 and 135 V. This should result in a 219:69 ratio in the 60-80% range and a 50:69 ratio that is 0.8 or greater. If tuning to meet 524.2 requirements, the 219/69 ratio should be on the low side of this range.

   An alternative to the above procedure is to select Scan in the Edit Parameters window (Figure 7) while monitoring ions 69, 219, and 50. The 219:69 and 50:69 ratios are displayed under the Relative Abundance heading and are updated with each scan. Highlight the Ion Focus setting and adjust its value using the slider bar. The effect of different Ion Focus values will be seen almost immediately in the ion ratios. These ratios will bounce around somewhat, but trends can be seen over a few scans. A good choice for the 50:69 ratio would be about 0.85.

7. Click OK and return to the Manual Tune screen. Under the Calibrate menu item, choose Adjust Abundances, which will automatically reset the electron multiplier to get ion abundances in the optimum range. Save the tune, choosing a new name for the tune file (for example, BFB1.U). Return to Instrument Control (View/Instrument Control) and be sure to select this tune file for the method used to acquire the BFB checkout chromatogram. Inject or purge an appropriate amount of BFB and evaluate the tune using the software tools provided (Figures 2 through 4). Assuming that it passes, assign this tune to the P&T/GC/MS volatiles method in use.

Figure 12 shows the spectrum (average of the three scans across the apex with baseline subtraction) for a 1-µL syringe injection (50 ng/µL split 50:1) of BFB using an ion focus value of 115 V. All other parameters (except for the electron multiplier) were set by the Autotune algorithm. This spectrum passes any of the tuning criteria listed in Table 1 but has a higher 174/95 ratio than was achieved using the standard BFB tune.

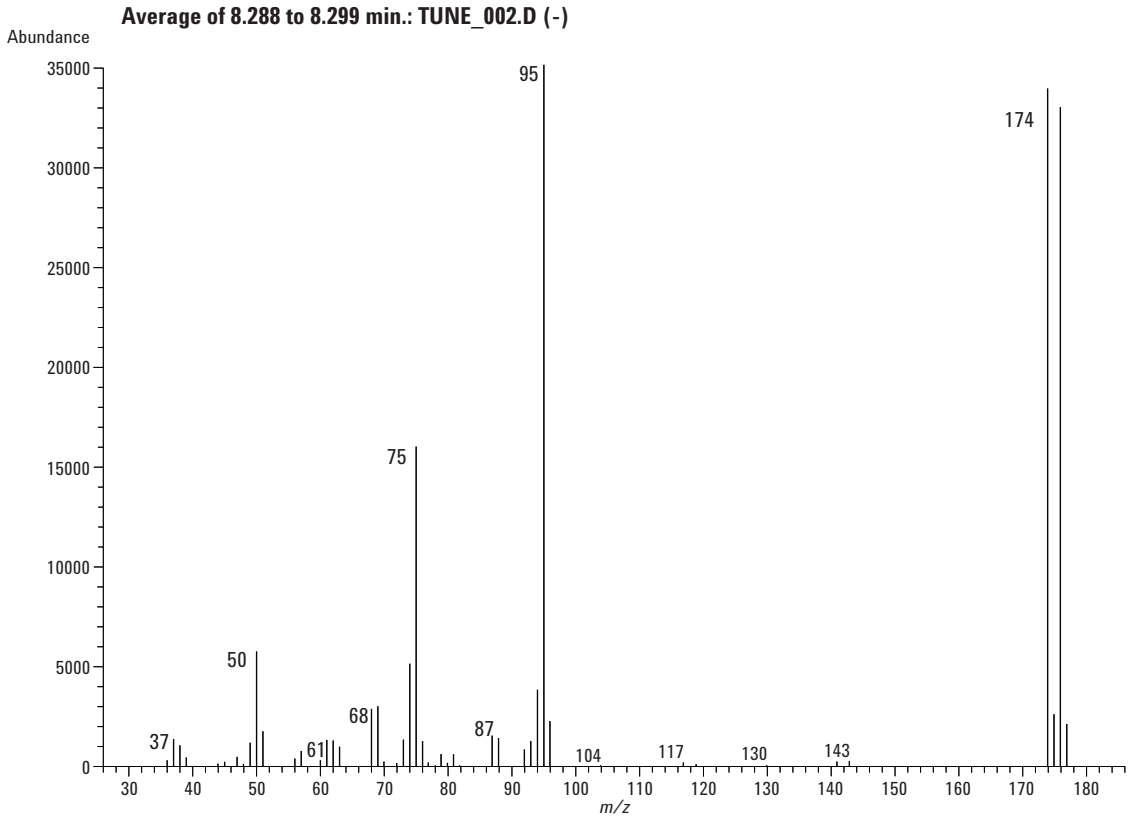

Figure 12. Average spectrum of BFB obtained after using the procedure described under Modified Autotune. After running a standard Autotune, the Ion Focus value was increased to 115 V.

The true test of a successful BFB tune is whether it holds up during repetitive VOC analyses and through normal instrument maintenance procedures. In one extreme test, the same BFB tune easily passed CLP-SOW criteria during a period when two different MSD sources were installed and four different filaments were used. On one Agilent 6890/5973 GC/MS instrument this procedure did not work until the MSD source was cleaned.

Finally, a note of caution is appropriate. While these techniques have worked well for the Agilent 6890/5973A and N GC/MSD systems, this does not imply that the same procedures are appropriate for older Agilent MSDs. Tuning frequency is dictated by the nature of the samples, choice of column and other factors such as column bleed and source cleanliness. If the source becomes too dirty, it must be cleaned in order to pass BFB tuning criteria, no matter which approach is taken.

Modified Autotune Summary

These steps summarize the procedure for modifying the standard Agilent 5973 Autotune to pass BFB tuning criteria. It is provided here as a quick reference guide for those who are already familiar with tuning procedures.

1. In the Manual Tune portion of the Agilent GC/MS ChemStation software, perform a standard Autotune.
2. In the Ramp Parameters window, change the Ion Focus Stop value to 140.
3. In the Acquisition & Display Parameters window, change ion 502 to 50.
4. In the Edit Parameters window click on Ion Focus and then on Ramp.
5. Adjust the Ion Focus value so that the 50/69 ratio is 0.8 or larger. The 219/69 ratio usually falls in the 60 to 80% range. When this PFTBA ion ratio is under 70%, the 174/95 ratio of BFB is usually under 100%.
6. In the Manual Tune window under the Calibrate menu item, adjust ion abundances.
7. Save the tune file with a new name, assign it to the method and verify that the tune passes by injecting a BFB sample according to the method requirements.

Conclusions

There are several ways to tune the Agilent 6890/5973 GC/MSD system to meet any of the USEPA BFB tuning criteria. However, factors such as source cleanliness, choice of column, flow rates and instrument-to-instrument variability make each GC/MSD system unique. Automated BFB and target tuning procedures are normally successful but the 174/95-ion ratio may not be high enough to meet laboratory needs. In our experience, the most robust and long-lasting BFB tunes were generated by the procedure outlined above under Modified Autotune. The procedure takes just a few minutes to complete.

References

1. *Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/Mass Spectrometry,* Method 524.2, revision 4.1, U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Cincinnati, OH (1995).
2. *Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS),* Method 8260B, revision 2 (1996).
3. *USEPA Contract Laboratory Program Statement of Work for Organics Analysis, Multi-Media, Multi-Concentration, OLM04.2,* USEPA Contract Laboratory Program, Office of Emergency and Remedial Response.

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Printed in the USA
November 6, 2001
5988-4373EN

Agilent Technologies

Analysis of Volatile Organic Compounds by Purge-and-Trap Gas Chromatography/Mass Selective Detection

Application

Environmental

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Abstract

Purge-and-trap sample concentration followed by gas chromatography with mass spectral detection is the most widely used technique for the analysis of volatile compounds in water. Using 5-mL water samples, most target compounds can be quantitated at 0.5 µg/L or lower. Analysis of 25-mL samples provides even greater sensitivity. Agilent Technologies' Purge-and-Trap/Gas Chromatography/Mass Spectral Detection system is recognized worldwide for its reliability, sensitivity and ease of use. Agilent also provides specialized environmental software, consumables, and gas chromatography columns for volatiles analysis. When combined with Agilent's service and support, laboratories have everything required to produce high-quality, reliable results.

Introduction

Without careful monitoring, many chemical by-products of modern industrial production can be released into the environment, often contaminating ground and surface water bodies upon which we all depend. In many parts of the world, public drinking water is chlorinated to kill disease-causing bacteria. Some byproducts of this process, such as the halogenated methanes, are unhealthy at high concentrations. Therefore, chlorinated drinking water must be routinely monitored for these compounds and others to ensure public safety.

Numerous analytical techniques have been developed over the last 25 years to analyze for volatile organic compounds (VOCs) in ground and surface water, wastewater, and drinking water. Today, the most widely used analytical technique in the world for VOC analysis is purge-and-trap (P&T) sample concentration combined with gas chromatography and mass selective detection (GC/MSD).

Purge-and-trap has been adopted so widely because of its ability to remove and concentrate most or all of the VOCs in water samples. When combined with the Agilent Technologies GC/MSD, the system can be used to quantitate VOCs at concentrations as low as 0.1 µg/L (ppb). The Agilent GC/MSD has become the standard system in most environmental laboratories worldwide because it offers unmatched sensitivity, selectivity, stability, and ease-of-use. This paper illustrates use of P&T/GC/MSD as a universal technique for the analysis of VOCs in water samples.

Experimental

Table 1 lists the instrumentation and some of the conditions that were used for the analysis of VOCs in water samples. The exact conditions need to be optimized for the kind of samples that are to be analyzed. Agilent Technologies provides the necessary instrumentation, supplies and services to help ensure that laboratories adopting this technology can successfully analyze their samples.

Table 1. P&T/GC/MSD Instrumentation

Gas chromatograph/mass spectrometer	Agilent GC/MSD
Environmental analysis software	Agilent EnviroQuant ChemStation software
Column	20-m × 0.18-mm × 1.0-µm J&W DB-VRX
GC Inlet	Split/Splitless operated in the split mode
Carrier gas	Helium
Purge-and-trap concentrator	Tekmar-Dohrmann
Trap	Vocarb 3000
Purge-and-trap automatic sampler	Tekmar-Dohrmann
Water sample size	5 or 25 mL (5-mL samples used for this work)

Standard solutions containing 60 common VOCs dissolved in methanol were purchased from AccuStandard, Inc. (New Haven, CT USA).

Results and Discussion

P&T sample concentration involves bubbling helium gas through a water sample (usually 5 mL or 25 mL) for several minutes. As the helium bubbles through the sample it sweeps dissolved VOCs out of the water and into a trap that contains one or more adsorbents. During purging, the trap is held at 35 °C allowing the VOCs to be adsorbed on the packing material. After a suitable purging time (typically 11 minutes), the trap is heated to desorb the VOCs which are swept with the helium carrier gas into the GC/MSD.

Figure 1 shows a chromatogram obtained by P&T/GC/MSD analysis of a 5-mL water sample containing 60 different VOCs, each at a concentration of 5 µg/L. The compounds are identified in Table 2.

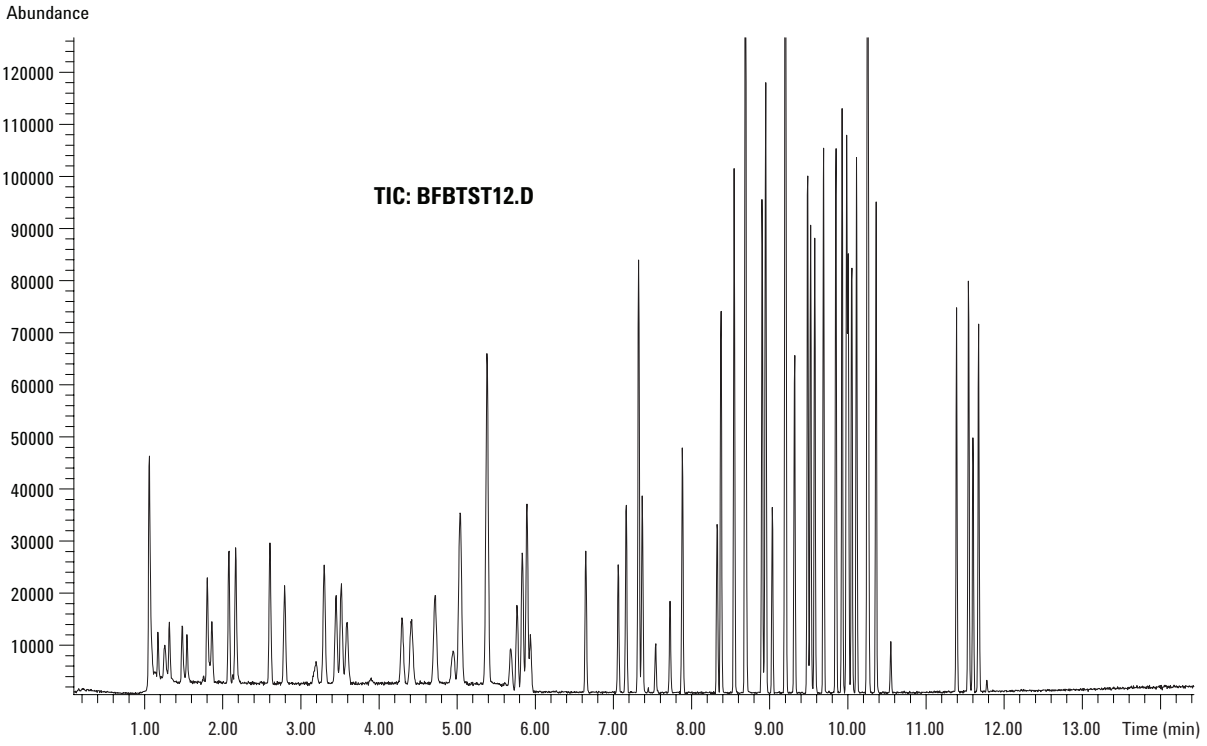

Figure 1. Chromatogram of the volatile organic compounds listed in Table 2. A 5-mL water sample containing each analyte at the 5-µg/L level was analyzed using the P&T/GC/MS system listed in Table 1. The internal standard and two surrogate compounds were present at 10 ppb and are designated in Table 2.

Table 2 Calibration Regression Coefficient r^2 Values

Number	Name	RT (min)	Quant Ion
1	Dichlorodifluoromethane	1.17	85
2	Chloromethane	1.25	50
3	Vinyl chloride	1.31	62
4	Bromomethane	1.48	94
5	Chloroethane	1.54	64
6	Trichlorofluoromethane	1.80	101
7	**1,1-Dichloroethene**	2.08	96
8	Methylene chloride	2.16	84
9	trans-1,2-Dichloroethene	2.60	96
10	1,1-Dichloroethane	2.79	63
11	cis-1,2-dichloroethene	3.30	96
12	Bromochloromethane	3.45	128
13	**Chloroform**	3.51	83
14	2,2-Dichloropropane	3.59	77
15	**1,2-Dichloroethane**	4.29	62
16	**1,1,1-Trichloroethane**	4.42	97
17	1,1-Dichloropropene	4.47	75
18	**Carbon tetrachloride**	4.95	117
19	**Benzene**	5.04	78
20	Fluorobenzene (Internal Standard)	5.38	96
21	Dibromomethane	5.76	93
22	1,2-Dichloropropane	5.83	63
23	Trichloroethene	5.89	95
24	Bromodichloromethane	5.94	83
25	cis-1,3-Dichloropropene	6.65	75
26	trans-1,3-Dichloropropene	7.06	75
27	**1,1,2-Trichloroethane**	7.16	83
28	Toluene	7.32	92
29	1,3-Dichloropropane	7.37	76
30	Dibromochloromethane	7.54	129
31	1,2-Dibromoethane	7.72	107
32	**Tetrachloroethene**	7.88	166
33	1,1,1,2-Tetrachloroethane	8.33	131
34	Chlorobenzene	8.38	112
35	Ethyl benzene	8.55	91
36	m&p-Xylene	8.69	106
37	**Bromoform**	8.70	173
38	Styrene	8.90	104
39	**1,1,2,2-Tetrachloroethane**	8.94	83
40	o-Xylene	8.95	106
41	1,2,3-Trichloropropane	9.03	75
42	Isopropylbenzene	9.20	105
43	4-Bromofluorobenzene (Surrogate)	9.20	95
44	Bromobenzene	9.32	156
45	n-Propylbenzene	9.49	91
46	2-Chlorotoluene	9.52	91
47	4-Chlorotoluene	9.58	91
48	1,3,5-Trimethylbenzene	9.69	105
49	t-Butylbenzene	9.85	119
50	1,2,4-Trimethylbenzene	9.93	105
51	s-Butylbenzene	9.98	105
52	1,3-Dichlorobenzene	10.00	146
53	1,4-Dichlorobenzene	10.05	146
54	4-Isopropyltoluene	10.11	119
55	1,2-Dichlorobenzene-d4 (Surrogate)	10.25	152
56	1,2-Dichlorobenzene	10.26	146
57	**1,4-Dichlorobenzene**	10.36	146
58	n-Butylbenzene	10.36	91
59	1,2-Dibromo-3-chloropropane	10.55	75
60	1,2,4-Trichlorobenzene	11.39	180
61	Naphthalene	11.54	128
62	Hexachlorobutadiene	11.61	225
63	1,2,3-Trichlorobenzene	11.67	180

Figure 1 and Table 2 show several places where the target compounds are not fully resolved in the chromatogram. Because different ions are used for quantitation, the overlapping peaks are no problem, as shown in Figure 2. In Figure 2a, a portion of the total ion chromatogram (TIC) has been enlarged showing peaks that overlap. As seen in Figure 2b, the extracted ion chromatograms used for quantitation of these compounds have peaks that are fully resolved. The GC/MSD system uses a combination of chromatography and ion selectivity to resolve all of the peaks, providing faster analyses with quantitation that is precise and accurate.

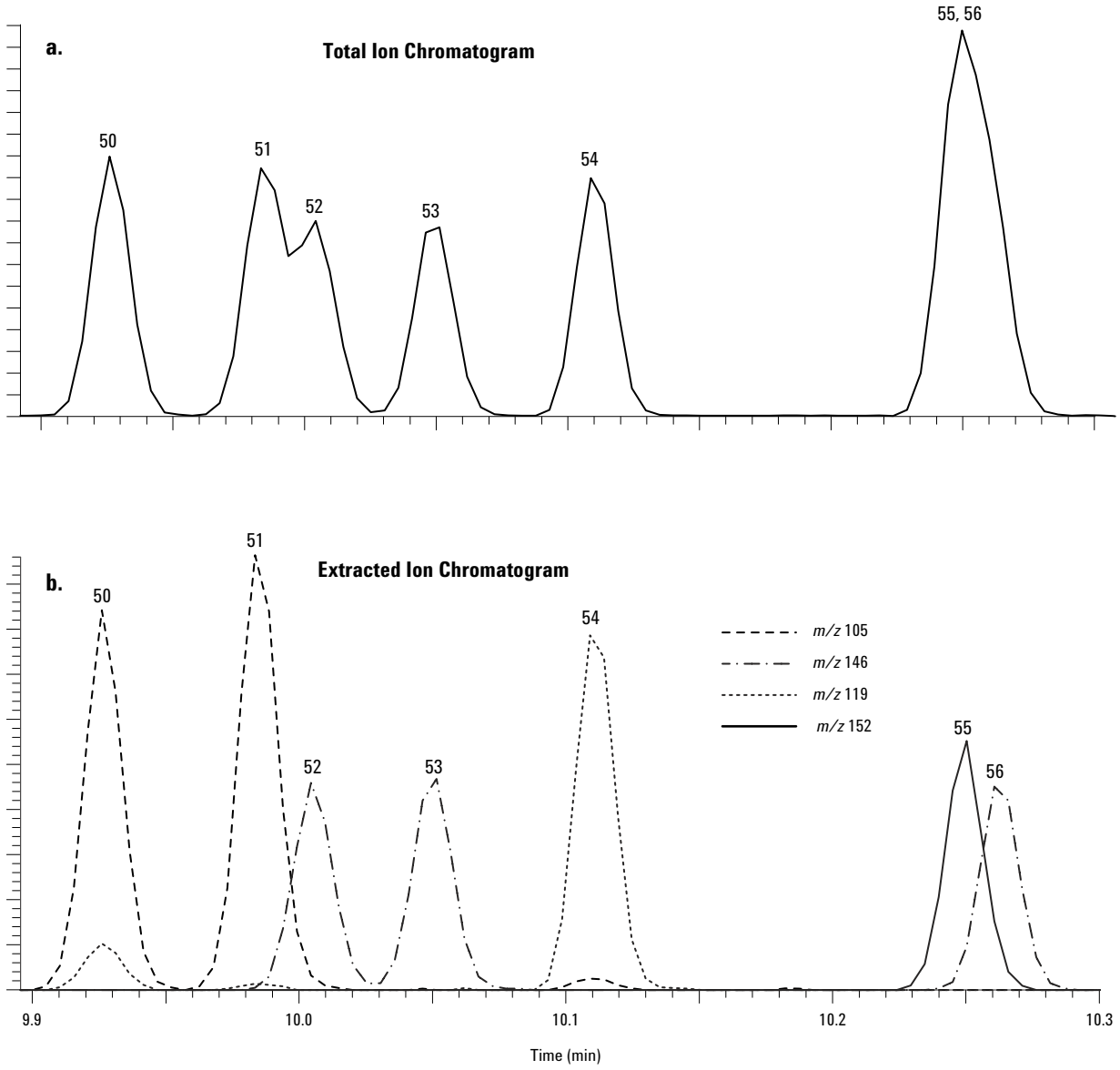

Figure 2. **a) A portion of the total ion chromatogram in figure 1. The peaks are identified in Table 2. s-Butylbenzene (51) and 1,3-dichlorobenzene (52) are partially resolved while 1,2-dichlorobenzene (56) and its deuterated analog (55) (used as a surrogate) are unresolved. b) The extracted ions used for quantifying these compounds show peaks that are fully resolved.**

Figure 3 shows calibration curves for chloroform (0.5 to 100 µg/L) and 1,2-dichloroethane (0.5 to 250 µg/L) that were generated by purging 5-mL spiked water samples. The Agilent MSD typically displays good linearity over four orders of magnitude. However, the region of linearity is compound dependent, i.e., not all compounds are linear over the same calibration range. Laboratories are usually able to generate linear calibration curves over two orders of magnitude for a large number of analytes, such as those listed in Table 2.

With the high sensitivity of the Agilent GC/MSD system, laboratories usually only need to purge 5-mL samples. However, when additional sensitivity is required, the P&T system can accommodate 25-mL samples which increases the sensitivity by a factor of 5 for most compounds.

Conclusions

Automated P&T/GC/MS is the most widely used technique for the analysis of volatile organic compounds in drinking water, waste water, factory effluent and ground water. Agilent Technologies provides the necessary instrumentation, GC columns, supplies and services required for laboratories to succeed with these analyses. Agilent's EnviroQuant software has been specifically designed for the needs of environmental laboratories.

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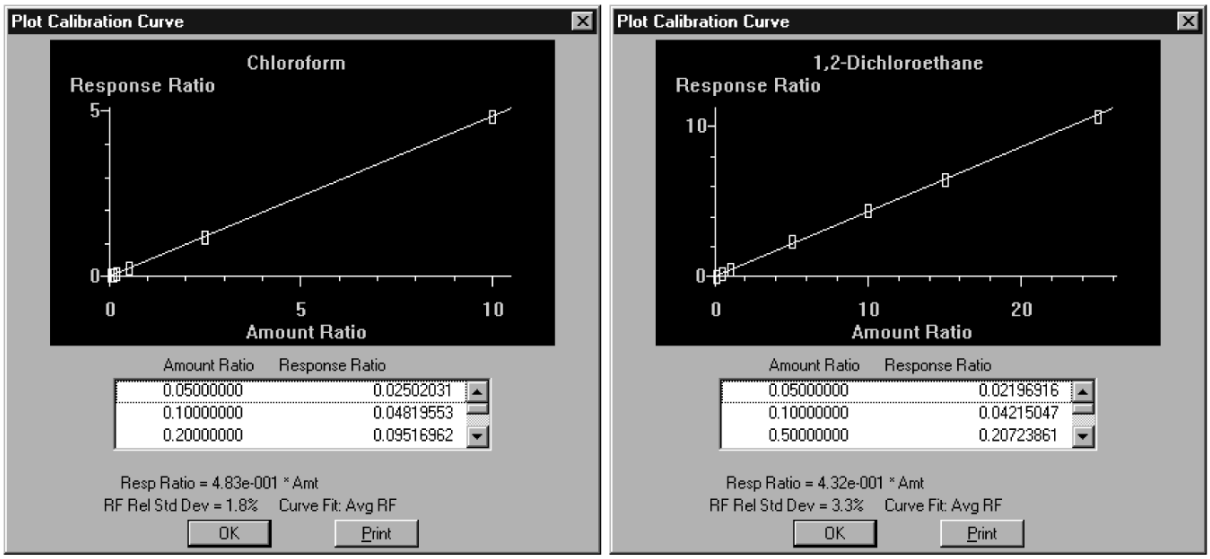

a. b.

Figure 3. Calibration curves for: a) chloroform (0.5-100 µg/L) and b) 1,2-dichloroethane (0.5-250 µg/L).

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Printed in the USA
November 6, 2001
5988-4463EN

Agilent Technologies

Using the Agilent 6890 Gas Chromatograph and Agilent 5973 Mass Spectrometer System for EPA Method 524.2

Application

Gas Chromatography

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Abstract

A system comprised of a purge and trap (P&T) concentrator, a gas chromatograph (GC), and a mass spectrometer (MS) was used to determine 61 volatile organic compounds (VOCs). All U.S. EPA method 524.2 criteria were met without using cryofocusing. The P&T and GC-MS conditions listed in tables 1 and 2 detail the instrument settings. The method development for analyzing 524.2 analytes was refined by members of the GC-MS Volatile Organics Analysis (VOA) group at Quanterra in Tampa, Florida.

Introduction

U.S. EPA method 524.2 is a general-purpose method used to identify and quantify volatile organic compounds (VOCs) in surface, ground, and drinking water. The method is applicable to a wide range of organic compounds including the four trihalomethane disinfection byproducts. The 61 VOCs in this note are a subset of the 84 VOCs that can be analyzed using method 524.2.

Compounds of sufficient high volatility and low water solubility are purged from a water sample using helium and trapped on a solid sorbant held at room temperature. At the end of the purge cycle, the trap is heated and, using helium, the compounds are desorbed onto the head of a gas chromatograph (GC) column. The GC column is temperature programmed, and the analytes are eluted into the mass spectrometer (MS) ion source. The MS is used for identification and measurement. The purge and trap (P&T)-GC-MS system is controlled from a PC.

Experimental

The program requirements for which the 524.2 analysis is used must meet local state regulatory guidelines as well as EPA method 524.2 acceptance criteria and U.S. Air Force compliance guidelines under the auspices of the Air Force Center for Environmental Excellence (AFCEE) program.

Quanterra maintains strict QA/QC procedures at all 12 facilities. Each location has a quality assurance officer (QAO) reporting directly to the corporate quality assurance director. Quanterra's network of 12 facilities in 10 states staffs over 700 employees and encompasses over 310,000 square feet of facility space, providing the capacity to handle any analytical need. Quanterra performs more than 1.5 million separate tests per year. A nationwide network of fully equipped labs, linked by advanced information management systems, assures a high standard of testing and consistent quality.

Quanterra's comprehensive quality management system (QMS) forms the foundation of their quality goals. Quanterra's QMS ensures that their clients receive high-quality analytical services that are timely and reliable, and that meet the intended purpose in a cost-effective manner. The QMS also applies to all Quanterra technical, business, and administrative functions. The principles and practices expounded in the QMS apply to all staff and are fundamental to the services they provide. As a result, Quanterra is continuously seeking ways to improve their products and services using the best technologies available. The AGILENT 6890 / AGILENT 5973 GC-MS system provides high-quality data and increased productivity.

The P&T instrumentation and conditions are listed in table 1. The Vocarb 3000 trap allows for higher desorb and bake temperatures. The high desorb temperature facilitates efficient desorption of target analytes, and the high bake temperature minimizes carryover between samples. The standard transfer line provided with the P&T was replaced with a Restek 0.53-mm SilcoSteel MXT 502.2 column. The use of the analytical column as the transfer line between the P&T and GC appears to improve peak symmetry for low-level standards. The transfer line is attached directly to the AGILENT 6890 GC injection port (direct capillary interface) and runs in the split mode. A purge rate of 50 mL/min appears to improve the recovery of analytes that are known to have poor purge efficiencies. The 50-mL/min purge flow did not have an adverse effect on the recovery of the gases and, as a result, produced method and program compliant data. Traditional trap packing materials (Tenax/charcoal/ silica) usually did not hold the gases at higher purge flow rates, resulting in poor recoveries. This problem was not observed when using the Vocarb 3000 trap. The original method's desorb and bake temperature of 180 °C is a limitation associated with traditional packing material (Tenax break down).

The GC-MS instrumentation and conditions are listed in table 2. Conditions were optimized for maximum sample throughput while meeting site-specific data quality objectives. The split ratio used allows the best combination of sensitivity and peak shape. With this configuration, it is advantageous to use the electronic pressure control (EPC) inlet (option available on the AGILENT 6890 GC). With the EPC inlet pressure on, the chromatography for the gases is improved, and analytes at the end of the temperature program have much sharper peak shape. EPC also gives much better reproducibility of analyte retention times.

Each 12-hour shift (site-specific requirements allow for a 12-hour clock for the tune verification) starts with verification of the fragmentation pattern of 4-bromofluo-robenzene (BFB) obtained from 25 ng on-column. A five-point calibration curve is then analyzed at concentrations of 500, 250, 125, 50, and 12.5 ng on-column. Once the calibration acceptance criteria is verified, a 100-ng (4 µg/L) laboratory control spike/laboratory control spike duplicate (LCS/LCSD) is analyzed followed by a method blank. Successful analysis of

Table 1. Purge and Trap Conditions

P&T	Tekmar LSC 3000
Automatic sampler	Tekmar ALS 2016
Trap	Vocarb 3000 Supelco part no. 2-4920
P&T-GC interface	Custom*
Sample size	25 mL
Purge temperature	35 °C
Purge rate	50 mL/min
Purge time	11 min
Dry purge time	1 min
Desorb preheat temperature	250 °C
Desorb temperature	260 °C
Desorb time	2 min
Bake temperature	270 °C
Bake time	6 min
Bake-gas bypass on time	1 min
Line/valve temperature	100 °C
Water management control (WMC) temperature	310 °C

*Standard transfer line replaced with approximately 0.7-m length of Restek MXT-502.2 SilcoSteel 0.53-mm id column

Table 2. Gas Chromatograph and Mass Spectrometer Conditions

Gas chromatograph	**Agilent 6890**
Inlet	EPC split/splitless
Mode	Split
Inlet temperature	200 °C
Pressure	13.9 psi
Split ratio	35:1
Split flow	24.2 mL/min
Gas saver	On at 2 min
Gas saver flow	20.0 mL/min
Oven	
Initial temperature	35 °C
Initial time	4 min
Rate	15 °C/min
Final temperature	200 °C
Final time	0.1 min
Equilibration time	0.5 min
Oven max temperature	240 °C
Column	**DB-624 fused silica capillary**
Agilent equivalent	Agilent part no. 121-1324
Length	20 m
Diameter	180 µm
Film thickness	1.0 µm
Initial flow	0.7 mL/min
Average velocity	37.0 cm/sec
Mode	Constant flow
Inlet	Front
Outlet	MS
Outlet pressure	Vacuum
Mass spectrometer	**Agilent 5973**
Solvent delay	1.1 min
EM voltage	2035 volts
Low mass	35 amu
High mass	260 amu
Threshold	200
Sampling	3
Scans/sec	3.25/sec
Quad temperature	150 °C
Source temperature	200 °C
Transfer line temperature	250 °C

the LCS/LCSD and blank are followed by 20 field samples. A typical instrument sequence, when initial calibration is not required, is shown in table 3. This new sample sequence starts with an instrument tune verification (BFB analysis) followed by the analysis of the continuing calibration verification (CCV) standard, If the CCV fails, the system is recalibrated. After the CCV, a 100-ng (4 µg/L) LCS/LCSD is analyzed followed by a method blank and 20 field samples. *The LCS/LCSD QC samples are a site-specific project requirement.*

Note: The AGILENT 5973 MS only required retuning every 4 to 6 weeks during large sampling events. During these events, 26 samples were analyzed every 12 hours, operating 6 to 7 days a week.

Results

The results from the BFB tuning analysis are shown in table 4, together with the EPA method 524.2 tuning criteria. If the BFB tuning criteria are not met, typically mass 50 was low or mass 176 was high. The problem was resolved by running the auto-tune option provided with the Enviro-Quant software followed by a reanalysis of the BFB solution. If the BFB still did not pass, the problem was resolved by replacing the trap. The AGILENT 5973 MS ran for over a year before there was a need to open the analyzer and replace the filaments. The source was cleaned while the analyzer was open, and a little scorching around the filament area was observed. SW-846 method 8260B and CLP-SOW Method OLC02.1 were also performed using this instrument, often containing high levels of target and non-target analytes. As a result, finding the source and its component parts in good condition was unexpected.

A list of target analytes for this project, together with their compound number and retention time (RT), are shown in table 5. The method detection limits (MDLs) shown are based on initial calculations per 40 CFR, Part 136, Appendix B. Prior to running client samples, an instrument detection limit (IDL) study was conducted. This comprised of a five-point calibration curve followed by the CCV, method blank, and seven replicates of the 0.5-µg/L standard for 7 consecutive days. The results between replicates within the same analytical

Table 3. Instrument Sequence

Sequence No.	**Description**
1	1 ppb BFB, with CCV
2	10 ppb CCV
3	4 ppb LCS
4	4 ppb LCSD
5	Method blank
6	Sample 1
7	Sample 2
⋮	⋮
	Matrix spike
	Matrix spike duplicate
	⋮
27	Sample 20

Table 4. BFB Tuning Criteria and Results

m/e	Ion Abundance Criteria	Ion Abundance Results
95	Base Peak, 100% relative abundance	100.00
50	15.00%-40.00% of mass 95	19.26
75	30.00%-60.00% of mass 95	46.39
96	5.00%-9.00% of mass 95	7.25
173	Less than 2.00% of mass 174	0.65
174	Greater than 50.00% of mass 95	85.07
175	5.00%-9.00% of mass 174	7.45
176	95.00%-101.00% of mass 174	100.70
177	5.00%-9.00% of mass 176	6.89

sequence demonstrated very little variation. Additionally, the results obtained between the day-to-day analytical sequences also exhibited very little variation. The IDL study and the MDL study yielded similar results with little or no statistical variation. All analyte MDLs are comfortably below the 0.5 µg/L reporting limit for this project. Lower detection limits could be achieved with lower split ratios.

The initial calibration for this set of analyses was done in August 1997 at the following five levels: 0.5, 2.0, 5.0, 10, and 20 µg/L. Response factors were calculated for each analyte at each level. The percent relative standard deviations (%RSDs) of these response factors, listed in table 5, are all less than 20 and meet the criteria for Table 5. Target Compound List with QA\QC (continued) linearity. Hexachlorobutadiene and naphthalene had trouble meeting the daily ICV/CCV acceptance criteria on a daily basis; these compounds are known as poor purgers. Fortunately, the site-specific QA requirements allows for the use of a quadratic calibration when the acceptance criteria for linearity is not met.

Table 5. Target Compound List with QA\QC

Compound Number	Compound Name	RT	MDL	Init Cal %RSD RRF limit 0-20	CCV %D limit ± 30	LCS %Rec limit 70-130	LCSD %RPD limit 0-20
Internal Standard							
36	Fluorobenzene	6.651					
Surrogates							
63	4-Bromofluorobenzene	10.919		5.23	-12.	99.8	2.0
33	1,2-Dichlorobenzene-d(4)	12.436		6.54	-1.9	105.	7.6
Target Analytes							
34	Benzene	6.322	0.18	8.64	3.9	94.8	6.8
64	Bromobenzene	11.065	0.15	3.72	-12	112.	5.5
27	Bromochloromethane	5.574	0.19	15.5	-1.3	104.	2.1
40	Bromodichloromethane	7.592	0.21	10.1	-0.9	100.	5.0
61	Bromoform	10.595	0.23	15.3	-21.	111.	5.3
6	Bromomethane	1.907	0.31	13.4	4.7	82.3	1.8
79	n-Butylbenzene	12.451	0.19	10.7	-11.	97.2	8.3
74	sec-Butylbenzene	11.897	0.19	9.47	-8.5	98.7	12.
72	tert-Butylbenzene	11.682	0.20	6.21	3.0	110.	12.
31	Carbon tetrachloride	6.091	0.17	10.0	-5.3	104.	7.8
55	Chlorobenzene	9.784	0.17	6.67	-4.0	105.	3.0
7	Chloroethane	2.007	0.18	6.51	9.9	82.8	4.2
29	Chloroform	5.699	0.18	7.47	6.1	97.0	1.8
4	Chloromethane	1.510	0.19	6.77	16.	75.6	8.4
69	2-Chlorotoluene	11.264	0.18	6.53	-9.4	103.	7.1
71	4-Chlorotoluene	11.369	0.17	7.55	-11.	105.	7.6
51	Dibromochloromethane	9.188	0.17	11.1	-12.	108.	0.04
81	1,2-Dibromo-3-Chloropropane	13.215	0.30	6.44	16.	NR	NR
52	1,2-Dibromoethane	9.287	0.25	13.3	9.2	95.1	8.0
39	Dibromomethane	7.404	0.24	14.0	8.4	111.	1.0

Table 5. Target Compound List with QA\QC (continued)

Compound Number	Compound Name	RT	MDL	Init Cal %RSD RRF limit 0-20	CCV %D limit ± 30	LCS %Rec limit 70-130	LCSD %RPD limit 0-20
Target Analytes (continued)							
80	1,2-Dichlorobenzene	12.451	0.19	7.44	-0.4	104.	3.2
76	1,3-Dichlorobenzene	11.996	0.20	8.36	-2.5	105.	3.3
78	1,4-Dichlorobenzene	12.090	0.17	8.84	0.3	99.8	2.8
3	Dichlorodifluoromethane	1.353	0.21	6.07	15.	83.3	9.1
22	1,1-Dichloroethane	4.475	0.22	7.82	11.	88.7	5.3
35	1,2-Dichloroethane	6.342	0.20	11.2	5.3	99.8	3.5
12	1,1-Dichloroethene	2.280	0.20	7.61	8.5	99.0	6.4
25	cis-1,2-Dichloroethene	5.275	0.22	8.63	-6.3	97.2	3.6
18	trans-1,2-Dichloroethene	3.842	0.17	10.9	13.	94.8	4.9
38	1,2-Dichloropropane	7.284	0.22	10.0	8.6	88.3	2.1
49	1,3-Dichloropropane	8.957	0.12	6.56	7.4	95.7	0.76
24	2,2-Dichloropropane	5.265	0.20	9.39	4.2	92.6	0.80
32	1,1-Dichloropropene	6.097	0.18	9.13	5.0	88.3	12.
42	cis-1,3-dichloropropene	8.053	0.19	10.5	2.3	95.0	0.64
46	trans-1,3-dichloropropene	8.618	0.14	10.6	5.1	95.0	3.3
56	Ethylbenzene	9.904	0.20	8.46	3.9	89.4	8.6
83	Hexachlorobutadiene	14.229	0.24	16.1	-4.5	90.6	5.7
62	Isopropylbenzene	10.778	0.17	9.23	0.6	98.4	10.
75	4-Isopropyltoluene	12.049	0.20	9.07	11.	102.	8.9
16	Methylene chloride	3.445	0.21	9.07	-3.5	109.	5.0
98	Methyl-t-butyl ether	3.884	0.20	10.4	8.3	97.7	3.0
84	Naphthalene	14.287	0.13	19.7	5.3	76.8	5.8
67	n-Propylbenzene	11.186	0.21	6.92	-16.	108.	7.7
60	Styrene	10.422	0.18	11.8	-0.3	96.4	5.8
57	1,1,1,2-Tetrachloroethane	9.868	0.21	8.27	-5.3	102.	10.
65	1,1,2,2-Tetrachloroethane	11.065	0.18	6.57	-2.4	101.	4.8
48	Tetrachloroethene	8.942	0.21	8.61	8.6	89.0	10.
45	Toluene	8.393	0.14	9.37	-6.7	102.	9.6
85	1,2,3-Trichlorobenzene	14.528	0.18	9.13	0.7	90.7	5.5
82	1,2,4-Trichlorobenzene	14.052	0.16	11.0	-1.7	91.7	3.6
30	1,1,1-Trichloroethane	5.893	0.24	8.34	-2.9	102.	6.3
47	1,1,2-Trichloroethane	8.795	0.17	12.3	4.3	105.	1.2
37	Trichloroethene	7.059	0.16	8.77	-4.6	100.	6.7
9	Trichlorofluoromethane	2.273	0.19	5.26	6.2	99.1	3.9
68	1,2,3-Trichloropropane	11.102	0.20	5.54	2.7	101.	8.8
73	1,2,4-Trimethylbenzene	11.729	0.17	8.38	2.0	95.7	7.6
70	1,3,5-Trimethylbenzene	11.363	0.18	9.42	0.3	94.7	9.4
5	Vinyl chloride	1.609	0.15	7.54	4.4	82.1	13.
59	o-Xylene	10.411	0.13	10.7	0.3	94.0	7.5
58	m-Xylene	10.019	0.17	8.15	2.8	92.6	9.3
58	p-Xylene	10.019	0.17	8.15	2.8	92.6	9.3

After the BFB tuning verification is performed, a CCV is run at the 10-ppb level. The method requires that each analyte response factor (RF) is ± 30% of its initial calibration value. These percent deviations (%Ds) are listed in table 5, and all analytes meet the method criteria. If one or more analytes do not meet this criteria, a new five-point calibration curve is run. The data presented here were run in September 1997, one month after the initial calibration. This system is very stable for long periods of time. A five-level calibration has only been necessary eight to ten times in the last 12 months. A total ion chromatogram (TIC) for the CCV is shown in figure 1.

This project requires analysis of a LCS and LCSD. Laboratory blank water is spiked at the 4-µg/L level and analyzed in duplicate. The recoveries for each analyte must be between 70% and 130% for each analyte. A duplicate aliquot of the LCS, referred to as an LCSD, is then analyzed. The relative percent difference (RPD) of this LCS and the LCSD must be less than 20% for each analyte. The LCS recoveries and LCSD RPDs are shown in table 5. All analytes met the site-specific acceptance criteria.

After all of the project-specific QA/QC requirements are met, actual field samples can be analyzed. Results for three samples are shown in table 6. The samples were taken from private wells in an Area of Concern (AOC) in the northeast United States. All ion profiles met the site-specific QC acceptance criteria and all other regulatory acceptance criteria for this AOC.

A TIC for sample 1 is shown in figure 2. The excellent peak shape is typical of the system performance in our laboratory.

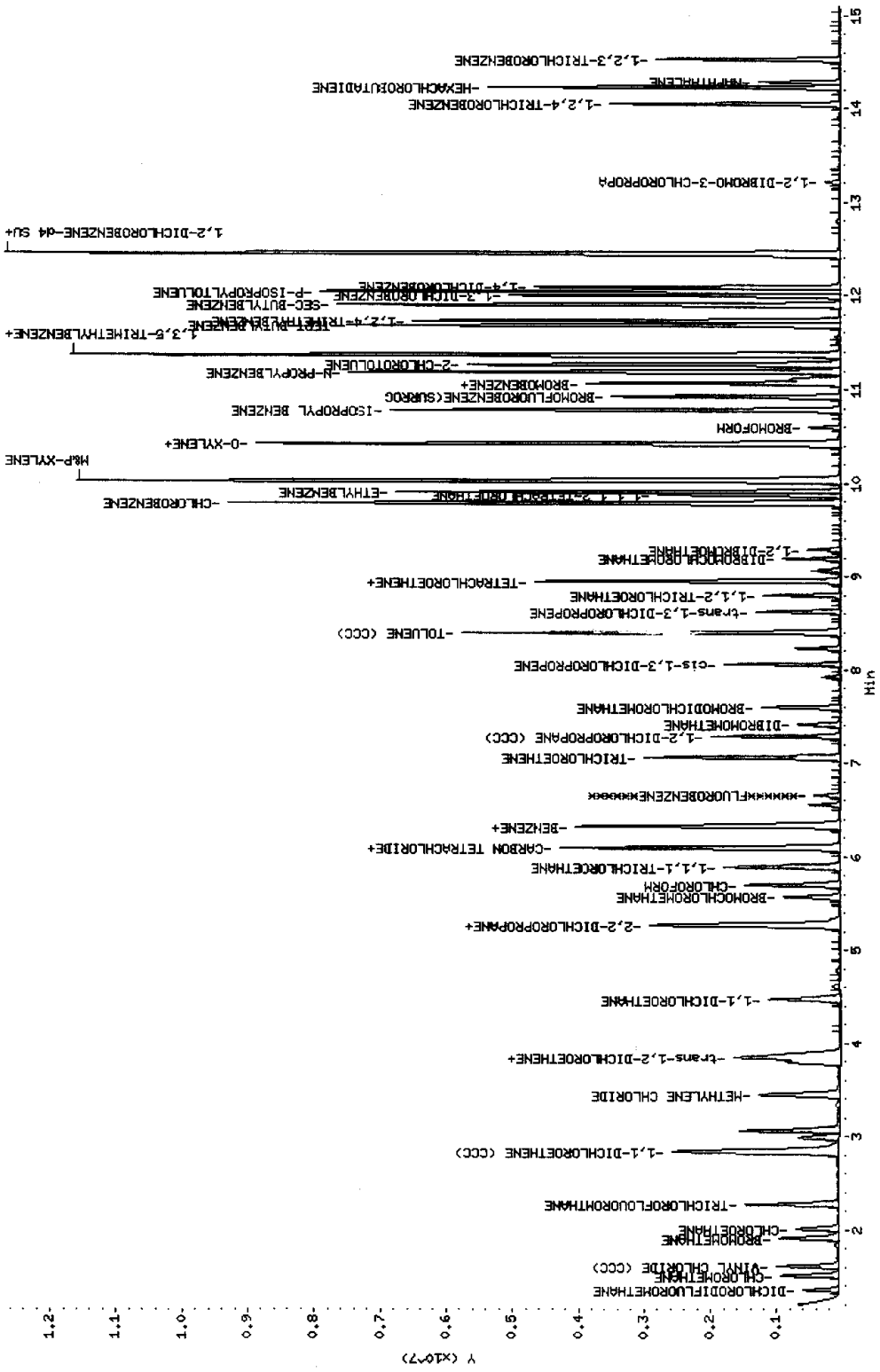

Figure 1. CCV total ion chromatogram.

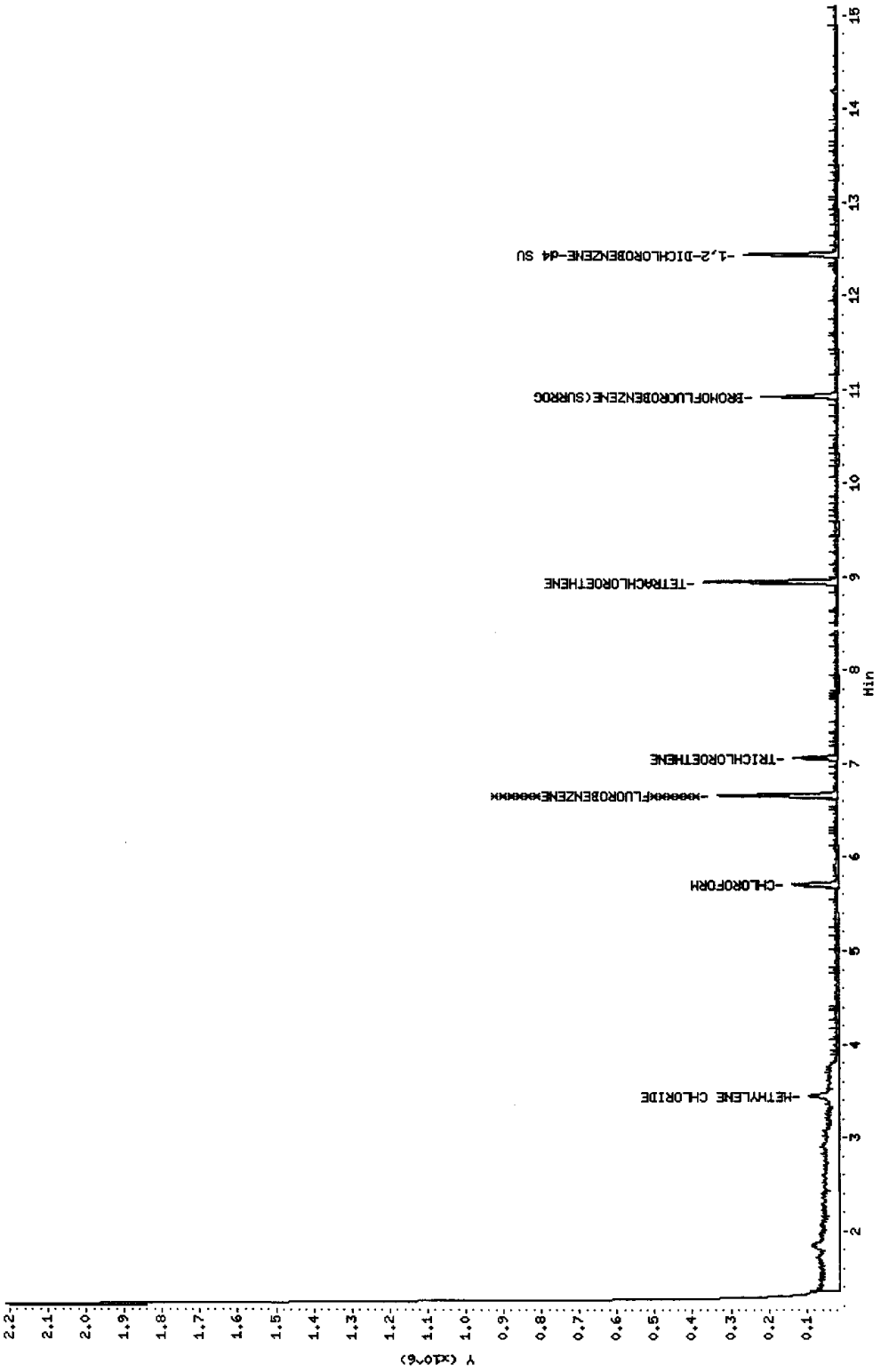

Figure 2. Total ion chromatogram for field sample one.

Table 6. Results of Sample Analyses

Compound Number	Compound Name	RT min	Sample1	Sample2	Sample 3
Internal Standard				*Area % Difference limit ± 30*	
36	Fluorobenzene	6.651	-25.6	-29.2	-18.65
Surrogate Standards				*% Rec 1.0 ppb limit 80-100*	
33	1,2-Dichlorobenzene-d(4)	12.436	98.7	99.5	95.1
63	4-Bromofluorobenzene	10.919	85.2	93.4	89.0
Target Analytes			*[ppb]*	*[ppb]*	*[ppb]*
16	Methylene chloride	3.445	0.55	0.56	1.0
29	Chloroform	5.699	1.0	3.7	1.0
37	Trichloroethene	7.059	0.54	< 0.50	< 0.50
40	Bromodichloromethane	7.592	< 0.50	5.9	2.3
48	Tetrachloroethene	8.942	1.2	< 0.50	< 0.50
51	Dibromochloromethane	9.188	< 0.50	8.4	5.1
61	Bromoform	10.595	< 0.50	2.7	3.2

Conclusions

The AGILENT 6890/AGILENT 5973 GC-MS can be used to perform EPA method 524.2. All calibration, verification, and quality control criteria of the method can be met on a routine basis. The system exhibits excellent stability, minimal downtime, and sufficient sensitivity to meet the requirements for this project. The system performance, combined with expert personnel and a rigorous QA/QC program, results in high sample throughout for method 524.2. The AGILENT 6890/ AGILENT 5973 GC-MS allows Quanterra to meet clients' expectations in a timely and cost-effective manner.

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Printed in the USA
April 26, 2001
5968-1257E

Agilent Technologies

Optimized Analysis of Gasoline (BTEX) in Water and Soil Using GC/FID with Purge and Trap

Application Note
228-324

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Abstract

Gas chromatography with purge and trap analysis using their HP-1 capillary column and the Agilent 5890 Series II gas chromatograph/flame ionization detector was done to determine gasoline components in contaminated water and soil in accordance with modified EPA Methods 8015/8020. Purge and trap and gas chromatograph parameters were optimized for accurate quantitation of gasoline range organics (aliphatics, aromatics, and oxygenates) and to increase analysis speed.

Key Words

- EPA Methods 8015/8020
- Gasoline
- BTEX
- Purge and Trap
- GROs
- GC/FID analysis
- LUST

Introduction

Modified EPA Methods 8015/8020 are used to determine gasoline and gasoline components in water and soil by capillary gas chromatography (GC) with a flame ionization detector (FID) or photoionization detector (PID). The hydrocarbons in gasoline encompass a wide range, from butane to decane and benzene to naphthalene, and cover a boiling point range of 50°C to 281°C. For such complex mixtures, an efficient purge and trap (P and T) system is required to concentrate samples for high-resolution gas chromatography. Detection is achieved using an FID, and quantitation is based on FID response to a gasoline standard. Other light petroleum products that can be determined in the same manner include paint stripper, Stoddard solvent, mineral spirits, petroleum naphtha, and aviation jet fuels using the pattern recognition technique.

The analysis of gasoline components, e.g., gasoline range organics (GROs), and benzene, toluene, ethylbenzene, and xylenes (BTEX) in particular is of great importance because BTEX is frequently used as a marker in the identification of gasoline-type products. Subsequently, the analysis of BTEX is often used to determine the composition and the origin of such products including weathered fuels leaking from underground storage tanks (LUST), spills in pipe lines, and run-off from surface transportation.

For the analysis of gasoline with BTEX, the sample is introduced into a sparge tube on the P and T autosampler or purge vessel or the P and T unit. The P and T concentrates the volatiles in the sample and transfers them onto the capillary column.

Parameters affecting the efficiency of P and T sample concentration include time and temperature for sample purge, dry purge, desorption of trapped volatile organics and trap baking. Most P and T system manufacturers recommend 11 minutes of purge or a total of 440 ml purge gas through the sample. Many laboratories use the manufacturer's set purge flow of 40 ml/min which corresponds to 11 minutes of purge time, to achieve a minimum of 440 ml purge gas through the sample. In this study a Vocarb-3000 trap was used because it can provide higher trapping efficiency and allow for higher desorption and baking temperature.

A typical analysis can usually be completed in 35 to 40 minutes. In this application both P and T parameters and GC conditions were optimized for accurate quantitation and analysis speed.

Experimental

Samples were concentrated using an Agilent 7695A P and T system with a Vocarb-3000 trap (part no. 5182-0775) and a 5-ml frit sparger (part no. 5182-0852). Using an HP-1 column (30 m x 0.53 mm x 5.0 µm, (part no. 19095Z-623), hydrocarbons were analyzed on an Agilent 5890 Series II GC with EPC and FID. Instrument requirements and optimal GC and P and T conditions are listed in **Table 1**.

Working solutions were prepared from diluting commercial gasoline, LUST-modified GROs (part no. 5182-0860), and internal standard and surrogate (part no. 8500-6007) with GC-grade methanol (Burdick and Jackson). Concentrations of GROs, gasoline, and jet fuel standards are listed in **Table 2**.

Samples were prepared from spiking 5 ml of organic-free reagent water using a 5-ml sample syringe with a luer connector (part no. 9301-1185) with standard solutions using 5-µl to l00-µl fixed needle syringes (HP part nos. 9301-0810, 9301-0818, 9301-0059, 9301-0063, respectively).

Results and Discussion

To obtain accurate and reproducible results, complete sample purging, managing water adequately from the P and T system, and preventing carry-over from the trap are essential. Many environmental laboratories analyze gasoline with BTEX using long sample purge (11 to 15 minutes), dry purge (2 to 4 minutes), trap desorb (2 to 4 minutes), and trap bake (10 to 20 minutes) times. Therefore, a typical run usually takes 40 to 48 minutes including 3 to 5 minutes for trap cool-down.

Figure 1 shows a GC/FID analysis of a gasoline standard and a GC/PID chromatogram of a GROs standard using an OI 4460A P and T system with a BTX trap and DB-1 column (30 m x 0.53 mm x 5 µm). GC and P and T conditions are listed in **Table 3**. Although the GC runs were completed in 27 minutes, the actual cycle time for each run was 37 to 40 minutes.

Table 1. Instrument Requirements and Optimized Conditions

A. Recommended Instrumentation

Gas chromatograph:	5890 Series II
Injection port:	Split/splitless inlet
Column:	HP-1, 30 m x 0.53 mm x 5.0 µm (Part no. 19095Z-623)
Detector:	FID
Injection technique:	7695A P and T
Data system:	3365 ChemStation and HP Vectra 486/100MX

B. Experimental Conditions

GC Parameters

Inlet:	220°C, split injection (split ratio 5:1)
Carrier:	Helium, 10 ml/min, constant flow (6.5 psi at 40°C)
Oven parameters:	40°C (3 min) at 7°C/min to 125°C to 250°C (3 min) at 35°C/min
Detector:	FID, 300°C; nitrogen makeup gas, 25 ml/min; H_2, 30 ml/min; and air, 350 ml/min PID, 250°C

P and T Parameters

Line temperature:	200°C	Purge time:	11 min
Valve temperature:	200°C	Dry purge time:	1 min
Mount temperature:	40°C	Desorb time:	2 min
MCS line temperature:	100°C	Bake time:	5 min
Purge ready temperature:	30°C	BGB time:	2 min
MCS desorb temperature	40°C		
Desorb preheat temperature:	245°C		
Desorb temperature:	250°C		
Bake temperature:	265°C		
MCS bake temperature:	300°C		

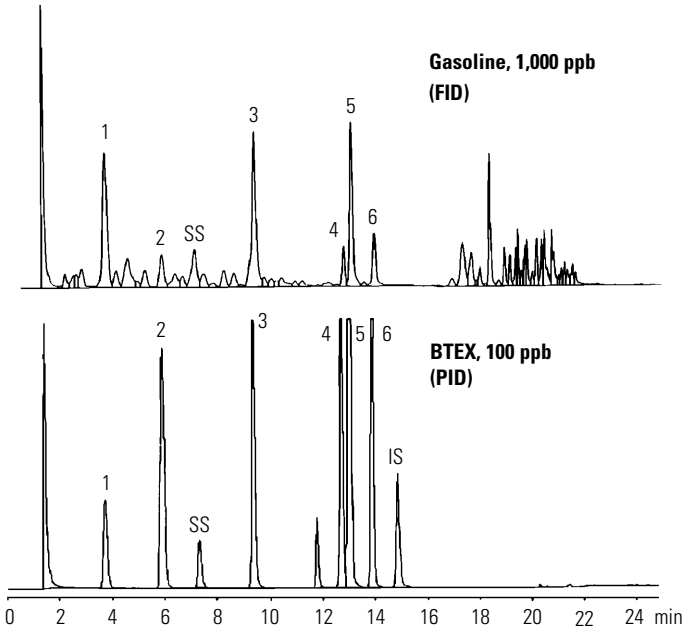


Figure 1. Typical chromatograms of gasoline and GROs standards using a DB-1 column under the GC and P and T conditions (Table 3) used in environmental testing laboratories (see Table 2 for peak identification).

Optimized GC Run Time

With the HP-1 column (30 m x 0.53 mm id x 5 µm) and a faster oven temperature, the GC run time was initially reduced to 21 minutes for GROs and gasoline (see **Figure 2**). Good baseline separations and sharp symmetric peaks **(Figure 2B)** were obtained for all GROs, including surrogate (α,α,α-trifluorotoluene) and internal (4-bromofluorobenzene) standard. The oven temperature program used was 40°C (3 min) at 7°C/min to 125°C to 250°C (3 min) at 35°C/min and a constant carrier flow of 10 ml/min. Under these conditions (**Table 1**), both pentane and MtBE were clearly separated from the large solvent peak (menthanol).

Even though the last GROs component (naphthalene) eluted below 200°C at 17.8 minutes, the oven temperature was increased to 250°C to bake out the high-boiling material purged from the sample. As a result, no carryovers were found even with repeated injections of gasoline standard in the 23,000-ppb level.

GC run times were further lowered by using a thinner-film HP-1 column and/or faster oven temperature programs. **Table 4** shows the benefits of using various column thicknesses, temperature ramps, and carrier flows to achieve the optimal GC run time of 17 minutes. Analytes generally elute faster from a thin-film column (**Figure 4**). In **Figure 3**, the thick-film column retained hydrocarbons longer initially until the faster oven temperature ramp (15°C/min) sped up the elution of all GROs components from the column. To avoid potential coelution (peaks 4 and 5), a comparative smaller carrier flow (4.5 ml/min) was used instead of the optimal 10 ml/min carrier flow. Reducing the GC run time, however, would be counterproductive because the total run time is dependent on the P and T cycle.

Table 2. Analytes in Working Standards

Standards	Peak No.	Components	Concentration
GROS mix	1	MtBE	100 ppm each
	2	Benzene	
	3	Toluene	
	4	Ethylbenzene	
	5	m-/p-Xylene	
	6	o-Xylene	
	7	1,2,4-Trimethylbenzene	
	8	1,3,5-Trimethylbenzene	
	9	Naphthalene	
	10	α,α,α-Trifluorotoluene (SS)	
	11	4-Bromofluorobenzene (IS)	
Gasoline standard		Gasoline	500 ppm
Gasoline		Gasoline	2,500 ppm
Jet fuel		Aviation jet fuel	1,000 ppm

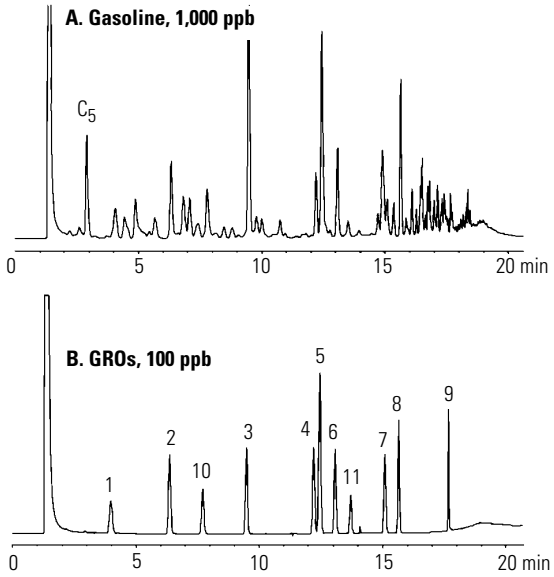


Figure 2. Chromatograms for gasoline and GROs standards using an HP-1 column under the optimal GC and P and T conditions listed in Table 1. (See Table 2 for peak identification.)

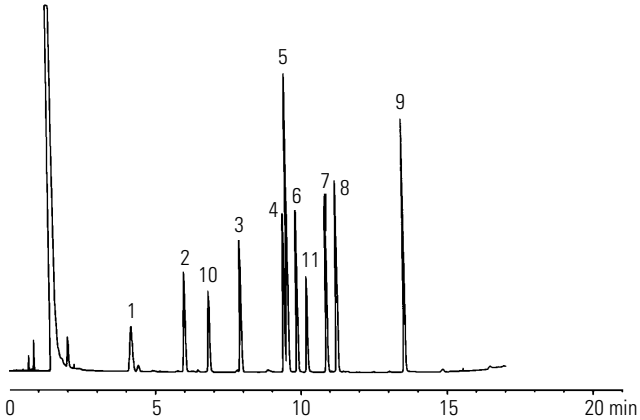
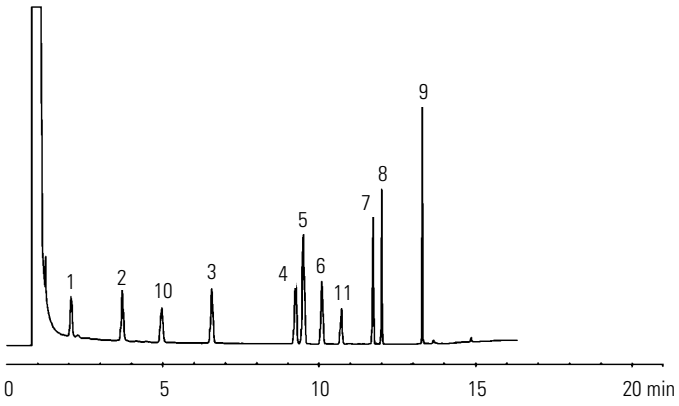


Figure 3. Chromatogram of GROs standards using a thick-film HP-1 (30 m x 0.53 mm x 5 µm) column. (See Table 2 for peak identification and Table 4 for GC conditions.)

Table 3. Typical GC and P and T Conditions for Gasoline and BTEX Analysis

GC Parameters			
Injection:	Direct injection		
Carrier flow:	Initially 10 ml/min, constant pressure mode		
Oven temperature:	50°C (hold 3 min) to 125°C at 5°C/min to 240°C (5 min) at 45°C/min		
Detector:	PID (250°C) in series with FID (300°C)		
P and T Parameters			
Trap:	BTX trap		
Purge temperature:	Ambient	Purge time:	11 min
Dry purge temperature	22°C	Dry purge time:	2 min
Desorb preheat temperature	150°C	Desorb time:	4 min
Desorb temperature	180°C	Bake time:	15 min
Bake temperature:	200°C		

Figure 4. Chromatogram of GROs standards using a thin-film HP-1 (30 m x 0.53 mm x 3 µm) column. (See Table 2 for peak identification and Table 4 for GC conditions.)



Optimized P and T Cycle Time

Further optimization of the run was dependent on obtaining the most efficient parameters for the P and T cycle. Each aspect of the cycle was optimized as follows.

Sample Purge

Experimentation showed 11 minutes of purge time, or 440 ml of helium purge gas, to be the most efficient time for analyses of gasoline and GROs because shorter purge times (8 minutes or 320 ml of purge gas) were not sufficient to purge all GROs from the sample solution. **Figure 5** shows a comparative analysis of the same GROs standard shown in **Figure 2B** using 8 minutes of purge time instead of 11 minutes of purge time. The conditions for both analyses were the same and are shown in **Table 1**. By comparison, hydrocarbon recoveries (including aromatics) for the GC runs with 8 minutes of sample purge were not as good particularly for the high-boiling fractions, such as trimethylbenzenes and naphthalene (compare peaks 7, 8, and 9 in **Figure 5** and **Figure 2B**). The naphthalene peak in **Figure 5** (8 minutes of purge) was remarkably small, and area counts were lower than 1% of that recovered in **Figure 2B**. Based on this finding, 11 minutes is the optimal sample purge time for the determination of gasoline with BTEX.

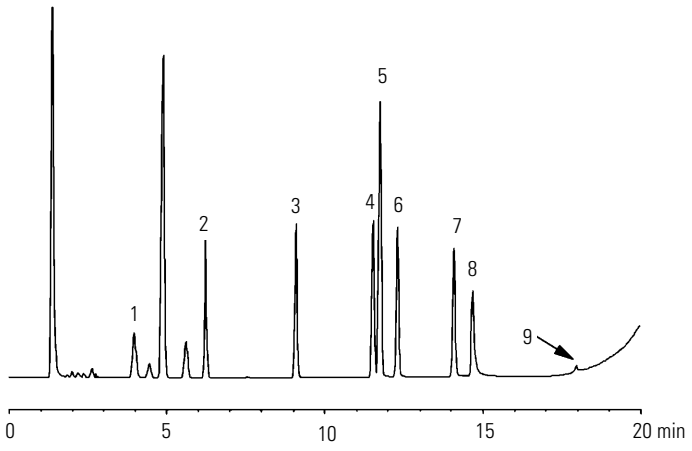
Dry Purge

During sample purge, a larger amount of water is purged along with the volatile organics and is collected on the trap sorbent. Sorbent material in the Vocarb-3000 trap is designed to minimize water trapping and reduce the release of excessive water onto the GC column during the thermal desorption process. A 1-minute dry purge of the Vocarb trap was selected because the early-eluting peaks (such as pentane, MtBE, and benzene in **Figure 2**) were not skewed by water released from the trap onto the column.

Desorption

According to Klee[1], a fast and reproducible desorption temperature is the key to good chromatography using the P and T concentration technique. The higher the desorption temperature and desorption rate, the faster the volatile analytes can be moved to the GC column, and the narrower the peak widths of the early-eluting analytes. Therefore, a short desorption time is preferred. In addition,

Figure 5. Chromatogram of GROs standard using an 8-minute sample purge. (See Table 2 for peak identification and Table 1 for GC and P and T conditions.)



Doherty[2] reported that peak heights and peak areas of volatile organics, including those in the GROs mix, were virtually unchanged when the desorb time changed from 4 minutes to 1 minute. Several manufacturers of P and T systems also recommend a 1-minute desorb time for the routine analysis of volatile organics. However, experimentation (**Figure 2**) using a 2-minute desorb time at 250°C accommodated sharp initial peaks as well as good separation. This study applied a 2-minute desorption time at 250°C to all analyses.

Trap Baking

Three different bake times were evaluated for the Vocarb-3000 trap (used a bake temperature of 265°C, recommended for the Vocarb-3000 trap): 10, 8, and 5 minutes. At each bake time, the gasoline sample (1000-ppb concentration) was run using an 11-minute purge time followed by a run of reagent water with no sample purge. Chromatograms of these two runs were evaluated for carryover. In all three cases (bake times of 5, 8, and 10 minutes), no carryover was observed for any gasoline component. Therefore, a 5-minute bake time at 265°C was selected as an optimal bake time for the analysis of gasoline and GROs aromatics.

For samples containing 46,000 ppb of gasoline, no carry over from the trapped analytes was observed at the 5-minute bake time. This is based on the comparison of chromatograms of reagent water (0-minute purge) run immediately after each sample. However, carry over from the purge vessel was found. Repeated rinsing of the purge vessel with reagent water reduced the amount of carry over but did not eliminate it. Therefore, after a high level sample is run, it is advisable to remove and clean the purge vessel prior to the next run.

Heavier petroleum products, such as diesel and jet fuel (**Figure 6**), that often contain volatile components are also detectable by this method. Again, carry over is a problem. Carry over was observed in the reagent water (used an 11-minute purge) run immediately after the jet fuel sample.

Carry over ranged from 10 ppb to 60 ppb jet fuel and was high enough to cause a false-positive identification in subsequent runs.

As demonstrated by **Figure 7B** (a chromatogram of reagent water, 0-minute purge, run immediately after a jet fuel sample), carry over from the Vocarb trap was found to be negiligible. Clearly the carry over was the result of contamination from the purge vessel (see **Figure 7A**). Although repeated rinsing reduced the amount of carry over, it did not eliminate it completely. Purge vessel carry over was eliminated completely when the purge vessel and the purge needle were removed and cleaned (see **Figure 7C**).

Table 4. GC Run Time of 17 Minutes

HP-1 Column Thickness	Oven Ramp	Carrier Flow Time
30 m x 0.53 mm x 5 µm	40°C (3 min) at 15°C/min to 250°C	4.5 ml/min (see **Figure 3**)
30 m x 0.53 mm x 3 µm	40°C (3 min) at 7°C/min to 95°C to 250°C (2 min) at 45°C/min	10 ml/min (see **Figure 4**)

Figure 6. Chromatogram of 1,000-ppb aviation jet fuel standard. (See Table 1 for GC and P and T conditions.)

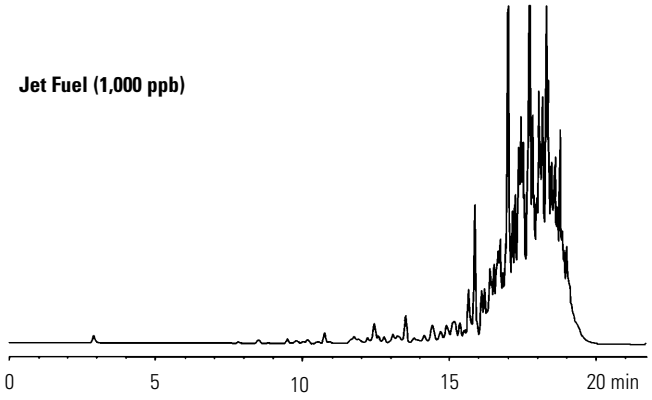
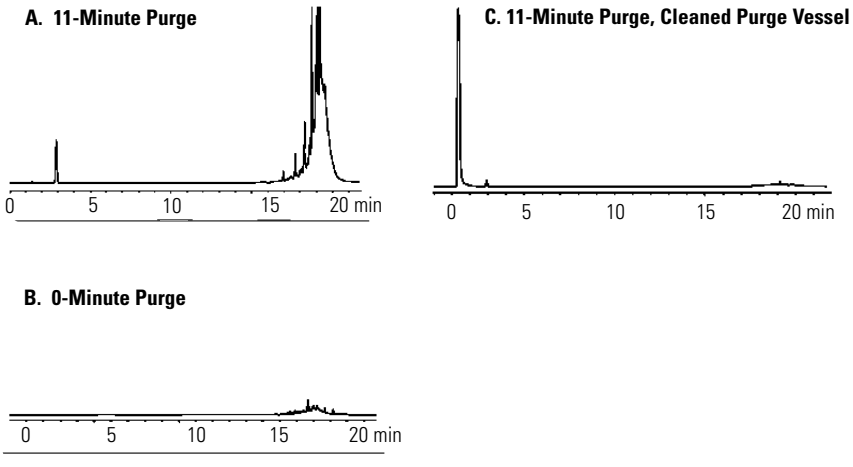


Figure 7. Chromatograms of reagent water following the analysis of the 1,000-ppb aviation jet fuel sample. (See Table 1 for GC and P and T conditions.) Note: The chromatograms were plotted on the same FID response scale.



Conclusion

Determination of optimized P and T parameters is critical in establishing optimized run times for the analysis of gasoline/BTEX. By reducing the P and T bake time to 5 minutes and selecting shorter dry purge (1 minute) and desorption times (2 minutes), the overall P and T cycle was shortened to 25-26 minutes. This is compatible with the run time of 21-22 minutes established for optimized GC conditions. When carry over from the purge vessel is controlled, this same application can be used successfully for the analysis of samples containing in excess of 46,000 ppb of gasoline and other volatile organics in light petroleum products.

Acknowledgment

The authors wish to than Mr. Robert Remlinger at the Pace Laboratory in Petaluma, California for providing his GC/FID and GC/PID chromatograms.

Reference

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2. Linda Doherty, "The analysis of volatile organics in drinking water with an OI 4460A purge and trap and an HP GC/MS system," Hewlett-Packard Company, GC/MS Application Note, August 1991, Publication No. (23) 5091-2477E.

Printed in USA 04/00

Printed on recycled paper
Publication Number 5963-9968E

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Ambient Headspace GC and GC-MSD Analysis of Non-Polar Volatiles in Water

Application

Gas Chromatography

February 2000

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Abstract

Ambient headspace is an ideal method for prescreening samples prior to purge and trap (P&T) analysis. Instrumentation is protected from high level contaminants and rework is reduced. The nature of the technique also makes it attractive for high sample volume applications, such as monitoring of process water in food/beverage manufacturing.

Key Words

ambient headspace, drinking water, GC-FID, GC-micro ECD, GC-AED, GC-MSD, GC-MSD screener report, prescreening, purge and trap, retention time locking (RTL), nonpolar volatile organics

Introduction

Chlorination is a common practice for the disinfection of water supplies. The reaction of chlorine with dissolved organics in the water results in the formation of non-polar halogenated compounds. The principle compounds formed are the trihalomethanes. Usually, bromide salts are also present in water, and both brominated and chlorinated compounds are formed. Water sources also may be contaminated with industrial solvents, such as benzene, tetrachloroethene and methyl tertiary butyl ether (MTBE).

The analysis of these compounds is important to suppliers of drinking water, food and beverage processing companies, and industrial operations that discharge waste water.

Government regulations require that these compounds be measured in drinking water at part-per-billion (ppb) levels. Techniques like P&T are used routinely for this analysis. While P&T allows analysis at very low levels, problems arise with samples containing unexpectedly high levels of volatiles. Instrument contamination and subsequent carryover result in reduced productivity and higher cost. Prescreening using headspace analysis can prevent instrument contamination problems. Lab productivity is also increased with prescreening, because the approximate concentration range of analytes is known before P&T. Re-work of samples outside the P&T calibration range is eliminated.

Ambient headspace is a fast, low-cost technique for analyzing non-polar volatiles in water. It can be used instead of normal heated headspace for prescreening. For non-government regulated analyses, ambient headspace can also be used for routine work.

This application note describes a method evaluated on several different instrument systems and detectors. The choice of configuration is based on the specific measurement requirements.

Experimental

Sample Preparation

Sodium sulfate (Fisher Scientific, 10-60 mesh) and 2-mL autosampler vials (Agilent part number 5182-0543) were baked and stored at 100 °C to prevent contamination with volatiles. One-milliliter disposable serological pipettes (Corning) and aluminum crimp caps (Agilent part number 5181-1215) were used as received. Distilled water for preparation of standards and blanks was purified by constant purging with carbon-filtered helium.

Water samples are prepared for analysis as follows:

1. Sodium sulfate is added to each autosampler vial to form a layer of approximately 4 mm in height.
2. One milliliter of water sample is added to the vial with a disposable pipette.
3. The vial is immediately capped and crimped.
4. The sample is vortexed for about 3 seconds.

Standards are prepared as above, except 1 µL of spiking solution in methanol is added to 1 mL of purified water just before step 3. Only 1 µL is used to minimize the amount of methanol added to the water. The concentration of individual compounds in the spiking solution is 1,000 times higher than the desired final concentration in the vial.

A standards kit of volatiles in methanol was obtained from Supelco (part number 4-8804, Bellefonte, PA). The 58 compounds are divided into six different mixes. Spikes were prepared using one mix per vial.

Instrument Conditions

Table 1 lists the instrument conditions used.

Results and Discussion

Retention Time Locking

The method is designed for use on a variety of instrument configurations. Configurations used were GC-FID, GC-micro ECD, GC-AED, and GC-MSD. To simplify data analysis and comparison across the various instruments, retention time locking (RTL) is employed. RTL is a technique that matches the retention time (RT) from column-to-column and instrument-to-instrument to approximately 0.03 minutes[1]. Using RTL, compounds are identified by searching a table of retention times that have been collected under locked conditions.

This method is locked to a table of RTs of 65 volatile compounds from EPA method 8260. The table was created by running mixtures of standards on GC-MSD to confirm RTs based on mass spectra. The table is locked using tetrachloroethene at 4.247 minutes as the locking compound. To match the GC-MSD retention times to atmospheric pressure detectors, Agilent's method translation software[2] (MTL) is used in combination with RTL.

The mass spectra of the 65 compounds with retention times were collected into a user library. A screener database (SCD) was then constructed from this library reference. An SCD is used to screen for compounds based on RT and ion ratios. Combining precise RT with mass spectral information in the search reduces both false positives and false negatives in identifications.

Identifications for GC-FID and GC-micro-ECD used an RT table (Table 2) constructed with the GC RTL software. For each compound entry, the table contains the RT, molecular formula, and CAS number. Each detected peak in the chromatogram is searched against the table and a list of possible identities is generated. The more accurate and precise the RT control, the shorter the list of possible compounds for each peak.

The list of possible compounds is reduced further by searching with element information in addition to retention time. The presence or absence of a specific element can rule out compounds from the list. When used with GC-AED, this filtering can be extended further by using element ratios[3].

GC Column

The HP-5MS column chosen for this method is not necessarily the best or most common choice for volatiles. The desire is to use a column that is already in use in most laboratories. This column also allows ease of changing between ambient headspace and liquid injections, because the column is suitable for both. The flow characteristics of the column are compatible with the MSD and all other GC detectors.

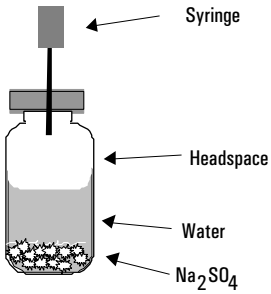
Inlet Liner

An injection port liner used with ambient headspace is small in volume compared to liners used for liquid injections. The sample is already a gas when injected, so there is no significant expansion. The small volume liner provides better peak shape for early eluting compounds that are not cold-trapped at the head of the column. A lower split ratio can be used, which results in better sensitivity. Liners of larger i.d. can be used sucessfully, but require higher split ratios to maintain peak shape.

Autoinjector

Ambient headspace is done using a gastight syringe. The largest volume syringe that can be used with the autoinjector is a 100-µL syringe (only half the volume can be injected). Note, the sampling depth of 20 mm is a critical parameter. This depth corresponds to drawing sample from the headspace and not from the water (Figure 1). Failure to set this parameter correctly will result in injecting 50 µL of salt solution into the inlet, causing instrument failure.

To minimize carryover between samples, the syringe is washed first with methanol and then water. Trace amounts of methanol in the syringe will give a peak on some detectors. The three water washes are required to minimize the residual methanol while allowing the maximum number of runs between solvent replenishment. If only trace level samples are being analyzed, the methanol wash can be eliminated, and the water washes can be reduced to one.



Software controlled variable sampling depth allows precise positioning of the syringe needle tip in the vial

Figure 1. Headspace sampling from a 2 mL autosampler vial.

Table 1. Instrument Conditions

Gas Chromatograph	**Agilent 6890 or 6850**
Injection Port	**Split/splitless**
Temperature	200 °C
Liner	Deactivated 1-mm i.d. (Restek 20973)
Carrier gas	Helium
Inlet pressure	20 psi (adjusted to lock), constant pressure
Split ratio	1:1
Column	HP-5MS, 30 m x 0.25 mm x 0.25 µm, part numbers 19091S-433 (for 6890) or 19091S-433E (for 6850)
Initial temperature	35 °C
Initial time	2 min
Temperature ramp	18 °C/min
Final temperature	70 °C
Final time	0 min
Ramp A	45 °C/min
Final temperature A	250 °C
Final time A	0 min
Autoinjector	**Agilent 7683**
Syringe	100-µL gastight injector, 5183-2042
Injection volume	50 µL
Solvent A	Methanol, 1 wash
Solvent B	Water, 3 washes
Sample rinses	None
Sample pumps	3
Injection speed	Fast plunger
Viscosity delay	5
Sampling depth	20 mm
FID Conditions	
Temperature	250 °C
Hydrogen	40 mL/min
Air	450 mL/min
Helium makeup	45 mL/min
AED Conditions	
Makeup gas	15 mL/min
Reagent gases	
Hydrogen	15 psi
Oxygen	10 psi
Temperatures	
Transfer line	250 °C
Cavity	250 °C
Solvent vent	None
5973 MSD Conditions	
GC inlet pressure	6.6 psi (adjusted to lock), constant pressure
Temperatures	
Source	230 °C
Quad	150 °C
Transfer line	260 °C
Mass range	35-300 amu
Scans	5.27/sec
Samples	2
Threshold	50
EM voltage	BFB.u tune voltage
Solvent delay	None
Micro-ECD Conditions	
Temperature	250 °C
Makeup gas	Nitrogen
Constant column + makeup flow	60 mL/min

Software for RTL Ambient Headspace on GC

Commercial software

GC ChemStation software *revision A.05.04 or higher*
GC RTL software *revision A.05.02*

User contributed software

GC RTL volatiles database
GC RTL autolocker
GC RTL autosearcher

Software for RTL Ambient Headspace on GC/MSD

Commercial software

MS ChemStation software *revision B.01.00 or higher*

User-contributed software

MS RTL volatiles screener database
MS RTL volatiles library

Table 2. Volatiles Ambient (HS)

FID RT	Compound Name	CAS No.	Molecular Formula	Weight	MSD RT	MSD Target & Qualifier Ions			
1.196	air, nitrogen	7727-37-9	N:2,	28.0	1.191	14	16	30	14
1.196	air, argon	7440-37-1	Ar:1,	40.0	1.191	40	42	40	40
1.217	dichlorodifluoromethane	75-71-8	C:1,Cl:2,F:2,	120.9	1.220	85	87	101	50
1.240	chloromethane	74-87-3	C:1,H:3,Cl:1,	50.5	1.244	50	52	49	47
1.240	water	7732-18-5	H:2,O:1,	18.0	1.242	17	19	16	16
1.261	vinyl chloride	75-01-4	C:2,H:3,Cl:1,	62.5	1.266	62	64	61	60
1.267	methanol	67-56-1	C:1,H:4,O:1,	32.0	1.267	31	29	15	30
1.313	bromomethane	74-83-9	C:1,H:3,Br:1,	93.9	1.317	94	96	93	95
1.331	chloroethane	75-00-3	C:2,H:5,Cl:1,	64.5	1.333	64	66	49	51
1.403	trichlorofluoromethane	75-69-4	C:1,Cl:3,F:1,	135.9	1.407	101	103	66	105
1.496	1,1 - dichloroethylene	75-35-4	C:2,H:2,Cl:2,	96.9	1.499	61	96	98	63
1.547	methylene chloride	75-09-2	C:1,H:2,Cl:2,	84.9	1.551	49	84	86	51
1.670	trans - 1,2 - dichloroethene	156-60-5	C:2,H:2,Cl:2,	96.9	1.673	61	96	98	63
1.725	MTBE methyl-t-butyl ether	1634-04-4	C:5,H:12,O:1,	88.1	1.702	73	57	41	43
1.744	1,1-dichloroethane	75-34-3	C:2,H:4,Cl:2,	99.0	1.745	63	65	83	85
1.931	cis - 1,2 - dichloroethene	156-59-4	C:2,H:2,Cl:2,	96.9	1.933	61	96	98	63
1.982	2,2-dichloropropane	590-20-7	C:3,H:6,Cl:2,	113.0	1.983	49	130	128	
2.001	bromochloromethane	74-97-5	C:1,H:2,Cl:1,Br:1,	129.4	2.002	77	79	97	61
2.008	chloroform	67-66-3	C:1,H:1,Cl:3,	119.4	2.009	83	85	47	48
2.247	1,1,1-trichloroethane	71-55-6	C:2,H:3,Cl:3,	133.4	2.246	97	99	61	63
2.283	1,2 - dichloroethane	107-06-2	C:2,H:4,Cl:2,	99.0	2.284	62	64	49	63
2.343	1,1 - dichloropropene	563-58-6	C:3,H:4,Cl:2,	111.0	2.345	75	39	110	77
2.402	benzene	71-43-2	C:6,H:6,	78.1	2.402	78	77	51	52
2.403	carbon tetrachloride	56-23-5	C:1,Cl:4,	153.8	2.406	117	119	121	82
2.805	1,2, - dichloropropane	78-87-5	C:3,H:6,Cl:2,	113.0	2.814	95	130	132	97
2.805	trichloroethene	79-01-6	C:2,H:1,Cl:3,	131.4	2.801	63	62	39	76
2.846	dibromomethane	74-95-3	C:1,H:2,Br:2,	173.9	2.840	93	174	95	172
2.902	bromodichloromethane	75-27-4	C:1,H:1,Cl:2,Br:1,	163.8	2.902	83	85	47	48
3.339	cis - 1,3 - dichloropropene	10061-01-5	C:3,H:4,Cl:2,	111.0	3.334	75	39	77	110
3.688	trans - 1,3 - dichloropropene	10061-02-6	C:3,H:4,Cl:2,	111.0	3.677	75	39	77	110
3.700	toluene	108-88-3	C:7,H:8,	92.1	3.689	91	92	65	63
3.761	1,1,2 - trichloroethane	79-00-5	C:2,H:3,Cl:3,	133.4	3.754	97	83	99	61
3.900	chloropicrin	76-06-2	C:1.Cl:3,N:1,O:2,	162.9	3.888	76	41	78	49
3.944	1,3 - dichloropropane	142-28-9	C:3,H:6,Cl:2,	113.0	3.937	117	119	82	47
4.077	chlorodibromomethane	124-48-1	C:1,H:1,Cl:1,Br:2,	208.3	4.066	129	127	131	48
4.214	1,2, - dibromoethane	106-93-4	C:2,H:4,Br:2,	173.9	4.203	107	109	79	81
4.247	tetrachloroethene	127-18-4	C:2,Cl:4,	165.9	4.245	166	164	129	131
4.671	chlorobenzene	108-90-7	C:6,H:5,Cl:1,	112.6	4.663	112	77	114	51
4.707	1,1,1,2 - tetrachloroethane	630-20-6	C:2,H:2,Cl:4,	167.9	4.701	131	133	117	119
4.836	ethylbenzene	100-41-4	C:8,H:10,	106.2	4.821	91	106	51	65
4.913	p - xylene	106-42-3	C:8,H:10,	106.2	4.904	91	106	105	77
4.914	m-xylene	108-38-3	C:8,H:10,	106.2	4.902	91	106	105	77
5.072	bromoform	75-25-2	C:1,H:1,Br:3,	252.8	5.060	173	175	171	93
5.137	o - xylene	95-47-6	C:8,H:10,	106.2	5.129	104	103	78	51
5.143	styrene	100-42-5	C:8,H:8,	104.2	5.110	91	106	105	77
5.317	1,1,2,2 - tetrachloroethane	79-34-5	C:2,H:2,Cl:4,	167.9	5.304	83	85	95	61
5.378	1,2,3 - trichloropropane	96-18-4	C:3,H:5,Cl:3,	147.4	5.365	75	110	77	61
5.413	isopropylbenzene	98-82-8	C:9,H:12,	120.2	5.404	105	120	77	79
5.505	bromobenzene	108-86-1	C:6,H:5,Br:1,	157.0	5.463	77	156	158	51
5.646	2 - chlorotoluene	95-49-8	C:7,H:7,Cl:1,	126.6	5.626	91	120	92	65
5.646	n - propylbenzene	103-65-1	C:9,H:12,	120.2	5.639	91	126	89	63
5.680	4 - chlorotoluene	106-43-4	C:7,H:7,Cl:1,	126.6	5.671	91	126	125	63
5.760	1,3,5 - trimethylbenzene	108-67-8	C:9,H:12,	120.2	5.746	105	120	77	119
5.933	tert - butylbenzene	98-06-6	C:10,H:14,	134.2	5.924	119	91	134	77
5.944	1,2,4 - trimethylbenzene	95-63-6	C:9,H:12,	120.2	5.928	105	120	77	119
6.032	1,3 - dichlorobenzene	541-73-1	C:6,H:4,Cl:2,	147.0	6.021	146	148	111	75
6.054	sec - butylbenzene	135-98-8	C:10,H:14,	134.2	6.043	105	134	91	77
6.076	1,4 - dichlorobenzene	106-46-7	C:6,H:4,Cl:2,	147.0	6.066	146	148	111	75
6.142	p - isopropyltoluene	99-87-6	C:10,H:14,	134.2	6.127	119	134	91	117
6.227	1,2 - dichlorobenzene	95-50-1	C:6,H:4,Cl:2,	147.0	6.213	146	148	111	75
6.341	n - butylbenzene	104-51-8	C:10,H:14,	134.2	6.320	91	92	134	65
6.514	1,2, - dibromo - 3 - chloropropane	96-12-8	C:3,H:5,Cl:1,Br:2,	236.4	6.494	157	75	155	39
7.011	1,2,4 - trichlorobenzene	120-82-1	C:6,H:3,Cl:3,	181.5	6.981	180	182	184	145
7.057	naphthalene	91-20-3	C:10,H:8,	128.2	7.026	128	127	129	51
7.163	hexachlorobutadiene	87-68-3	C:4,Cl:6,	260.8	7.146	225	227	223	190
7.181	1,2,3 - trichlorobenzene	87-61-6	C:6,H:3,Cl:3,	181.5	7.151	180	182	184	145

Sample Preparation

In the analysis of trace volatile compounds, it is critical to maintain low blank levels. The sample vials, reagent water, sodium sulfate, and laboratory environment must be free of contamination by volatiles. Store the vials and sodium sulfate in a laboratory glassware oven at 100 °C. Prepare the reagent water by purging distilled water with carbon-filtered helium in a gas-washing bottle at room temperature. Purge the water continuously to keep it ready for immediate use. Contamination via laboratory air typically is due to use of solvents in the lab or by cross-contamination from garments of lab personnel. Be careful choosing the sample preparation area.

Experiments were carried out to determine the relative effects of temperature and "salting out" on the headspace extraction efficiency. Raising the temperature of the autosampler tray to 60 °C increased the recovery of most compounds. However, the addition of sodium sulfate provides similar efficiency at room temperature. In practice, the sodium sulfate and vials are allowed to cool to room temperature. Sodium sulfate is added to each vial to a height of approximately 4 mm.

Blanks and samples are treated similarly. A 1-mL aliquot is pipetted into a vial containing sodium sulfate and crimped immediately. Spikes are prepared the same way, but 1 µL of spiking solution is added with a 5-µL GC syringe just before crimping. Note, when the tip of the syringe is placed into the water, agitation is minimized.

The caps are crimped tightly enough that they cannot be rotated by hand. Baking the caps at 100 °C caused improper sealing and resulted in leaks. Therefore, the crimp caps are used unbaked.

Vortexing for 3 seconds is sufficient to transfer the volatiles to the headspace. If a vortex mixer is not available, vigorous manual shaking for 15 seconds will suffice.

GC-FID

Figure 2 shows the FID chromatogram of a 20-ppb standard spike of volatiles mix 4. The FID response to the volatiles varies significantly with halogen content. Bromochloromethane and 2,2-dichloropropane are not resolved. Peak 7, tetrachloroethene at 4.247 minutes, is the locking peak used for RTL.

In this method, the split ratio is initially set to 1. A spike containing the mixture from Figure 2 is run and the peak shape of peaks 2 and 4 are inspected for tailing. If they tail, the split ratio is increased until the tailing is just minimized. In this specific setup, the split ratio was set to 2.

The chromatogram shows that the FID can provide a broad-based screen for nonpolar volatiles in the low ppb range.

Figure 3 shows the FID chromatogram and the MSD total ion chromatogram (TIC) of a 20-ppb standard spike of methyl-t-butylether (MTBE) in blank water. MTBE is often found in groundwater due to oxygenated gasoline leaking from underground storage tanks. MTBE can be detected at low ppb levels using either detector.

In both Figures 2 and 3, a large methanol solvent peak is present. This is due to the 1-µL methanol-based spiking solution.

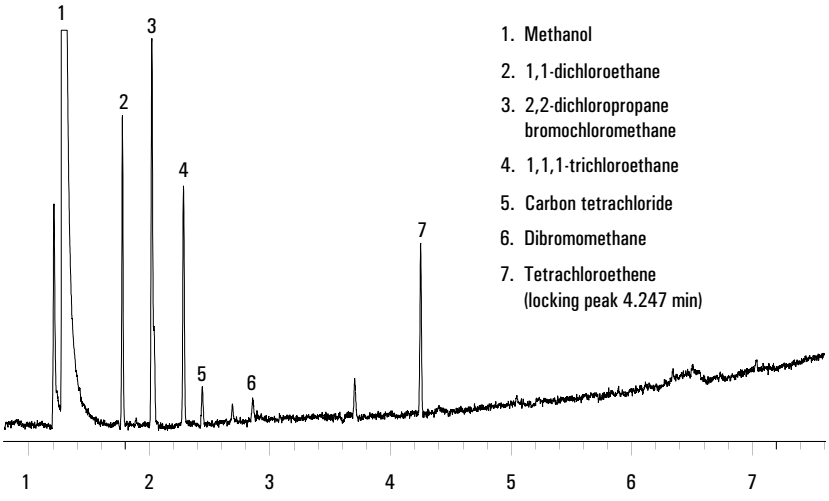


Figure 2. 20 ppb spiked standard (mix 4) in blank water by FID.

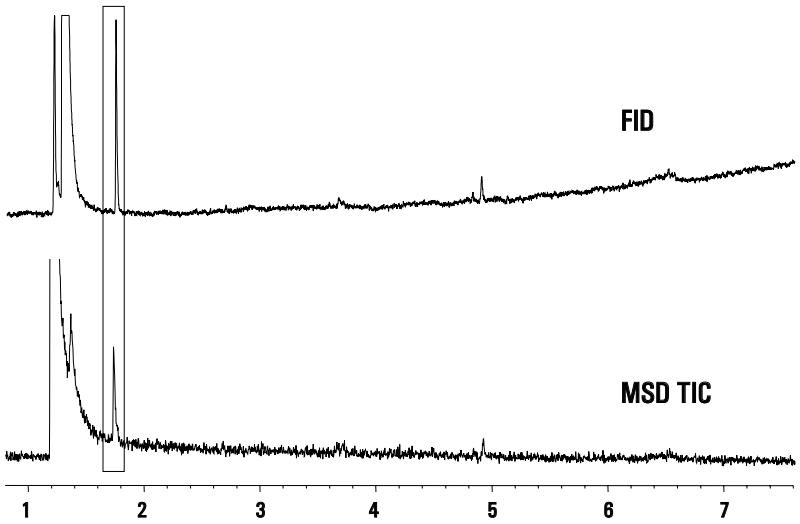


Figure 3. 20 ppb MTBE spike in blank water.

GC-MSD

The TICs of the six standard mixes spiked into blank water are shown in Figure 4. Using the MSD data, the following steps are taken:

1. Determine identity and retention time for each compound.
2. Create a spectral library of the 58 compounds.
3. Create a screener database by combining the results of steps 1 and 2.

There are nine pairs of compounds that overlap chromatographically. However, use of extracted ions differentiates all of the overlapped peaks. Peak identification with the screener software is accomplished using precise retention time, extracted ions, and spectral cross-correlation. The process used by the screener software is as follows:

1. Takes the retention time of the first compound in the database and extracts the target and qualifier ion chromatograms.
2. Integrates the ion chromatograms over a user specified time search window.
3. Compares the ratio of each qualifier ion to the target ion.
4. If the ratios fall within user specified criteria, the compound is marked as a "hit".
5. The results from steps 2 through 4 determine how the compound is reported.
6. Perform a cross-correlation between the sample spectrum and the library spectrum to aid in confirmation.
7. Repeat this process for each compound in the Screener Database.
8. Combine the results into a user definable report format and print the report.

Figure 5 shows the GC-MSD screener report for a tap water sample. Of the 65 compounds in the screener database, four were reported. A "?" in the status column indicates that the target ion was found, but that one or more of the qualifier ratios did not meet criteria. The out-of-range qualifiers are listed in the report. An "X" in the status column means that the target ion was found and that all of the qualifier ratios met criteria. The number in the "XCR" column indicates the quality of the match of the sample spectrum to the library spectrum, with 1.0 being a perfect cross-correlation.

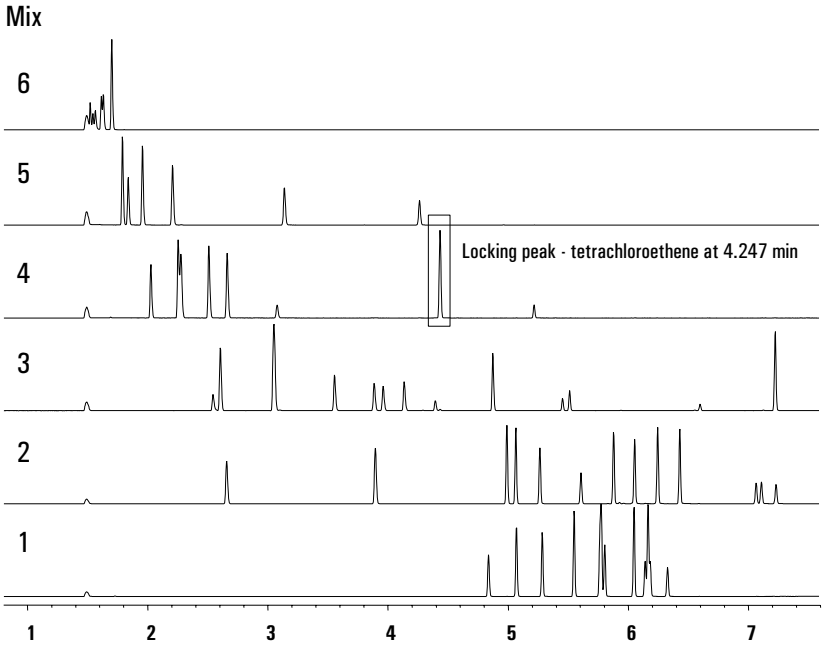


Figure 4. Six VOA calibration standard mixes by MSD.

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 Screen Report    (Not Reviewed)

  Data File : D:\DATA\AHS_MSD2\Sample1.D                  Vial: 6
  Acq On    : 10 Aug 1999  17:20                      Operator: Mikeski
  Sample    : Sample1 unfiltered  #2                    Inst    : GC/MS Ins
  Misc      :                                         Multiplr: 1.00
                                                   Sample Amount: 0.00
  MS Integration Params: RTEINT.P

 Screen File: AMHSMOD1.RES              Extraction Window: +/- 0.150 min
 Screen Database: AMHSMOD1.SCD          Qualifier Mode   : Absolute
                                        Qualifier %      : 20
                                        Zero qualifiers  : Included
                                        Subtraction Mode : Relative Areas


                                                    Target      Qualifiers
    Compound               Status ExpRT  Delta m/z   Resp. Out of Range  XCR
-------------------------------------------------------------------------------
 17 bromochloromethane        ?   2.002 +0.008  49      476 130,128     0.07
 18 chloroform                x   2.009 -0.003  83     4817             1.00
 27 bromodichloromethane      x   2.902 -0.000  83     3091             0.98
 33 chlorodibromomethane      x   4.066 +0.007 129      801             0.94


Screen Report Mon Aug 16 12:12:48 1999
```

Figure 5. GC-MSD Screener report.

As an example, the extracted ion chromatograms for chlorodibromomethane found in a tap water sample are shown in Figure 6. In this case, the search window was 0.1 minutes. The ratios of ions 127, 131, and 48 to the target ion 129, met criteria. In Figure 7, the sample spectrum matches the chlorodibromomethane library spectrum, resulting in a high XCR.

The combination of precise RT, qualifier ion ratios, and cross-correlation gives high confidence in chlorodibromomethane being present in the sample.

GC-AED

Figure 8 shows the chromatograms resulting from ambient headspace analysis on four different GC systems. Note how closely the RTs match system to system as a result of RTL.

The AED is useful in this type of analysis for the following reasons:

1. The carbon 193 nm chromatogram is very sensitive (about five times better than the FID).
2. The AED carbon channel responds to all compounds that contain carbon, even those that exhibit little or no response in the FID (examples: CO_2, CS_2, CCl_4).
3. The response factors for each element are independent of compound structure, allowing quantitation without having to run standards for all compounds.
4. With proper calibration, the element mole ratios (empirical formulae) can be calculated for unknown compounds.
5. The specificity of the AED differentiates the individual halogens in unknowns.

The chromatograms in Figure 8 show that the C 193 channel provides low-level general-purpose screening for volatiles. The chlorine and bromine channels clearly indicate which compounds contain each halogen.

Combining GC-AED with GC-MSD provides the broadest possible screening capability. The AED will show the presence of any volatile, its element content, and the concentration of the compound based on element response factors. GC-MSD identifies the volatile based on spectral information. This approach maximizes the speed and efficiency with which unknown compounds can be identified and quantitated.

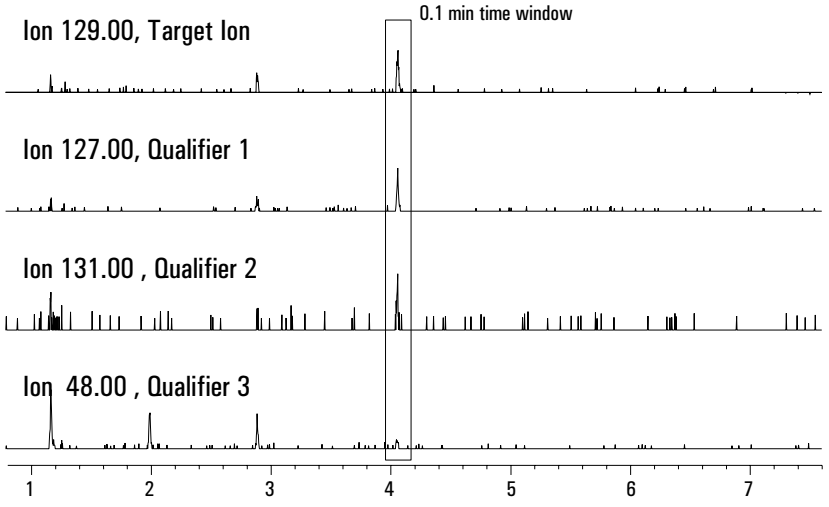

Figure 6. Extracted ions used by Screener to look for chlorodibromomethane.

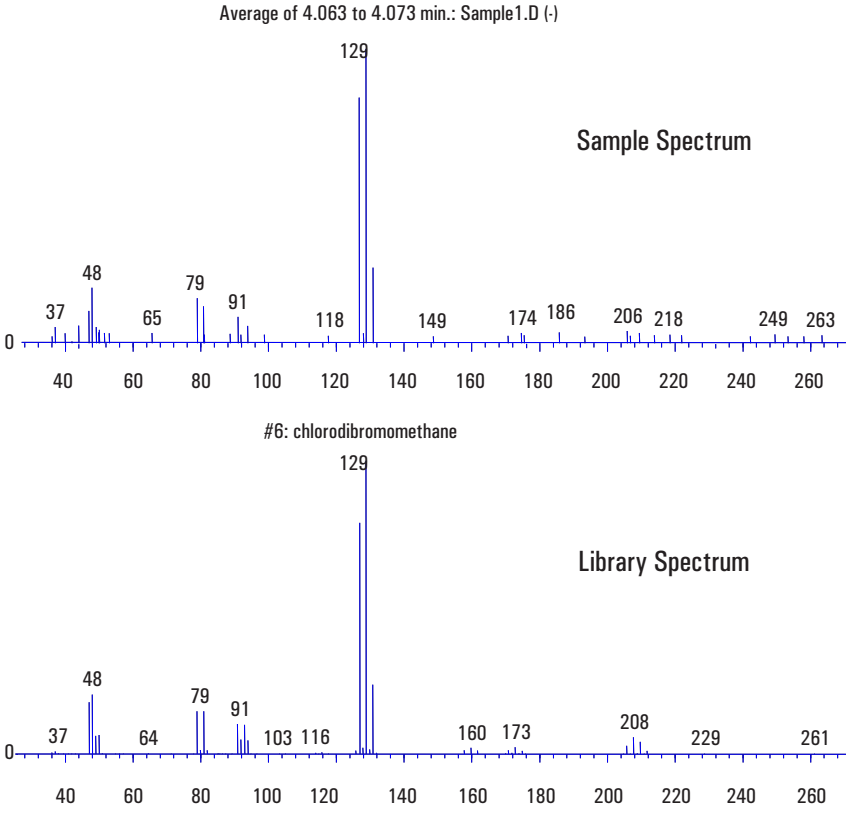

Figure 7. Sample and library spectra used by Screener for cross correlation.

GC-micro ECD

Also shown in Figure 8 is the analysis performed with GC-micro ECD. The signal-to-noise ratio is very high for those compounds that are responsive on ECD. The Agilent micro-ECD is uniquely suited for the detection of ultra low-level polyhalogenated compounds. It has a high response factor for polyhalogenated compounds and a low response factor for other compounds, minimizing interferences.

The micro-ECD peaks in Figure 8 were searched against the GC RTL volatiles database. A portion of the search report is shown in Figure 9. For each peak, the possible identities and information useful for GC-MSD analysis are given.

The sensitivity of the micro-ECD is demonstrated in Figure 10, where the polyhalogenates in mix four are easily detected at 20 parts per trillion. The detection limit observed with the

micro-ECD is comparable to that seen in routine P&T methods.

To further demonstrate the detection capability of the micro-ECD, Figure 11 shows the chromatogram from Figure 8 with an expanded Y axis. This tap water sample has >75 discernable peaks. One interesting compound detected was chloropicrin (trichloronitromethane). Chloropicrin was used as a chemical warfare agent in World War 1. However, its presence at ppt levels in drinking water is not surprising, as it is a known disinfection byproduct[4]. The identification of the compound was confirmed by GC-MSD with single-ion monitoring on multiple masses.

Figure 11 shows the same tap water after passage through a commercial spigot filter. The filter lowers the level of detected compounds by a factor of 100 to 300 fold.

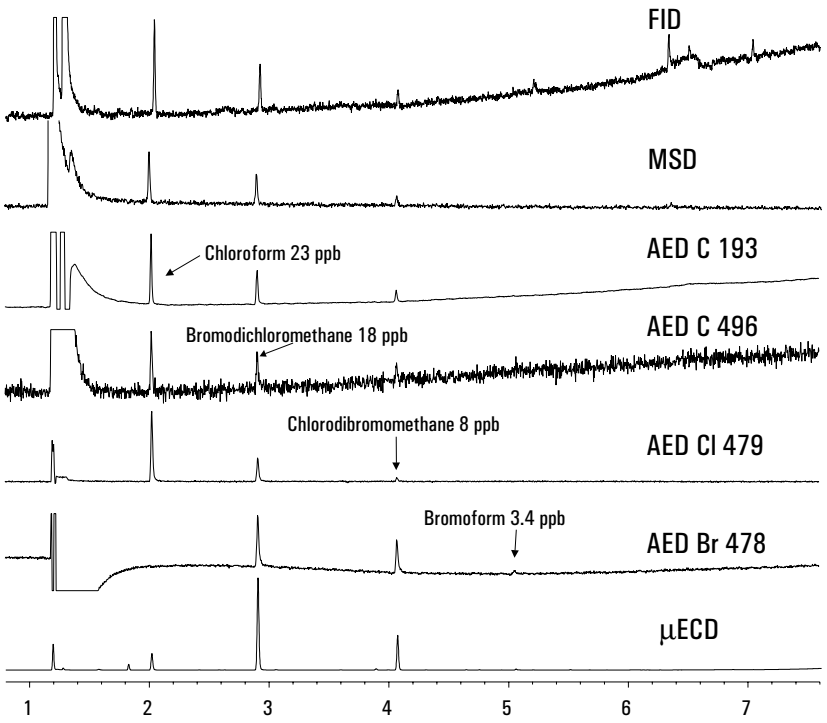

Figure 8. Local tap water sample RT locked on four instrument systems.

Precision

The precision of the technique is illustrated in Figure 12. The raw area repeatability for 10 consecutive vials of a tap water sample is 6.1% RSD. Note that this is measured with a peak present in the ppt concentration range. The retention time precision is also very good, a result of the Agilent 6890 oven and pneumatics performance.

The precision over an extended period of time also was tested. A series of 15 samples spiked with 200 ppb benzene was prepared in blank water and run in groups of five. The first group was run immediately as a control. The second group was left at room temperature and run 4 hours later. The last group, also held at room temperature, was run 24 hours later. The raw area repeatability for all 15 vials was 10% RSD. This includes the uncertainty introduced with the 1-µL spiking process. The maximum deviation of the retention time of benzene was 0.002 minutes.

```
Data File:  D:\HPCHEM\1\DATA\AHS_ECD2\NIXONU_2.D
Sample Name:  Nixon U #2
Instrument 1 9/30/99 8:44:34 AM

==================================================================
          Results of Table Auto-Search for Signal 1
==================================================================

Search results for 2.019 +/- 0.015 minutes
                                                        Weight
 2.008  chloroform                                      119.38
  CAS No.      67-66-3
  Mol_Formula  C:1,H:1,Cl:3,
  MSD RT       2.009
  MSD Target & Qualifier Ions  83   85   47   48

Search results for 2.905 +/- 0.015 minutes
  RT  Compound Name                                     Weight
 2.902  bromodichloromethane                           163.83
  CAS No.      75-27-4
  Mol_Formula  C:1,H:1,Cl:2,Br:1,
  MSD RT       2.902
  MSD Target & Qualifier Ions  83   85   47   48
```

Figure 9. GC RTL autosearch report.

Linearity

The linear dynamic range (LDR) of the technique was measured and is shown in Table 3. Five concentrations of nonpolar halogenated volatiles covering the range of 0.02 to 200 ppb were analyzed using the micro-ECD. The correlation coefficients for six compounds are all 0.99 or better, demonstrating that the technique is linear. The upper end of the LDR in this case is limited by saturation of the micro-ECD. This data, taken with that of other detectors, indicates the linear range of the sampling technique extends at least from 0.02 ppb to 2000 ppb. In practice, the LDR is determined by the detector used.

Table 3. µECD Linearity, 0.02-200 ppb includes error in spiking 1 µL

Compound	Corr. Coef.
Chloroform	0.994
1,1,1-trichloroethane	0.999
Carbon tetrachloride	0.999
Dibromomethane	0.990
Tetrachloroethene	0.998
Bromoform	0.996

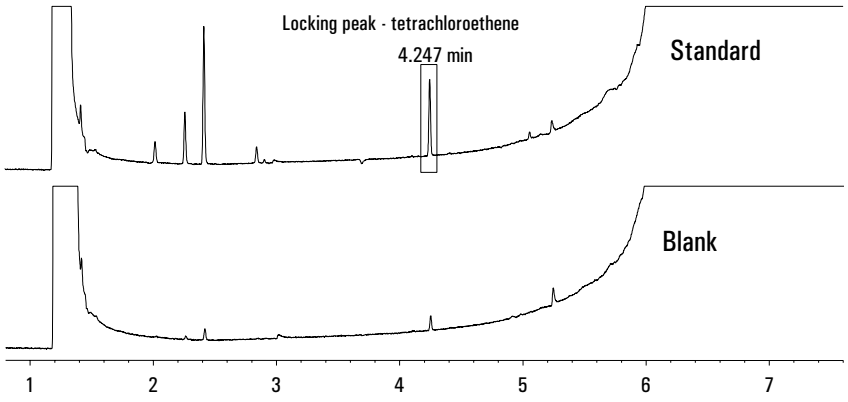

Figure 10. 20 ppt mix 4 in blank water by µECD.

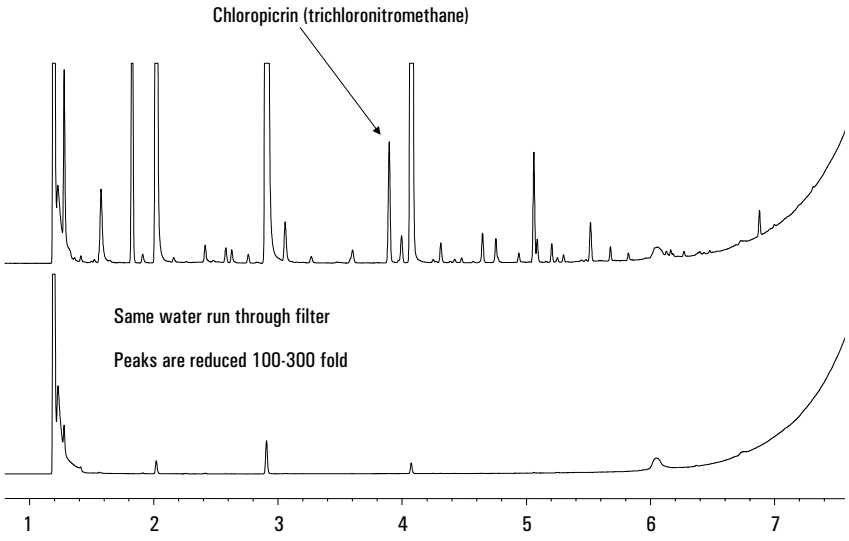

Figure 11. Local tap water sample by µECD.

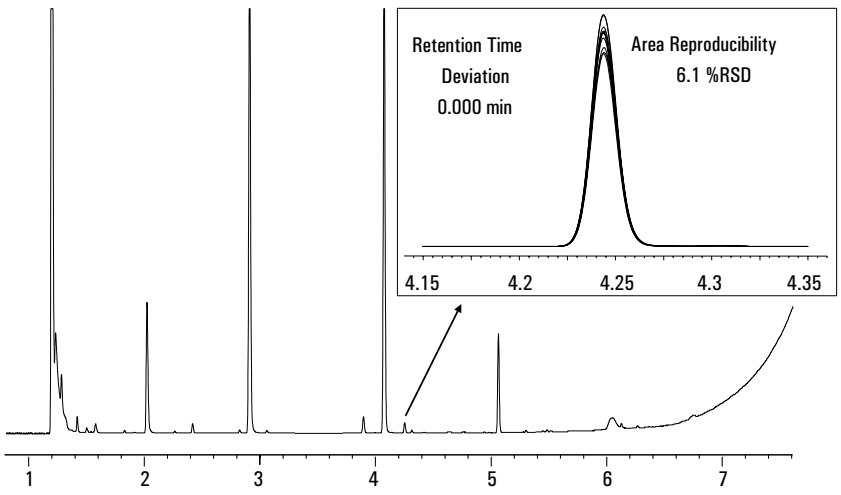

Figure 12. Chromatograms from 10 analyses of tap water, overlaid all in the same scale.

Conclusions

Ambient headspace is a fast, low cost, simple, and robust technique for the analysis of nonpolar volatile organics in water. The technique is easily implemented on an Agilent 6890 or 6850 GC. Given the broad range of detectors available for these GCs, the sensitivity, selectivity, and linear dynamic range can be matched to analyst's needs.

Ambient headspace is an ideal method for prescreening samples prior to P&T analysis. Instrumentation is protected from high level contaminants and rework is reduced. The nature of the technique also makes it attractive for high sample volume applications, such as monitoring of process water in food/beverage manufacturing.

References

1. B. D. Quimby, L.M. Blumberg, M. S. Klee, and P. L. Wylie, "Precise Time-Scaling of Gas Chromatographic Methods Using Method Translation and Retention Time Locking" Application Note 228-401, Publication number 5967-5820E, May 1998 (Downloadable from agilent.com)
2. Agilent's GC Method Translation software (available free from the Agilent Technologies Web site, http://www.chem.agilent.com/cag/servsup/usersoft/main.html#mxlator
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Printed in the USA 3/2000
5968-9455E

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Analysis of EDB and DBCP in Water with the Agilent 6890 Series Gas Chromatograph and Agilent 6890 Micro-Electron Capture Detector — EPA Method 504

Application

Gas Chromatography

August 1997

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Abstract

The pesticides 1,2-ethylene dibromide (EDB) and 1,2-dibromo-3-chloropropane (DMCP) were analyzed by dual-column gas chromatography with dual micro-electron capture detectors (Agilent 6890 micro-ECDs) after micro-extraction with hexane in accordance with U.S. EPA method 504.

Stability, sensitivity, and linearity of the micro-ECD were significantly better than the classical ECD. Relative standard deviation (% RSD) for the entire method was less than 7% over a concentration range greater than two orders of magnitude with method detection limits of 0.003 µg/L or lower.

Key Words

Micro-ECD, 6890 GC, EPA Drinking Water Method 504, ethylene dibromide, 1,2-dibromo-3-chloropropane, GC/ECD analysis

Introduction

Ethylene dibromide (EDB) and 1,2-dibromo-3-chloropropane (DBCP) are volatile pesticides and suspect carcinogens. The U.S. EPA regulates maximum contaminant levels (MCLs) for these compounds in drinking water supplies at very low levels (EDB at 0.05 µg/L and DBCP at 0.2 µg/L). Both EDB and DBCP can be determined by performing a micro-extraction with hexane and analyzing the extract by gas chromatography using an electron capture detector (ECD), as described in EPA Method 504.[1]

EPA method 504 reported method detection limits (MDLs) of 0.01 µg/L for both pesticides.[1,2] Results using an Agilent 6890 GC with the micro-ECD show that these analytes can be determined down to 0.01 µg/L with MDLs of less than 0.003 µg/L. The micro-ECD had a stable baseline and was linear from 0.010 to 1.14 µg/L.

Table 1. Experimental Conditions

Sampler	Agilent 7673, 10-µL syringe, 2-µL splitless injection
Inlet	Split/splitless; 200 °C, pulsed splitless mode (20 psi for 1 min)
Carrier	Helium, 6 psi (40 °C); 3.5 mL/min constant flow (each column)
Column	(A) 30 m, 0.53-mm id, 0.8-µm film DB-608, an equivalent of HP-608 (part number 19095S-023)
	(B) 30 m, 0.53-mm id, 1.0-µm film RTX-1701, an equivalent of HP-PAS 1701 (part number 19095S-123)
Oven	40 °C (4 min); 10 °C/min to 240 °C
Detector	330 °C; Makeup gas: nitrogen, constant column and makeup flow (60 mL/min)

Experimental

Samples and standards were prepared as described in EPA drinking water method 504.[1] All analyses were performed using a 6890 Series GC with a single split/splitless inlet and dual micro-ECDs. Instrument conditions are listed in table 1.

A water sample (35 mL) was extracted with 2 mL of hexane. From that extract, 2 µL were injected into the 6890 Series GC in the splitless mode. A "Y" connector was used to split the sample equally between two polar but dissimilar columns. Column A (an equivalent of the HP-608 column), which provided separation of EDB and DBCP without interference from trihalomethanes, was used as the primary analytical column. Column B (an equivalent of the HP-1701 column) was used as the confirmation column. These columns were previously installed and used in the GC system to analyze pesticides and arochlors according to U.S. EPA CLP and 8080/8081 methods.

Results and Discussion

A common problem in determining EDB and DBCP in drinking water by gas chromatography/electron capture detection (GC/ECD) is interference from chlorination disinfection byproducts such as trihalogenated methanes. For example, dibromochloromethane (DBCM), commonly found in drinking water supplies in relatively high concentrations, can elute very close to EDB and thus can be misidentified as EDB.

Using the optimized GC conditions listed in table 1, EDB was clearly separated from significant levels of DBCM on both columns. Typical chromatograms of a hexane extract of a calibration standard are shown in figure 1. Both EDB and DBCP are well separated from possible interference, including DBCM and dibromomethane (DBM).

Micro-ECD Linearity

Linearity of the 6890 micro-ECD was determined by preparing standards from 0.005 to 1.14 µg/L in reagent water. The standards were extracted according to EPA method 504 and analyzed by gas chromatography. Typical average response factors (based on peak heights), relative standard deviations (% RSD) of response factors (RFs), and correlation coefficients of the linear curves are listed in table 2.

Figure 2 shows linear calibration curves for EDB and DBCP with correlation coefficients better than 0.999 (see table 2). The % RSD of RFs was 4% to 7%, over a concentration range greater than two orders of magnitude (0.005 to 1.14 µg/L). This easily met method 504 requirements for 20% RSD for a similar concentration range. The micro-ECD continued to meet these requirements over a period of 2 to 3 months with little or no maintenance required except for routine septum and liner changes.

MDLs, Precision, and Accuracy

Method detection limits (MDL) were calculated according to EPA method 504 by analyzing seven replicate extracts of a low-level standard (0.02 µg/L). As shown in table 3, the MDLs were 0.002 and 0.003 µg/L for EDB and DBCP, respectively. These MDLs were three- to five-fold below those reported by EPA method 504

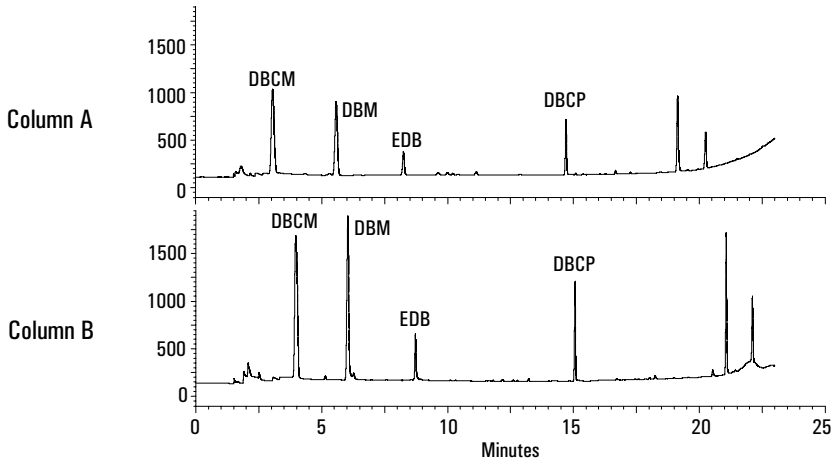

Figure 1. Hexane extract of a midpoint calibration standard (EDB/DBCP = 0.286 µg/L each).

Table 2. Typical Linearity on Column A*

Analyte	EDB	DBCP
Average response factor (RF)	4.66E-06	2.06E-06
Standard deviation, RF	2.19E-07	1.45E-07
%RSD, RF	4.69%	7.01%
Correlation coefficient	0.9992	0.9997

* Seven-level calibration at 0.0057, 0.020, 0.0571, 0.114, 0.286, 0.571, and 1.141 µg/L

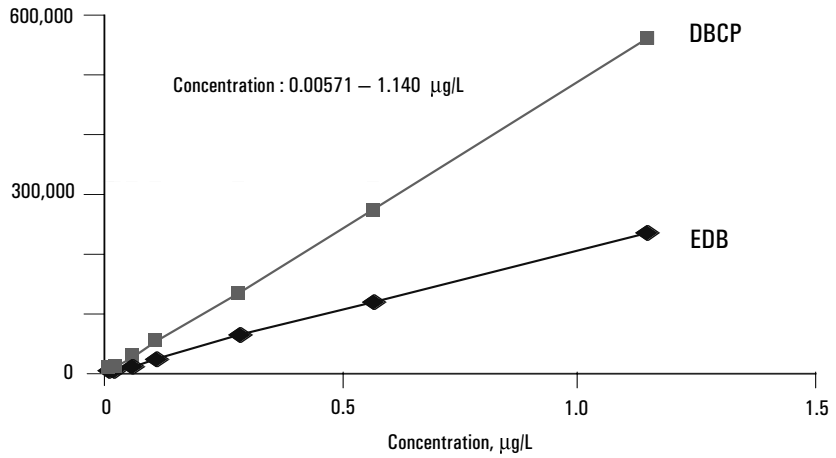

Figure 2. Typical calibration curves on column A

Table 3. MDLs, Precision, and Accuracy

Analyte	EDB	DBCP
Spiked concentration, µg/L	0.02	0.02
Number of replicates	7	7
MDL, µg/L	0.002	0.003
Spiked concentration, µg/L	0.20	0.20
Number of replicates	6	6
Average concentration, µg/L	0.202	0.205
Reproducibility, % RSD	5.3%	5.4%
% Recovery	101%	103%

and a Collaborative Study by K. W. Edgell and J. E. Longbottom.[2]

Six extracts of reagent water samples fortified with 0.20 µg/L of EDB and DBCP were analyzed. Both precision and accuracy were excellent, with reproducibility at 5% RSD and recovery of around 100% (see table 3).

Ruggedness of the 6890 Micro-ECD

For the detector to meet the low detection limit requirements, the chromatographic baseline must be clean and stable. In this study, the 6890 micro-ECD provided a clean baseline with no negative deflections during continuous operation over a period of 3 months. A variety of samples were also analyzed, including soil pesticide extracts that contained many late-eluting compounds (see figure 3). The 6890 micro-ECD showed rapid recovery even though this instrument had been switched from a drinking water method (EPA method 504) to solid waste methods (EPA method 8080/8081 and CLP method for pesticides and arochlors[3]), and back again.

EPA method 504 requires a continuous calibration (using a midlevel standard) for each 12-hour shift of operation or every 10- to 20-sample analyses. The retention times and the responses for these continuous calibration runs must match those from the initial calibration run with specific limits. The difference in responses (%D) between the later calibration run and the initial run must be less than 15%.

Table 4 presents the results of the sequence runs on the 1st, the 15th, and the 27th day of a month when samples were continuously analyzed according to EPA method 504. Responses of the 6890 micro-ECD proved to be quite stable over 3 to 4 weeks of continuous operation. The %D of EDB and DBCP did not vary by more than 10%, easily meeting the method requirement of 15%.

Conclusion

The Agilent 6890 Series GC with the micro-ECD can detect low levels of EDB and DBCP in drinking water and water supplies. All EPA method 504 criteria were easily met, yielding MDLs of 0.003 µg/L or less, reproducibility of 7% or less, and a linearity with correlation better than 0.999 over a concentration range greater than two orders of magnitude.

The system performance was stable for a long time (3 months), despite switching methods between EPA method 504 and CLP method for pesticides and arochlor. Stability, sensitivity, and linearity of the 6890 micro-ECD were significantly improved over the classical 6890 ECD.

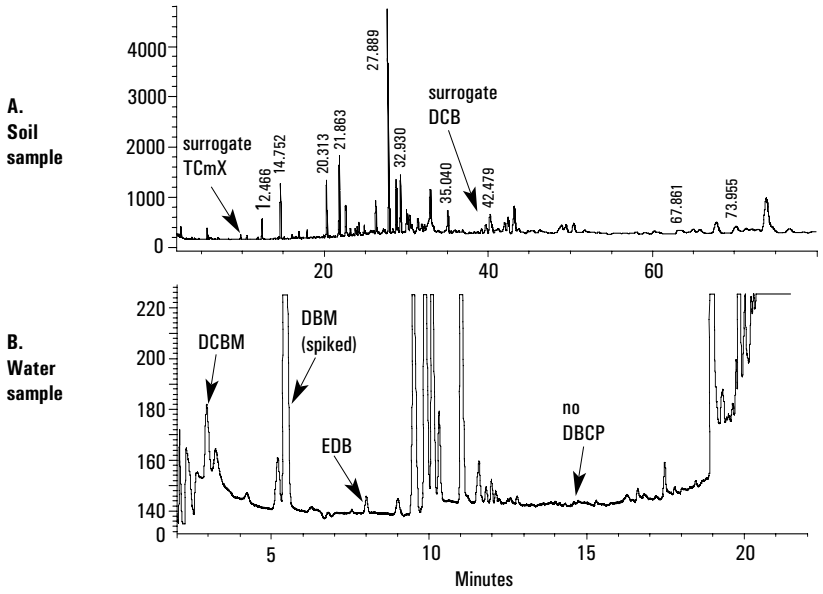

Figure 3. Typical chromatograms of sample extracts*

*The soil sample was analyzed according to EPA CLP method for pesticides along with 30 to 40 other samples in a sequence run.[4] No target pesticide was detected in this particular sample. The water sample was analyzed along with 20 other water samples based on EPA method 504 on the next day after the 6890 system was switched from the CLP method. No DBCP was found in any sample, and EDB was detected in only 3 to 4 samples. EDB in this sample was at the 0.01- to 0.02-ppb level. These chromatograms were plotted on different scales. Note the high signal for the soil sample. This demonstrates that it was possible to shift very quickly from analyzing dirty soil samples to analyzing low-level water samples using the 6890 system with micro-ECD .

Table 4. System Performance

	Run	Retention Time		Responses		%D	
	No.	EDB	DBCP	EDB	DBCP	EDB	DBCP
Day 1 Sequence							
Initial calibration	7	8.16	14.62	28486	70242		
Continuous calibration	19	8.16	14.62	29118	72434	2.2%	3.1%
Continuous calibration	30	8.16	14.61	28969	74268	1.7%	5.5%
Day 15 Sequence							
Initial calibration	7	8.11	14.58	30878	64439		
Continuous calibration	18	8.10	14.56	31684	66978	2.6%	0.8%
Continuous calibration	29	8.12	14.58	31241	71009	1.2%	6.9%
Continuous calibration	34	8.12	14.59	31219	70276	1.1%	5.8%
Continuous calibration	50	8.13	14.59	31689	72829	2.6%	9.6%
Continuous calibration	60	8.12	14.59	31627	72974	2.4%	9.8%
Day 27 Sequence							
Initial calibration	6	8.13	14.59	32203	76362		
Continuous calibration	19	8.13	14.59	31557	74711	–2.0% –	2.2%
Continuous calibration	28	8.13	14.59	31855	75417	–1.1% –	1.2%

* %D = (initial response — continuous calibration response) / initial response

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Printed in the USA 3/2000
5966-0495E

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Analysis of Formaldehyde and Acetaldehyde in Air by HPLC using DNPH Cartridge

Noriko Shimoi and Hiroki Kumagai

Environment

Abstract

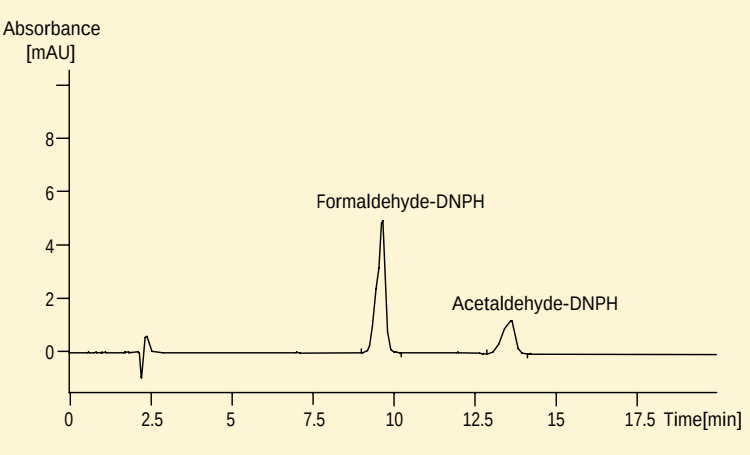
The monitoring of aldehydes, especially formaldehyde and acetaldehyde, is important for the monitoring of air pollution and acid rain problems. These aldehydes are analyzed by HPLC using 2,4-dinitorophenylhydorazine (DNPH) as the derivatization reagent. The cartridge of silica gel that was impregnated with DNPH (DNPH cartridge) is commonly used for the sampling and concentrating of aldehydes in air.
This application brief describes the analysis of formaldehyde and acetaldehyde in the air using DNPH cartridge.

Analyzed Compounds

Formaldehyde and acetaldehyde in air as DNPH derivatives.

Sample

Air of some location in Japan.



Conditions

Column
250 mm ˘ 4.6 mm i.d. Inertsil ODS 80A
Mobile Phase
CH_3CN/H_2O = 50/50
Temperature
40 °C
Injection vol
25 µl
Diode array detector
A—365/8 nm, reference off

Figure 1
Chromatogram of DNPH derivatives of formaldehyde and acetaldehyde

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Sample Preparation

Sampling was performed by sucking air through the DNPH cartridge (ozone scrubber was inserted before DNPH cartridge) with a pump. Sampling time was 24 hours at a flow rate of 0.1 l/min. The actual sampling volume was measured by the flow meter.

Method performance

Limit of Detection: formaldehyde 0.25 µg/m^3, acetaldehyde 0.35 µg/m3 (calculated from 3σ of blank values)
Repeatability of RT over 6 runs < 0.1 %
Repeatability of area over 6 runs < 0.5 %

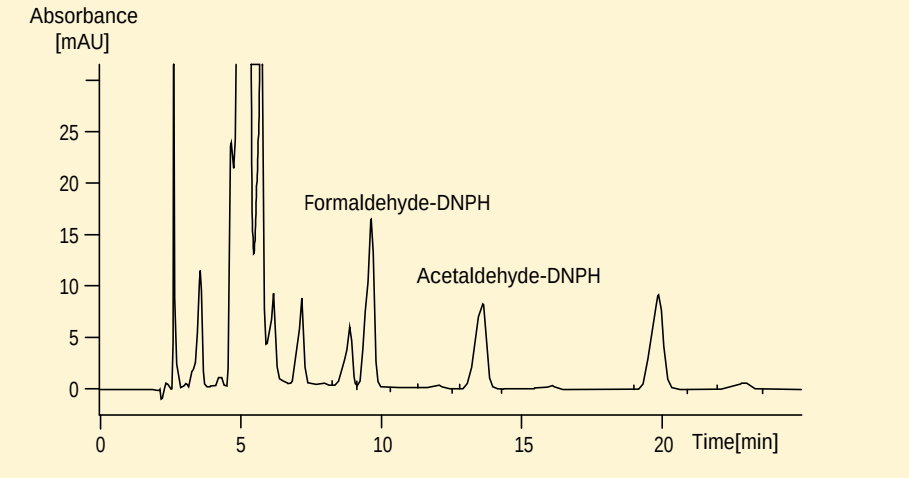


Figure 2
Chromatogram of aldehydes in the air of city A (Japan)

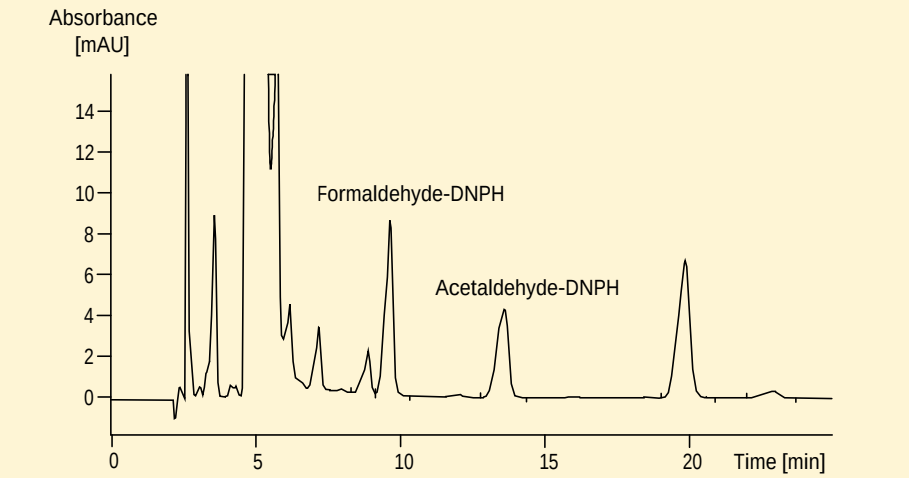
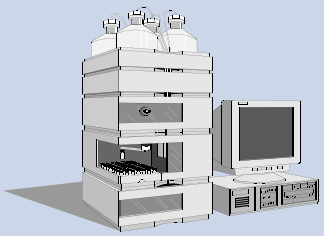


Figure 3
Chromatogram of aldehydes in the air of city B (Japan)

Equipment

Agilent 1100 Series
- degasser
- binary pump
- autosampler
- thermostatted column compartment
- diode array detector

Agilent ChemStation + software



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Released 06/97
Publication Number 5965-9796E

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Semi-volatiles Applications

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Addressing the Challenges of Analyzing Trace Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) Using LC/QQQ

Application

Food, Environmental

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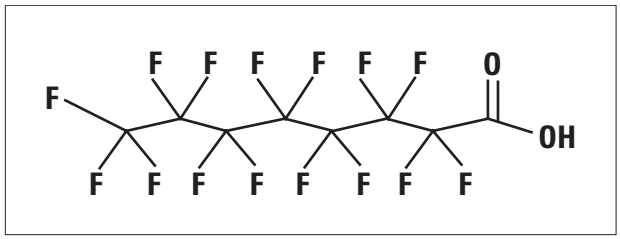
Abstract

An approach to the difficult task of quantifying trace quantities of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in complex matrix was developed using liquid chromatography and tandem mass spectrometry (LC/MS/MS). The technique uses isotopically labeled analytes for accurate quantitation (0.4 to 400 pg on column). It is important to recognize that if using the linear chain sample as standard for calibration, the quantitation results of real-world samples (branched and linear isomers mixed) will be off by as much as 40%.

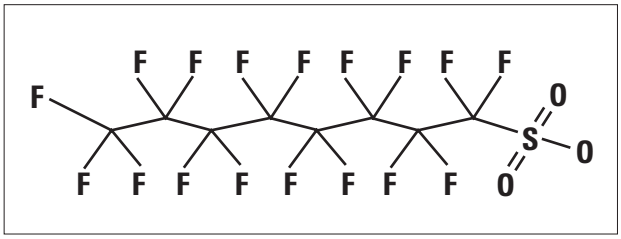
Introduction

Perfluorooctanoic acid (PFOA) is an industrial surfactant and a necessary processing aid in the manufacture of fluoropolymers [1]. Fluoropolymers have many valuable properties, including fire resistance and the ability to repel oil, stains, grease and water. One of the most common uses of PFOA is for processing polytetrafluoroethylene (PTFE), most widely known as Teflon®. PFOA is also a by-product from direct and indirect contact with food packaging (for example, microwave-popcorn bags, bags for muffins or french fries, pizza box liners, boxes for hamburgers, and sandwich wrappers), and in the fabrication of water- and stain-resistant clothes.

Perfluorooctanesulfonic acid (PFOS) is usually used as the sodium or potassium salt and is referred to as perfluorooctane sulfonate. See Figure 1.



PFOA



PFOS

Figure 1. Chemical structures for PFOA and PFOS. Note that both have C8 chains.

Analytical Methodology for PFOA/PFOS

- LC/MS/MS is the preferred detection methodology due to its high sensitivity and specificity in complex matrices.
- Multiple reaction monitoring (MRM) is used to quantitate, using two or more product ions for confirmation.
- The detection limit is typically in the range 1 to 100 pg/mL (ppt), requiring high-sensitivity detection.
- On-column or off-line solid-phase extraction (SPE) and concentration are needed to achieve low-level detection (1 pg/mL).

Measuring PFOS and PFOA

Issue 1: ***What transitions should be used to give the best accuracy when quantifying with a linear standard?***

Quantification of PFOS and PFOA is usually based on a linear standard, but actual samples show a series of branched isomers together with the linear isomer. The ratio of these isomers varies based upon biodegradation and industrial processes in their formation; therefore, it is unlikely that a standard can be formulated to mimic the actual sample. The relative intensities of the MRM transitions will vary based upon branching, making some transitions better than others. Branching impacts ionization efficiency and CID energy; therefore, it affects the accuracy of analytical measurement [2].

Issue 2: ***Can isotopically labeled standards in matrix be used to measure nonlabeled PFOS and PFOA?***

Most biological and environmental matrices have background levels of PFOS and PFOA; although matrix-matched calibrations are providing good results, the accuracy can be enhanced. The method of standard additions is a protocol to address this issue, but it adds several additional injections to the analysis. Matrix may have varying amount of background. Standard addition is not practical in analyzing many different matrices. Solvent calibrations do not correct for matrix effects.

Experimental

Sample Prep

- All solvent standards were prepared in methanol.
- Plasma extracts were prepared by acetonitrile precipitation and centrifuging, with the upper layer taken and spiked with known concentrations of PFOA or PFOS.

LC

- Agilent 1200 Rapid Resolution LC system
- ZORBAX Eclipse Plus C18 Rapid Resolution HT column 2.1 cm × 50 mm, 1.8-µm particles (P/N 959741-902)
- 20-µL injection, 0.4 mL/min column flow
- 0 to 100% B in 10 min, A = water with 2 mM ammonium acetate; B = MeOH

MS/MS

- Agilent QQQ
- Negative-ion detection
- 3500 V_{cap}, drying gas 9.5 L/min at 350 °C, nebulizer 45 psi
- Fragmentor voltages, collision energy (CE), and ion transitions are experimentally determined

Multiple Reaction Monitoring (MRM)

Figure 2 displays a cross-section of the Agilent 6410 QQQ above a hypothetical sequence of spectra characteristic of ion transitions within the instrument.

The ions are generated in the source shown at the far left of the figure. The precursor ion of interest is then selected from this mixture and isolated through the Q1 quadrupole, which acts as a mass filter. This is similar to selected ion monitoring (SIM). After Q1, characteristic fragments that are specific to the structure of the precursor ion are generated in the collision cell (Q2, although not a quadrupole). By using the Q3 quadrupole, these fragments are then selected for measurement at the detector. This is a selective form of collision-induced dissociation (CID), known as tandem MS/MS. By setting Q3 to a specific fragment ion existing in the collision cell, the chemical or background noise is almost totally eliminated from the analyte signal, therefore, significantly increasing the signal-to-noise ratio. Ion 210 is called the precursor ion and ions 158 and 191 are product ions. Each transition (210→191 or 210→158) is a reaction for a particular target. Typically, the QQQ is used to monitor multiple analytes or mass transitions, therefore, the term MRM. The 158 could be considered the quantitation ion, because it is the

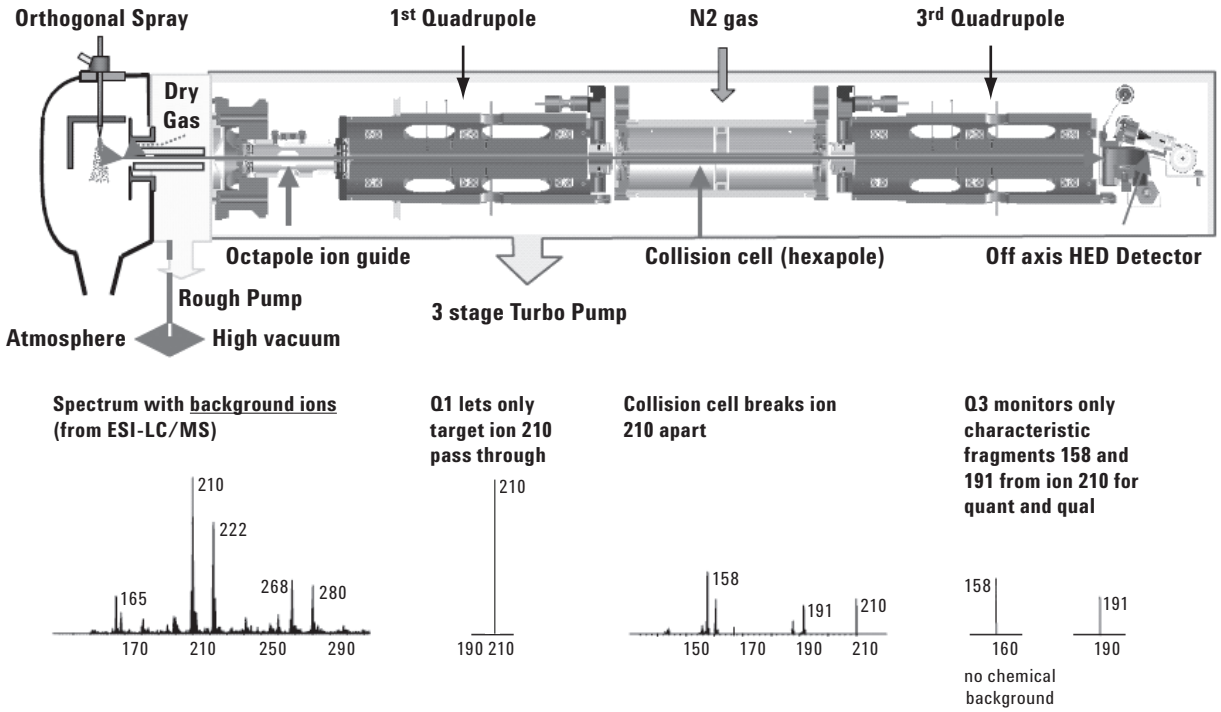


Figure 2. A cross-section of the Agilent 6410 QQQ above a sequence of spectra characteristic of ion transitions within the instrument for a hypothetical sample (*not* PFOA or PFOS). Note that the final spectrum is very clean, containing only the desired target ions. (HED = high-energy dynode electron multiplier)

most intense, and 191 could be used for confirmation by using the area ratio of the 191 qualifier to the 158 quantifier ion as a criterion for confirmation. With MRM, most chemical noise is eliminated in Q1, and again in Q3, allowing us to get ppt detection.

The fragmentor is the voltage at the exit end of the glass capillary where the pressure is about 1 mTorr. Fragmentor and collision energies need to be optimized. A fragmentor that is too small won't have enough force to push ions through the gas. A fragmentor that is too high can cause CID of precursor ions in the vacuum prior to mass analysis, thereby reducing sensitivity. The actual voltage used is compound-, mass-, and charge-dependent, and therefore needs to be optimized to get the best sensitivity. The CE in the collision cell needs to be optimized in order to generate the most intense product ions representative of each target compound. Collision cell voltage will depend on the bond strength, the molecular weight of the compound, and the path by which the ion is formed (directly from the precursor ion or through a series of sequential intermediates). Typically each product ion will exhibit a preferential collision energy that results in the best signal abundance.

The experimental operations required to arrive at optimal conditions are exemplified by the series of experiments shown in Figures 3 to 5.

Optimization of the fragmentor voltages for the $[M-H]^-$ ions of PFOA (*m/z* 413) and PFOS (*m/z* 499) are shown in Figure 3.

Note that there is little signal detected for PFOA at the optimal fragmentor voltage for PFOS (200 V). Ions 413 and 499 are called precursor ions. PFOA is relatively fragile; its precursor signal drops off at 160 V. PFOS shows that it is harder than PFOA to break apart; the best fragmentor voltage for PFOS is 200 V.

The appropriate collision energies for product ions *m/z* 369 $[M-CO_2H]^-$ and *m/z* 169 $[C_3F_7]^+$ are experimentally determined and used to quantify PFOA. See Figure 4.

In each case the collision energy producing the most intense peak for each ion is chosen for the analysis. PFOA takes little collision energy to break into ion *m/z* 369 (6 V for highest intensity).

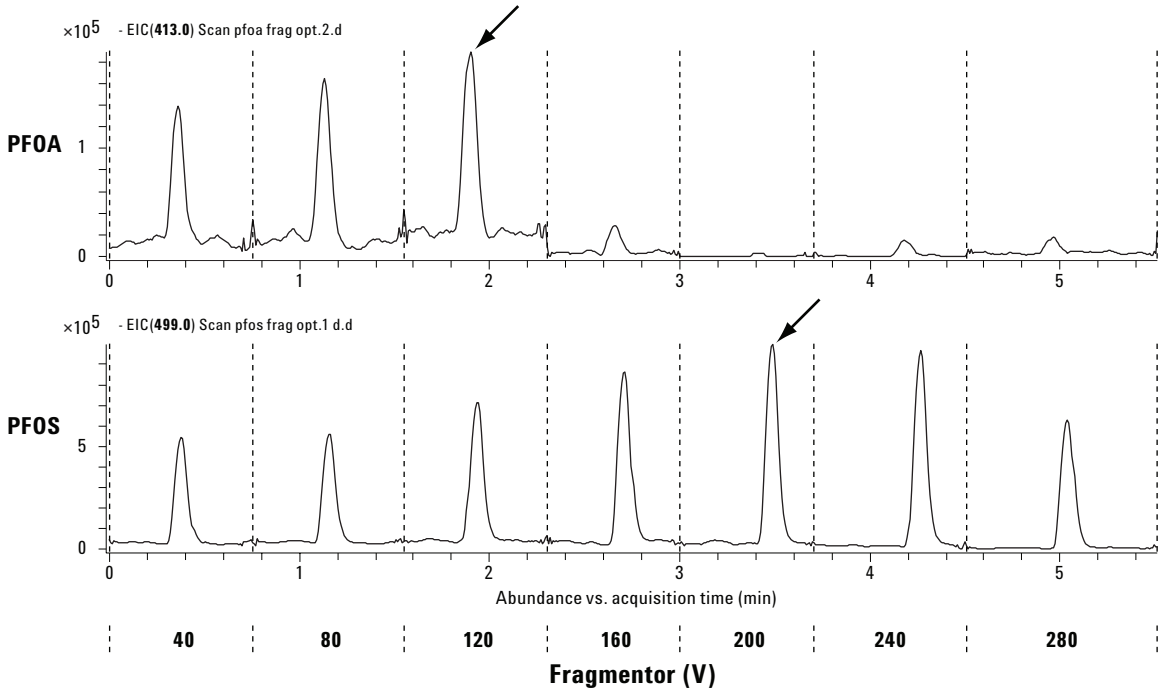


Figure 3. Determination of optimal fragmentor voltage using sequential plots of signal intensity versus applied voltage.

To maximize the intensity of the ion at *m/z* 169, the collision energy needs to go to 16 V.

The QQQ software can switch collision energies very rapidly. So in a method, the optimal collision voltage can be selected for each ion transition.

In the same manner, the appropriate collision energies for PFOS product ions at *m/z* 169, 99, and 80 are experimentally determined and used for its quantitation. The optimal collision energies for the three ion transitions are 45, 50, and 70 V. See Figure 5.

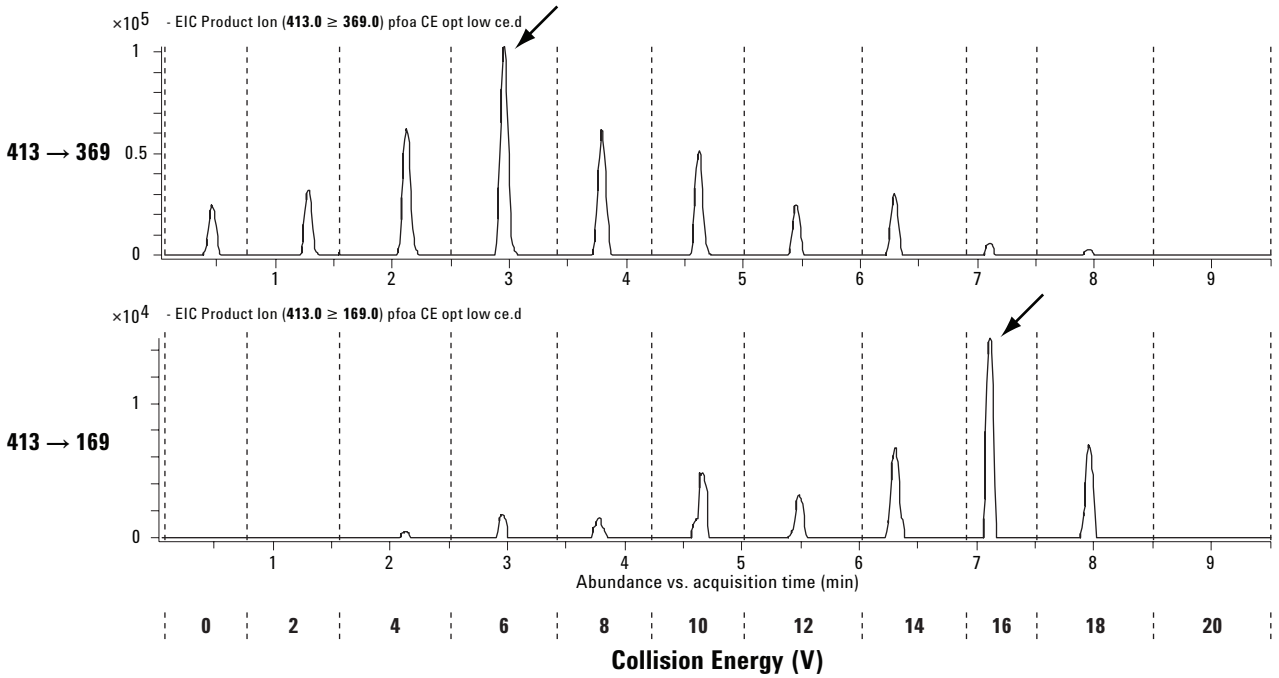


Figure 4. Signal intensity as a function of collision energy for PFOA product ions *m/z* 369 $[M\text{-}CO_2H]^-$ and *m/z* 169 $[C_3F_7]^+$.

Notice the big difference in collision energy between PFOA (6 to 16 V) and PFOS (45 to 70 V). We have seen from fragmentor optimization that PFOA is relatively fragile compared to PFOS, in which the optimum fragmentor voltages are 120 and 200 V for PFOA and PFOS, respectively. The CE reinforces that aspect.

Example calibration curves for the specified product ions used to quantitate PFOA and PFOS are shown in Figure 6. The analyst can also sum the intensities of these MRM transitions to get a calibration curve.

These five ion transitions exhibit linear correlation coefficients > 0.998, and are good for quantitation over three orders of magnitude. Notice that the lowest amount on column is 0.4 pg.

Regarding issue 1: ***What transitions should be used to give the best accuracy when quantifying with a linear standard?***

This is addressed using Figures 7 to 9.

Figure 7 exhibits chromatograms from these representative transitions for PFOA and PFOS for the linear standard and samples containing branches (10-min gradient).

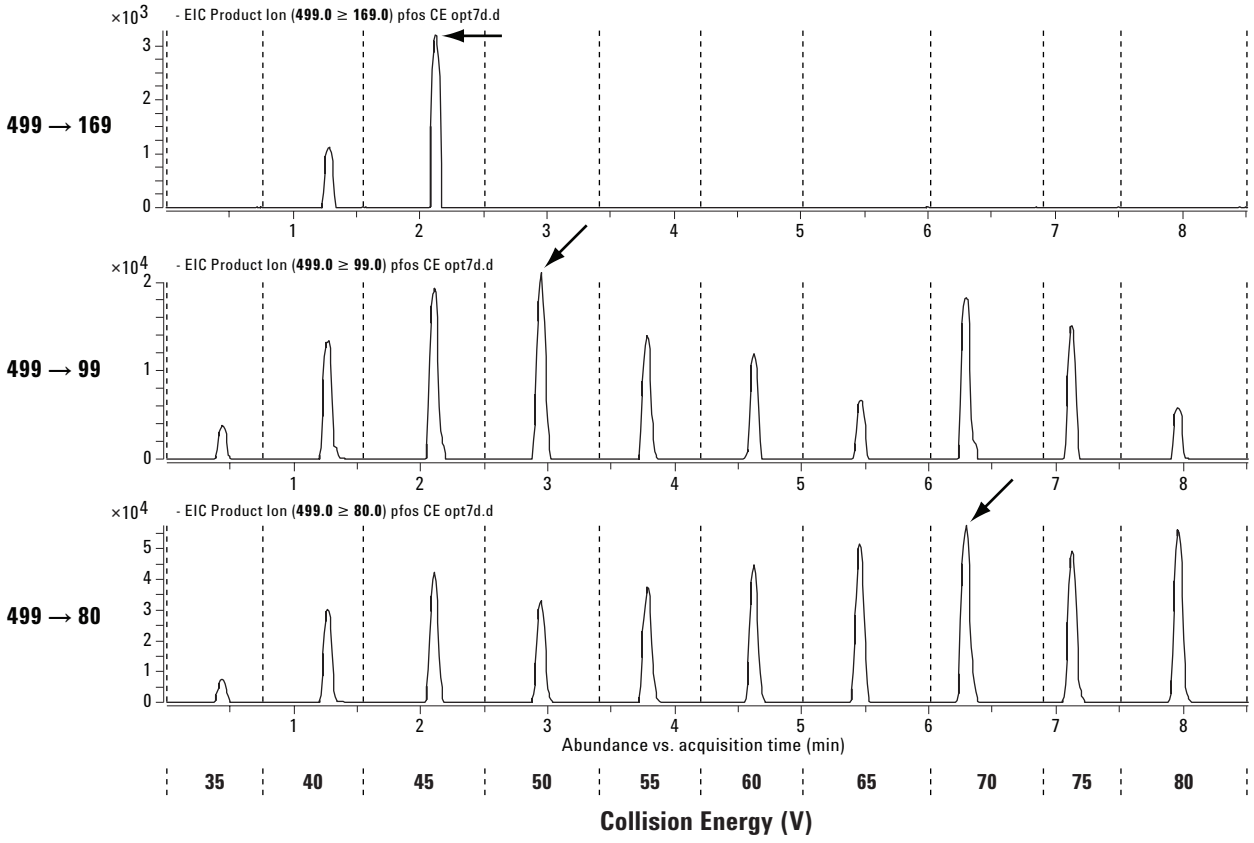
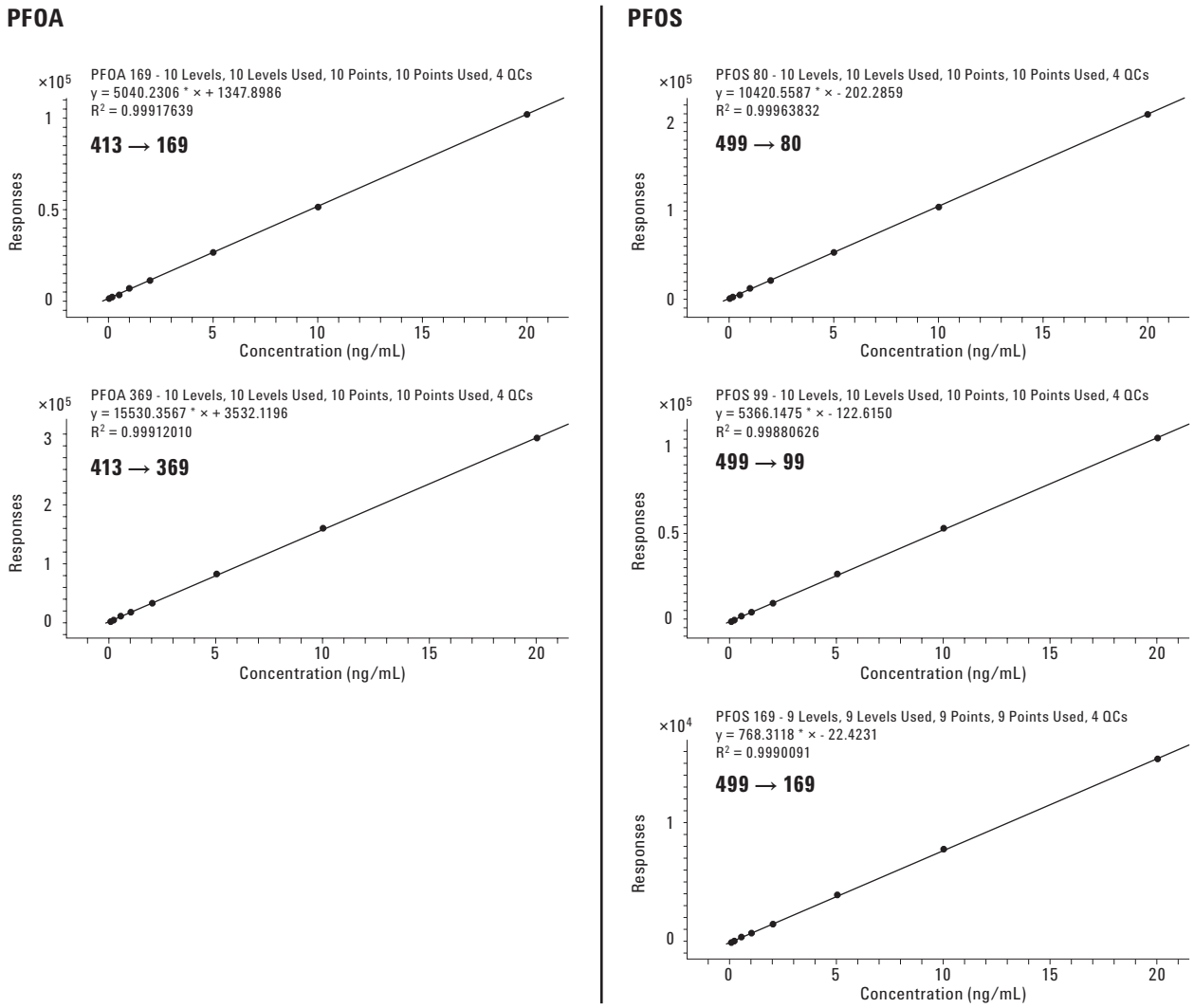


Figure 5. Signal intensity as a function of collision energy for PFOS product ions at *m/z* 169, 99, and 80.



Concentration range 0.02 to 20 ng/mL (0.4 to 400 pg injected on column)

Figure 6. Calibration curves for the product ions used to measure PFOA and PFOS.

Real-world samples have been detected with branched isomers due to manufacturing processes, metabolism, and degradation processes. The top chromatogram of Figure 7 shows only linear chain compounds from a standard. The bottom chromatogram is an actual sample from the environment. It shows additional peaks (shoulders) in the chromatogram resulting from branched isomers.

We examine those peaks in greater detail in Figure 8.

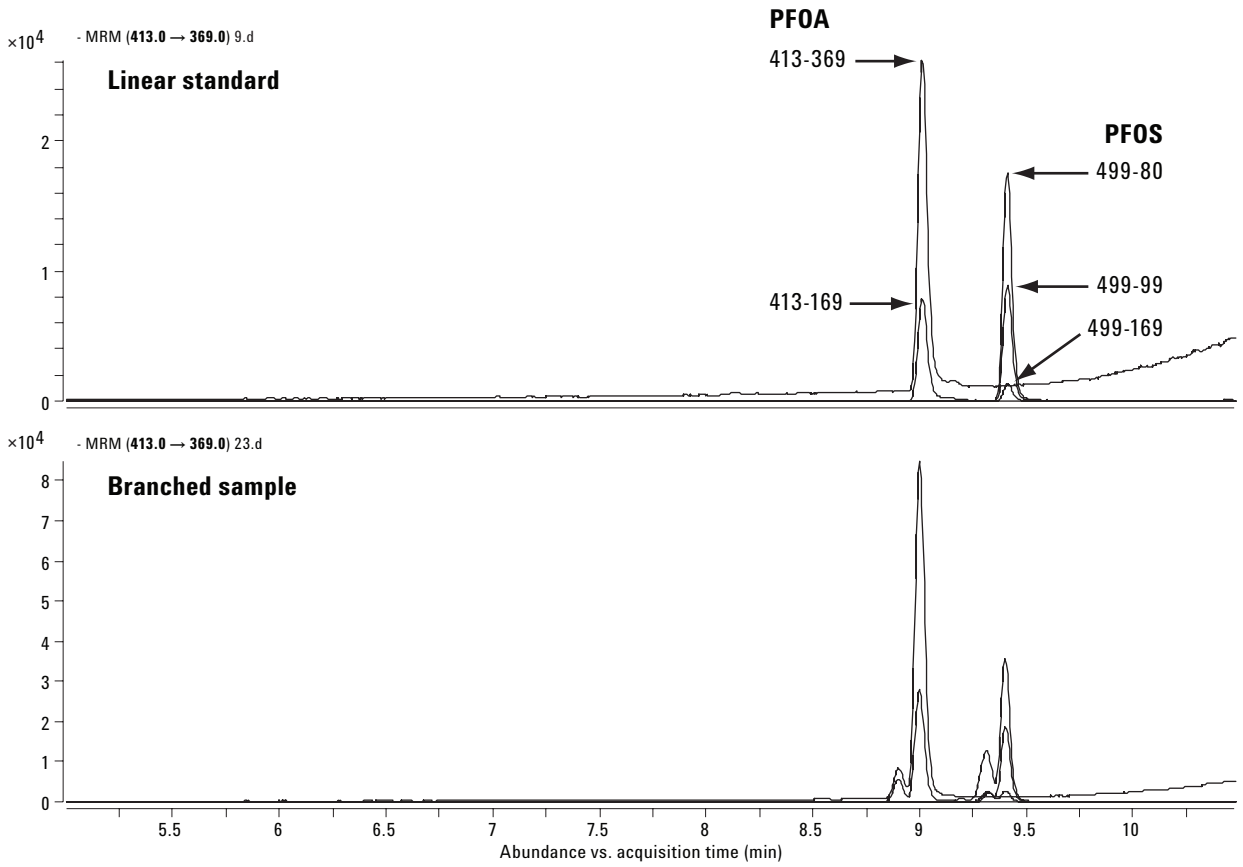


Figure 7. MRM chromatograms for PFOA and PFOS for both linear and branched samples.

The relative abundances for each MRM transition are dependent on the branching locations and the specific mass transitions. Figure 8 shows a 10-minute run. The chromatography can separate the linear from the branched isomers. The branched sample is typically a C7 chain with a methyl side group (isooctyl isomer). The most interesting part of the analysis is that the ion ratios for the branched compounds are very different from the linear chain compounds [3, 4, 5]. For linear PFOA, the ion at *m/z* 169 is about 30 to 40% of ion 369. The branched isomer shows that the ratio changed to 90 to 100%. For linear PFOS, the ion at *m/z* 99 is about 50% of ion 80 and is 500% of ion 169. The branched isomer shows that ion 99 is only 20 to 30% of ion 80, and 100% of ion 169. This is a cause of concern in terms of quantitation accuracy. This shows that CID stability is very different when the analyte is branched.

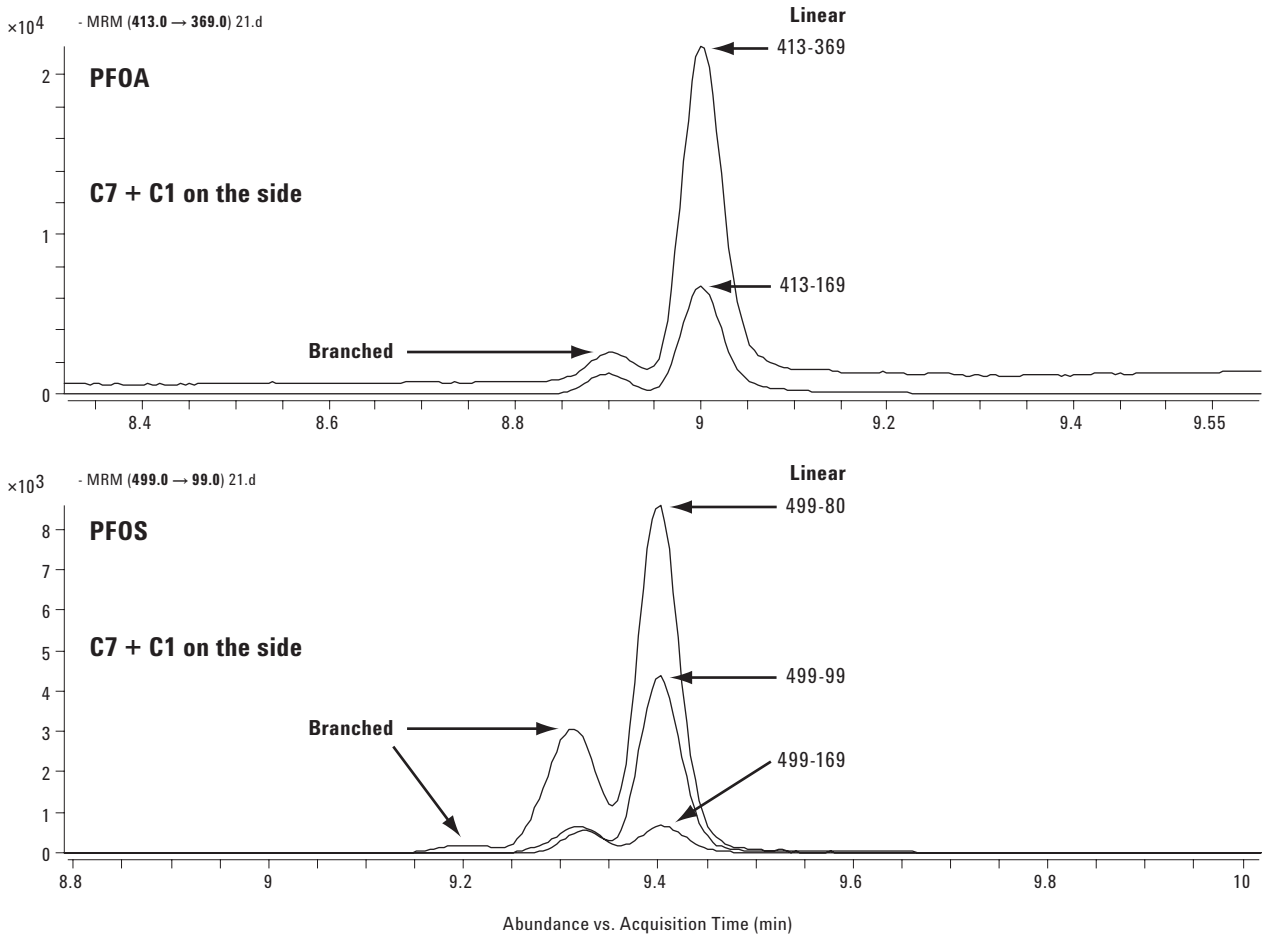


Figure 8. MRM chromatograms for PFOA and PFOS for both linear and branched samples.

Another variable in the analysis is the gradient time. Figure 9 compares the effect of a 3-min versus 10-min gradient.

In the fast gradient case (on the right), the branched isomers (dashed lines) are not resolved from the linear isomers (solid lines), resulting in a significant error in the measured value (most noticeable for PFOS).

The two chromatograms on the left are the same two that are shown in Figure 8. They are used here for comparison against the unresolved analytes shown on the right (3-min run). Although we would like to cut down on the analysis time, the branched and linear isomers need to be resolved in order to get accurate quantitation results.

Two samples of the same concentration. One sample is the pure linear isomer; the other sample has a mixture of branched isomers. If their MRM responses (ion ratios) are the same, they would show the same results as when the isomers are not resolved. This example shows that the responses are not the same when the isomers are not resolved. If you add the responses of the side chain analyte and the linear chain analyte of the same sample, the area of each ion transition is different from the pure linear chain analyte ion transition, as seen in the two chromatograms on the right, most apparent is for PFOS. If using the linear chain sample as standard for calibration, the results of real-world samples (branched and linear isomers mixed) will be off by as much as 40% (see Table 1). The quantitation falls apart.

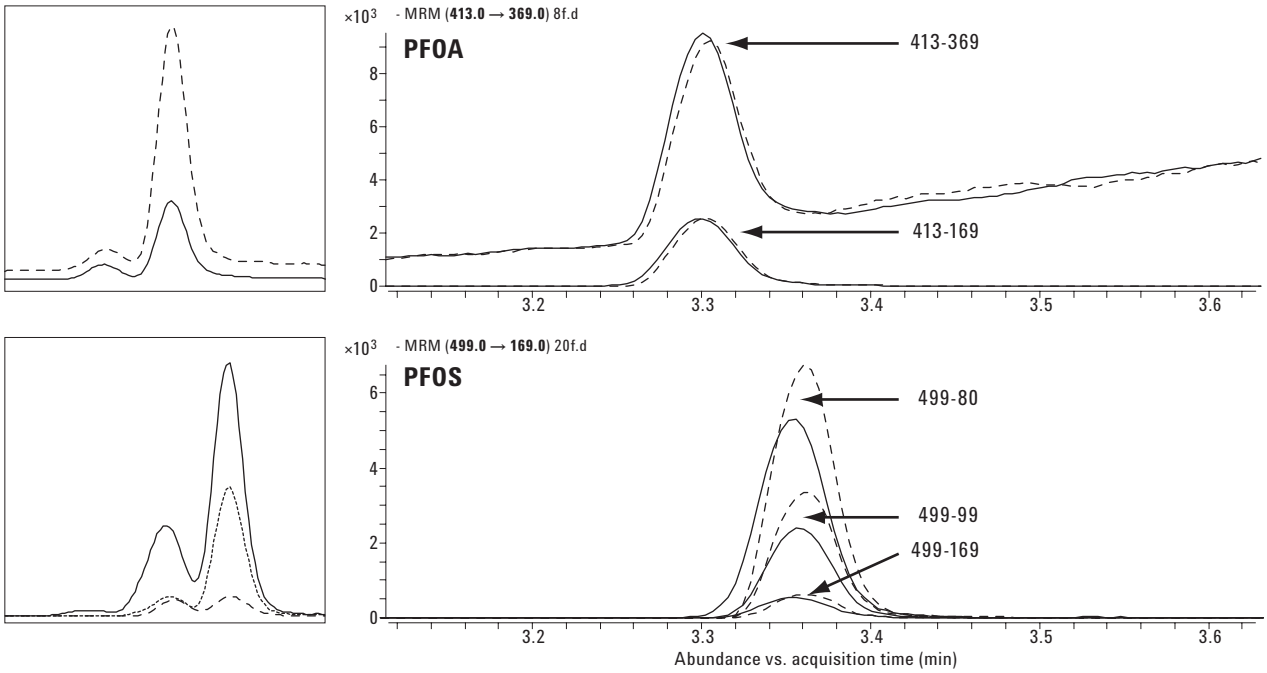


Figure 9. Comparison of PFOA and PFOS MRM chromatograms produced using both 10- and 3-minute gradients. The 3-minute gradient chromatograms are on the right.

The effect of measurement accuracy (*not* ion ratios) of total PFOA and PFOS in branched samples against *a linear standard for each MRM transition* is shown in Table 1.

Table 1. Measurement Accuracy (Target Is 100%) as Function of Compound, Transition, and Run Time

Compound	MRM transition	Percent response (n = 8)	
		10-min run	3-min run
PFOA	**413→369**	**105.9**	**108.2**
	413→169	**96.4**	**89.4**
PFOS	**499→169**	**102.5**	**112.2**
	499→99	75.0	73.3
	499→80	59.3	61.1

The best MRM ions are in bold type. The best results for PFOA can be obtained by averaging the results for the two MRM ions together.

Ion ratios can cause quantitation failure. For PFOA, it does not matter if it's a 3-min run or a 10-min run: the ion 369 transition response is always higher and the ion 169 transition response is always lower. The errors are larger for the 3-min run. The variations are greater for PFOS. In literature, PFOS analysis monitors the ion 80 transition, but it exhibits a large variation. It can be as low as 60%, as seen in Table 1. 499 → 169 is a good transition for quantitation. It is much more accurate, but it is less sensitive compared to 499 → 80 transition.

Regarding issue 2: *Can isotopically labeled standards in matrix be used to measure non-labeled PFOS and PFOA?*

This is addressed using Figures 10 to 12.

Observations regarding the effect of different matrices on signal responses are shown in Figure 10. The taller trace represents the response of PFOA in methanol. The response is lower as the same amount of PFOA is added into a plasma extract.

The matrix effect (common using electrospray ionization) can lead to signal suppression or enhancement; therefore, matrix-matched calibrations are required for accurate quantitation. Due to varying background levels of PFOS and PFOA in matrix, it may not be feasible to use matrix-matched calibrations for quantitating PFOS or PFOA concentrations in study samples. Also, the method of standard additions is not a practical alternative for many matrices with varying levels of target analytes.

As a practical alternative, measuring PFOA using isotopically labeled matrix-matched standards was examined. Results are shown in Figures 11 and 12.

Figure 11 shows that isotopically labeled standards can provide a good linear calibration curve over the quantitation range of 0.02 to 20 ng/mL (0.4 to 400 pg on column). Excellent linear correlation coefficients (≥ 0.9994) were obtained.

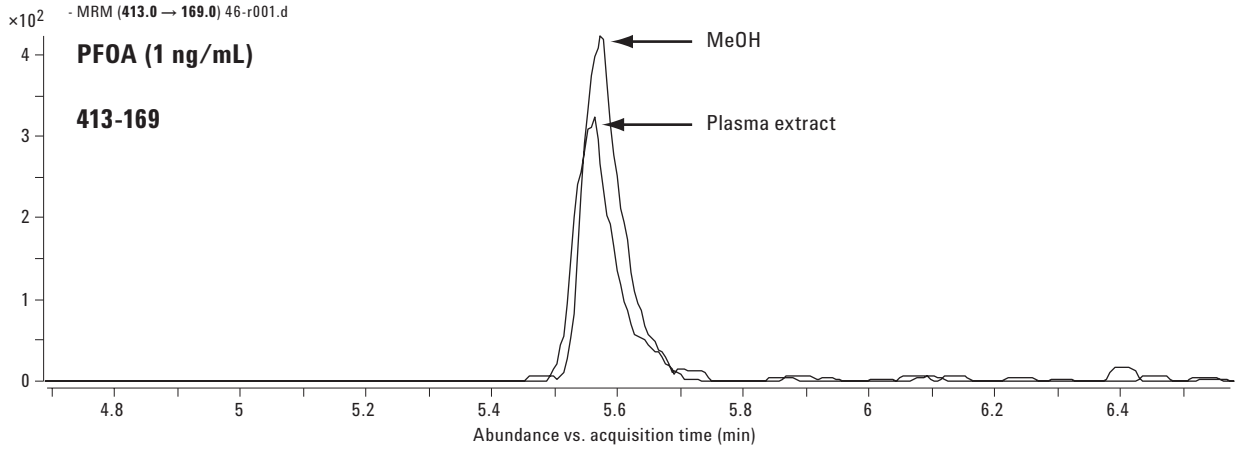
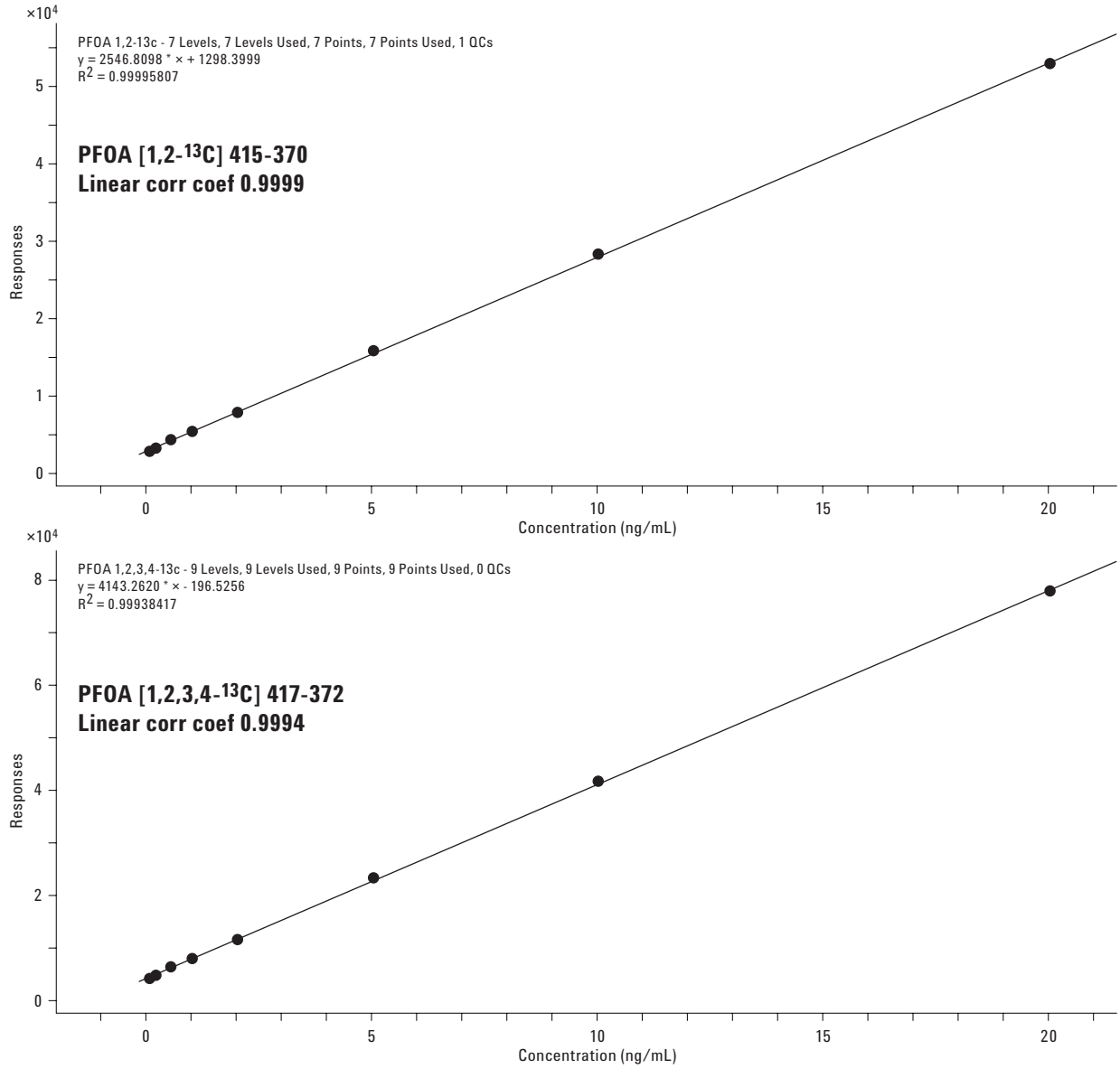


Figure 10. PFOA responses in MeOH and plasma extract at the same concentrations.



Calibration in plasma from 0.02 to 20 ng/mL

Figure 11. Linear correlations for PFOA using two different isotopically labeled calibration standards.

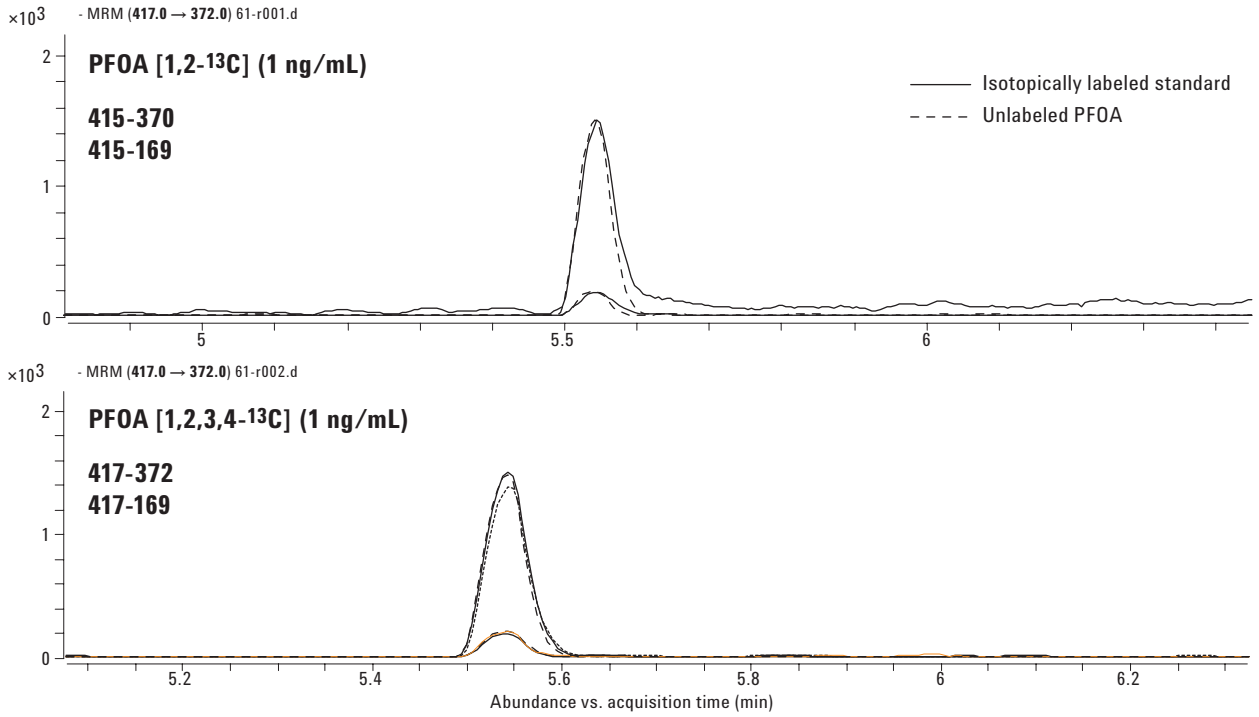


Figure 12. Both isotopically labeled PFOA compounds show good correlation to the unlabeled PFOA. The same transitions for the labeled and native forms of the PFOA were used.

Table 2. Comparison of Different Matrix-Matched Calibrations for Measuring PFOA in Plasma

	Calibration standard	Matrix for calibration	Plasma sample response (Std Dev)
1	PFOA	MeOH	71 (± 33 %)
2	PFOA [1,2-^{13}C]	Plasma	100.4 (± 3.1 %)
3	PFOA [1,2,3,4-^{13}C]	Plasma	97.3 (± 5.1 %)

Matrix-matched calibrations using isotopically labeled PFOA work well.

For row 1, the calibration standard used MeOH as the solvent, and the plasma sample exhibited a 71% response due to matrix suppression. Therefore, we cannot use a calibration standard in MeOH to quantitate samples in matrix; the variation can be as large as 30%. Rows 2 and 3 show that if the calibration is done using an isotopically labeled compound in matrix, the actual plasma sample yields accurate results: 100 and 97%.

Conclusions

- The Agilent LC/QQQ is an excellent instrument for quantifying trace target compounds in complex mixtures.
- The best ion transitions for analysis need to be determined experimentally.
- Fragmentor voltages and collision energies require experimental determination and optimization.
- Using MRM in the QQQ helps achieve the lowest detection limits in complex matrices.
- Branched PFOA/PFOS can affect quantitation accuracy as much as 40% unless it is corrected.
- Matrix suppression can cause the quantitation to be off by as much as 30%. Isotopically labeled analytes work well for accurate quantitation in spite of varying background levels of PFOA/PFOS in matrices.

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2. T. J. Wallington, M. D. Hurley, J. Xia, D. J. Wuebbles, S. Sillman, A. Ito, J. E. Penner, D. A. Ellis, J. Martin, S. A. Mabury, O. J. Nielsen, and M. P. Sulbaek Andersen, "Formation of $C_7F_{15}COOH$ (PFOA) and Other Perfluorocarboxylic Acids During the Atmospheric Oxidation of 8:2 Fluorotelomer Alcohol," *Environ. Sci. Technol.*, 40 (3), 924–930, 2006
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Acknowledgement

Partial support and the standards for this study were provided by the Environmental Lab of the 3M Company (St Paul, MN).

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Printed in the USA
April 23, 2008
5989-7790EN

Agilent Technologies

Fast USEPA 8270 Semivolatiles Analysis Using the 6890/5973 inert GC/MSD with Performance Electronics

Application

Environmental Analysis

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Abstract

The analysis of semivolatiles using EPA Method 8270 presents challenges due to the simultaneous measurement of acids, bases, and neutrals over a wide concentration range. Due to productivity demands, laboratories want to run faster while maintaining linearity and sensitivity for even the most active compounds. The 6890/5973 *inert* GC/MSD system with Performance Electronics is designed to meet the criteria for fast analysis, while minimizing activity and maintaining linearity.

Introduction

USEPA Method 8270 for semivolatiles analysis is used to concurrently measure a mixture of acids, bases, and neutrals. Most laboratories analyze for 70–100 compounds with a chromatographic run time of 25–40 min. Laboratories want to reduce this run time for productivity increases. The calibration range required for the analysis varies depending on a particular laboratory's statement of work (SOW). Historically, a range of 20–160 ng has been used. With the increased sensitivity of newer gas chromatograph/mass spectrometer (GC/MS) systems, laboratories are moving toward lower minimum detection limits (MDLs) and pushing the calibration range down to 1 ng.

The Agilent 6890/5973 *inert* GC/MSD (Gas Chromatograph/Mass Selective Detector) system with Performance Electronics was designed to meet the demand for faster runs and lower MDLs. Faster scan rates without loss of signal are now possible. This allows the use of smaller diameter columns, such as 0.18-mm id, resulting in shorter runs while maintaining sufficient data points across narrower chromatographic peaks.

The inert source allows for less material injected onto the column while maintaining mass spectrometer performance. Injection volume, therefore, can be matched to the 0.18-mm column. Performance comparisons using the inert source were published previously [1, 2].

This application note will demonstrate the use of the Agilent 6890/5973 *inert* with Performance Electronics for USEPA Method 8270. Smaller id columns with faster scan rates yield run times of 15 min while meeting Method 8270 criteria.

Experimental

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890
Inlet	EPC Split/Splitless
Mode	Pulsed splitless, 0.5 µL injection
Inlet temp	250 °C
Pressure	21.48 psi
Pulse pres	40.0 psi
Pulse time	0.20 min
Purge flow	50.0 mL/min
Purge time	1.00 min
Total flow	54.0 mL/min
Gas saver	Off
Gas type	Helium
Inlet Liner	Agilent splitless, single taper, 4-mm id, p/n 5181-3316
Oven	240 V

Oven ramp	**°C/min**	**Next °C**	**Hold min**
Initial		55	1.00
Ramp 1	25	100	0.00
Ramp 2	30	280	0.00
Ramp 3	25	320	4.60

Total run time	15 min
Equilibration time	0.5 min
Oven max temp	325 °C
Column	Agilent Technologies DB-5.625, p/n 121-5622
Length	20.0 m
Diameter	0.18 mm
Film thickness	0.36 µm
Mode	Constant Flow = 1.0 mL/min
Inlet	Front
Outlet	MSD
Outlet pressure	Vacuum
MSD	Agilent Technologies 5973 *inert* with Performance Electronics
Drawout lens	6-mm Large Aperture Drawout lens, p/n G2589-20045
Solvent delay	1.90 min
EM voltage	Run at DFTPP tune voltage - 153 V = 1012 V
Low mass	35 amu
High mass	500 amu
Threshold	10
Sampling	1
Scans/s	5.92
Quad temp	150 °C
Source temp	230 °C
Transfer line temp	280 °C
Emission current	DFTPP tune @ 25 µA

Calibration Standards were obtained from Accustandard, New Haven, CT, (p/n M-8270-IS-WL-0.25x to 10x). They contain 74 target compounds at nine concentration levels with six ISTDs at 40 ppm.

Pulsed splitless injection was used to minimize residence times of analytes in the liner, thereby reducing loss of active compounds. The column flow rate alone, without using a pulsed injection, would take too long to sweep the 900-µL liner volume.

The inlet liner (p/n 5181-3316) is the most commonly used liner for Method 8270 analysis. It does not contain glass wool which would contribute to active compound degradation. Other liners can be used and a detailed discussion of these can be found in Reference 1.

The Agilent 6890 240 V oven was necessary for the 25 °C/min Ramp 3 used.

A 120 V oven will achieve 20 °C/min at higher temperatures and could be used, resulting in slightly longer run times.

The DB-5.625 column was recently introduced in the dimensions listed. A 0.5-µL injection volume is well suited to this column. The excellent resolution from this column allows a higher than normal initial temperature, 55 °C vs 40 °C. This higher temperature shortens cool-down time by more

than 5 min, resulting in productivity increases for the laboratory. Benzo[b]fluoranthene and benzo[k]flouranthene met Method 8270 resolution requirements at the 80-ppm calibration level and lower, using the operating parameters in Table 1.

Previous work has shown improved linearity across a wide calibration range using a 6-mm drawout lens instead of the standard 3-mm lens [1]. Although not shown here, that comparison was repeated on this Performance Electronics system and is still valid. The 6-mm lens is also included in Agilent Kit p/n G2860A.

The 5973 *inert* was tuned using the automatic DFTPP target tune. The following steps were taken before executing DFTPP tune to insure that Method 8270 DFTPP criteria were met on injection.

1. Using the Tune Wizard, set the Mass 50 Target Abundance to 1.3% and the Emission Current to 25, as shown in Figures 1a–1f.
2. Edit the tuning macro as follows:
   a Copy atune73.mac from the MSDChem\msexe folder.
   b Paste the copy of atune73.mac into the MSDChem\msexe folder. The file name should be Copy of atune73.mac. This preserves an original copy of the file.
   c Open atune73.mac in Notepad. Refer to Figures 2a–2h.
   d Click Edit>Find and type samples in the Find What box.
   e Click Find Next.
   f Change the samples value from 3 to 1.
   g Change the averages value from 3 to 6.
   h Save the file and Close Notepad.

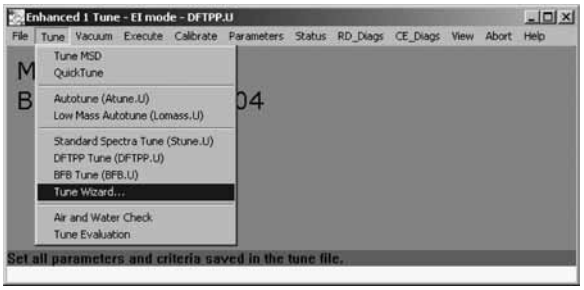

Figure 1a. Starting the Tune Wizard.

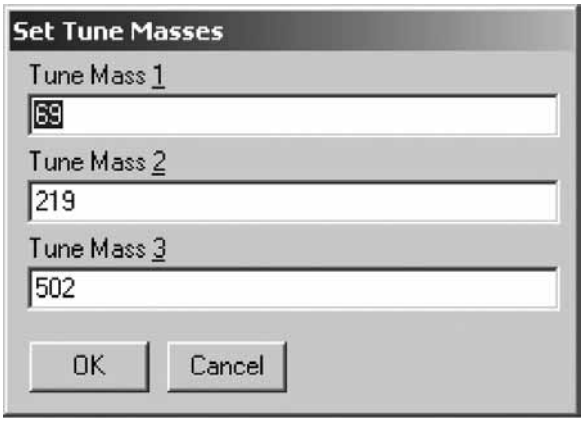

Figure 1b. Accept these masses.

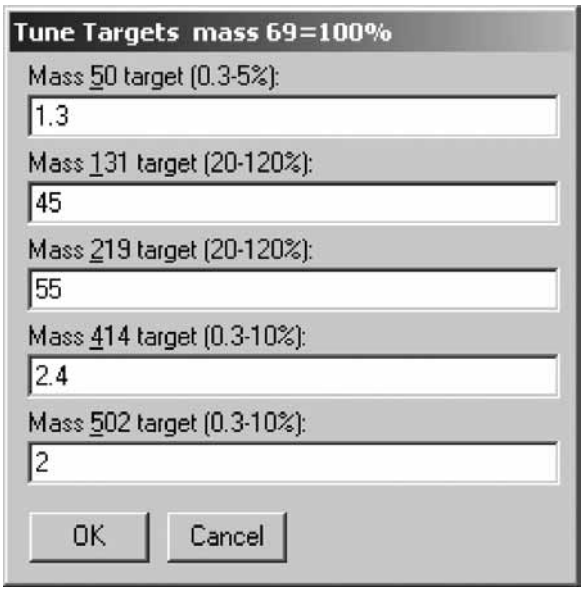

Figure 1c. Set Mass 50 target to 1.3.

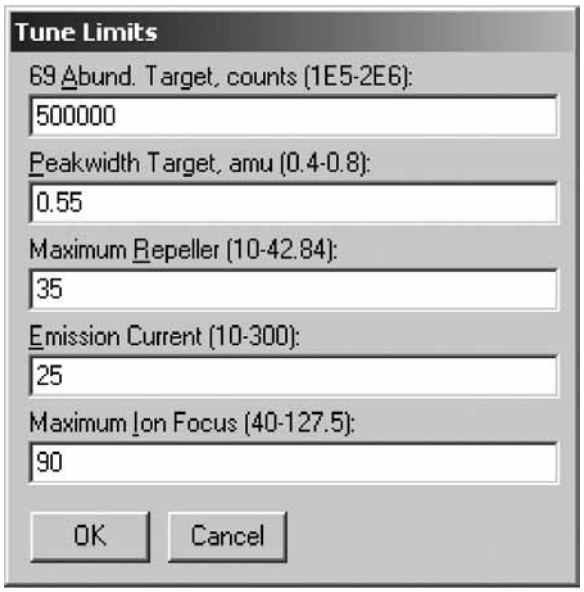

Figure 1d. Set Emission Current to 25.

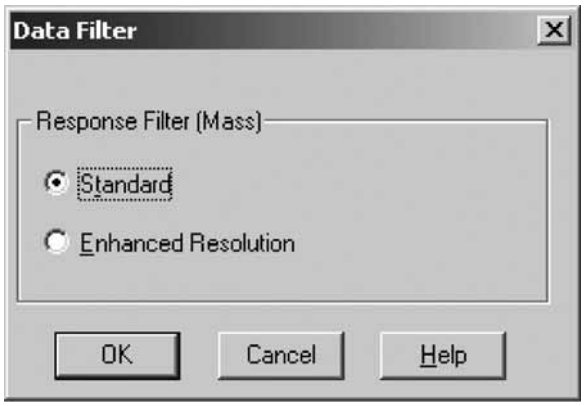

Figure 1e. Accept Standard.

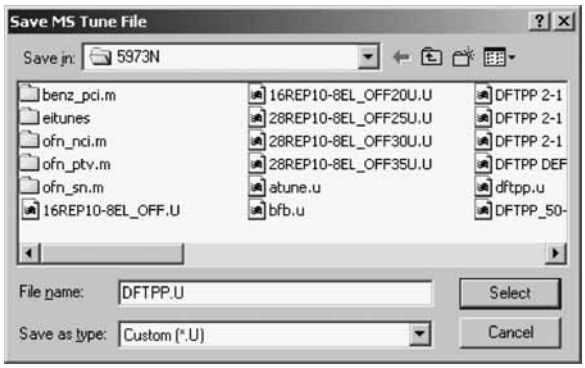

Figure 1f. Type in DFTPP.U if not present and click Select to save.

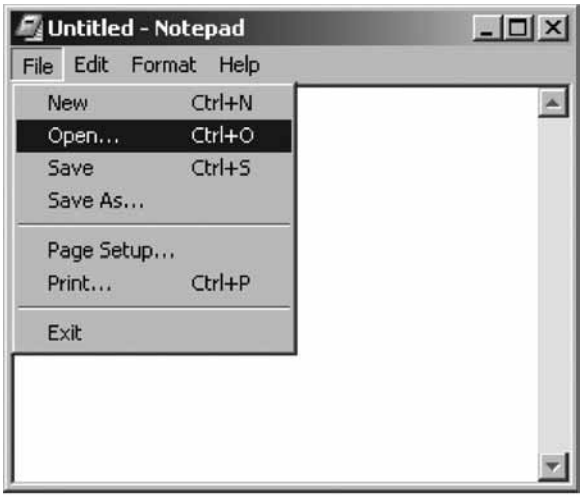

Figure 2a. Select File>Open in Notepad.

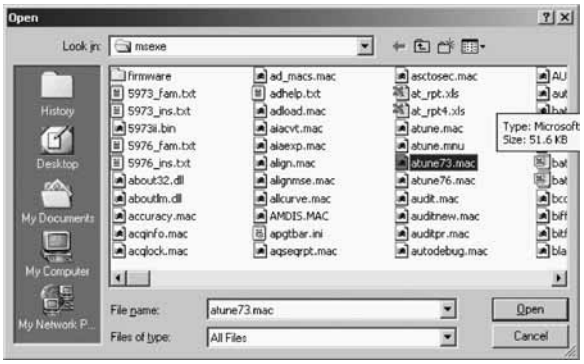

Figure 2b. Select atune.73.mac and click Open.

Figure 2c. Select Edit>Find.

Figure 2d. Type samples into the Find What box, then click Find Next.

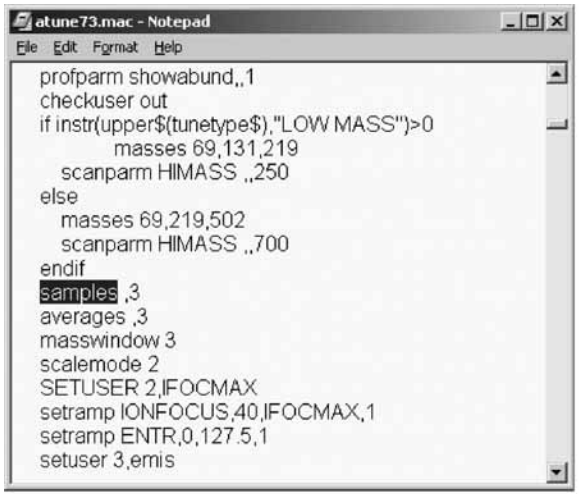

Figure 2e. Results of Find samples.

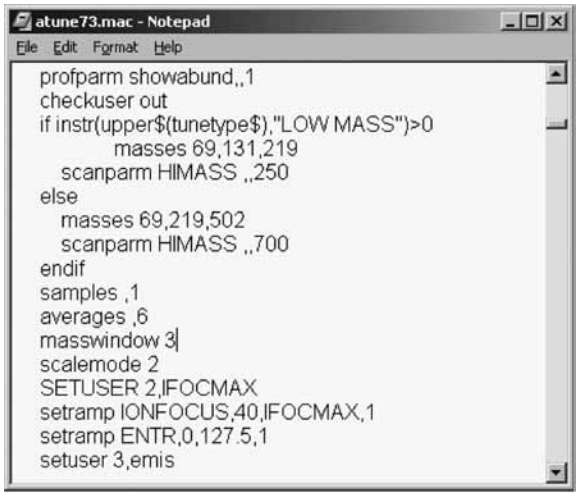

Figure 2f. Change samples from 3 to 1 and averages from 3 to 6.

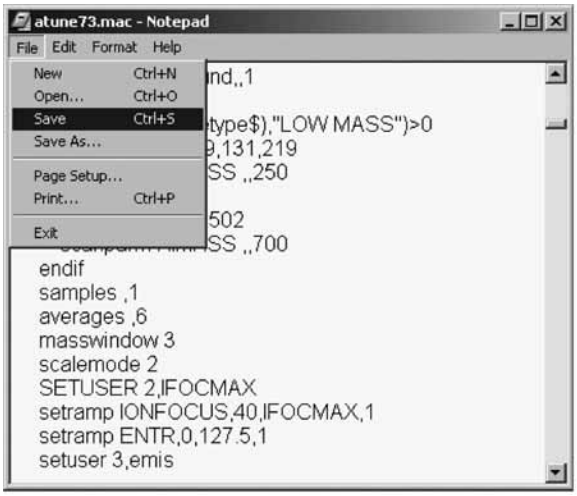

Figure 2g. Select File>Save (do not use Save as).

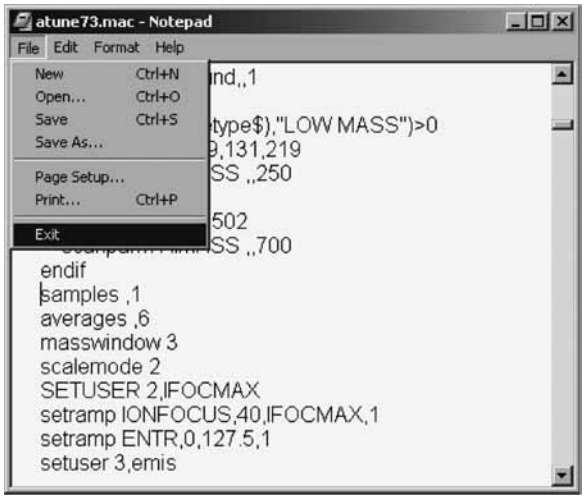

Figure 2h. Select File>Exit to close Notepad.

Previous work has shown improved linearity across a wide calibration range using a 25-µA emission current instead of the 35-µA default. The tuning macro was changed so that the sampling rate during tuning matched the sampling rate during data acquisition. The system was tuned at 2^1 and data were collected at 2^1. These changes resulted in reliably passing Method 8270 criteria on injection of DFTPP.

Remember that the tune macro changes are also reflected if an Autotune is done. The copy of atune73.mac contains the macro without the changes.

The sampling rate for data acqisition was changed-from the usual 2^2 to 2^1, while preserving sufficient sensitivity. The resultant 5.92 scans/s typically yield 10 data points across the peaks that have a width of 1.8 s.

Results

The system was calibrated at nine levels: 1, 2, 5, 20, 50, 80, 120, 160, and 200-ppm. The TIC (Total Ion Chromatogram) for the 5-ppm level is shown in Figure 3. The peak shape is excellent and the run time is less than 15 min. The benzo[b]fluoranthene and benzo[k]flouranthene resolution can be seen at about 11.4 min. Each calibration level contained 74 compounds together with 6 ISTDs at 40 ppm.

The RRF (relative response factor) was calculated automatically for each compound by the GC/MSD ChemStation software. Linearity was determined by calculating the %RSD (percent relative standard deviation) of the RRFs across the calibration range for each compound. This is also done automatically by the software in conjunction with Excel.

USEPA Method 8270D specifies criteria for suitable RRFs and %RSD. Minimum system performance is determined by four active compounds, the SPCCs (system performance check compounds) and is measured by the average RRF.

Table 2 lists the Method 8270D SPCC criteria and the performance of the 5973 inert. The 5973 *inert* data easily exceeds the 8270D criteria, and are very good considering the low end of the calibration range. This performance margin allows more samples to be run before system maintenance is necessary.

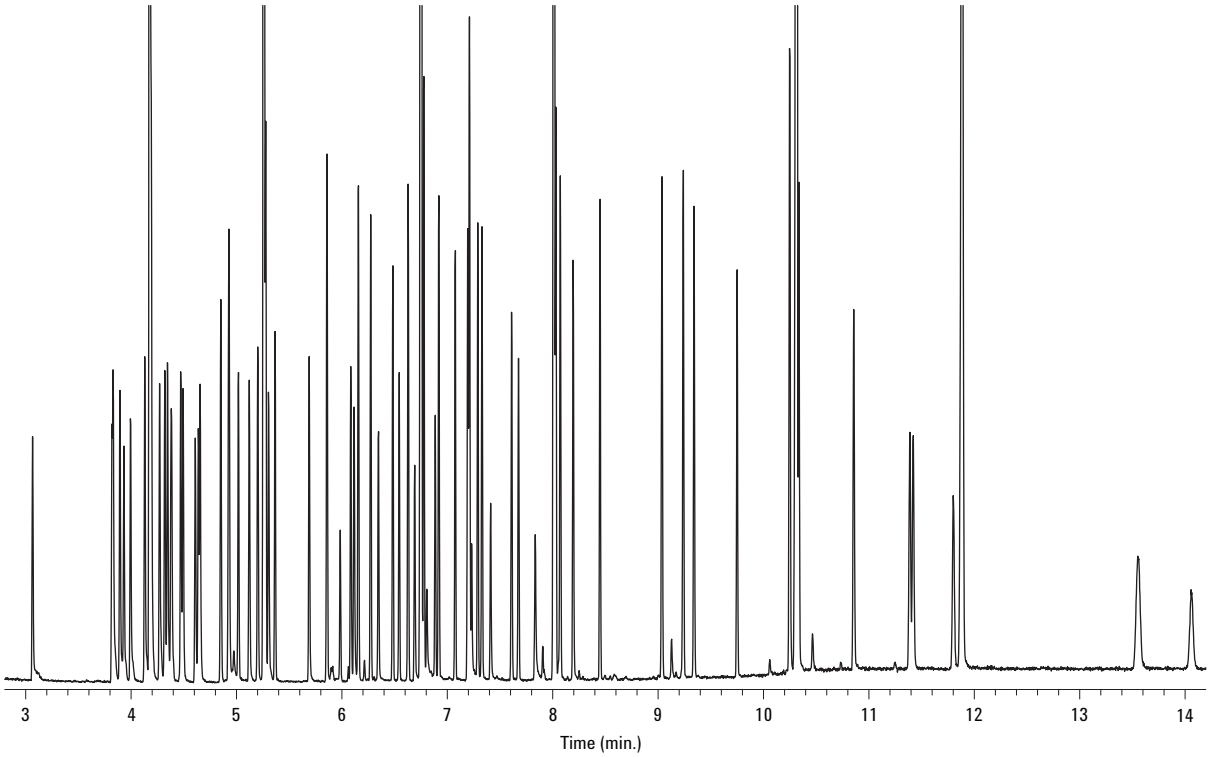

Figure 3. TIC for 5 ppm Method 8270 Semivolatiles.

Table 2. SPCCs and Comparison of Average RRF

	8270D Criteria	1–200 ng 5973 *inert*
N-Nitroso-di-n-propyl amine	0.050	0.963
Hexachlorocyclopentadiene	0.050	0.216
2,4-Dinitrophenol	0.050	0.133
4-Nitrophenol	0.050	0.139

Linearity is shown in Table 3. Method 8270D specifies that this group of Calibration Check Compounds (CCCs) meet a 30% RSD criteria. The %RSD is calculated across the RRFs determined at each calibration level. All CCCs pass criteria using a calibration range of 2–200 ppm. Across a 1–200 ppm range, pentachlorophenol does not pass due to its known activity.

Table 3. CCC %RSD of RRFs from 1–200 ppm and 2–200 ppm

	1–200	2–200
Phenol	6	6
1,4-Dichlorobenzene	7	6
2-Nitrophenol	6	6
2,4-Dichlorophenol	5	4
Hexachlorobutadiene	6	4
4-Chloro-3-methylphenol	5	5
2,4,6-Trichlorophenol	12	10
Acenaphthene	11	10
Diphenylamine	8	8
Pentachlorophenol	36	24
Fluoranthene	8	7
Benzo[a]pyrene	3	3

The excellent system linearity shown here is due to many factors including tuning, the large aperture drawout, and the Performance Electronics. The new electronics allow using a scan rate of 2^1, while maximizing sensitivity. This improved signal/noise together with more data points across a peak yields easier and more reproducible peak integration.

Conclusions

The Agilent 6890/5973 *inert* with Performance Electronics shows improved sensitivity at faster scan rates. The faster scan rates allow using 0.18 mm id columns for faster runs and shorter cool-down times. Analysis of 74 analytes and 6 ISTDs can be accomplished in less than 15 min. EPA Method 8270D tune criteria can be routinely achieved. SPCC performance and CCC linearity can be met over a wider calibration range than that historically used. Productivity increases are possible through shorter runs, faster cool-down, easier peak integration, and use of a wider calibration range.

References

1. M. Szelewski, B. Wilson and P. Perkins, “Improvements in the Agilent 6890/5973 GC/MSD System for Use with USEPA Method 8270”, Agilent Technologies, publication 5988-3072EN, www.agilent.com/chem.
2. M. Szelewski, “Fast Semivolatiles Analysis using the Agilent Technologies 6890/5973 *inert* GC/MSD”, Agilent Technologies, publication 5989-0207EN, www.agilent.com/chem.

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Printed in the USA
August 4, 2004
5989-1510EN

Agilent Technologies

Robustness of Eclipse PAH Columns for HPLC Analysis of Polycyclic Aromatic Hydrocarbons

Application

Environmental and Food

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Abstract

The Eclipse PAH (polycyclic aromatic hydrocarbon) column contains a rugged stationary phase suitable for a variety of PAH analyses. Their longevity, reproducibility, and scalability were demonstrated. The large number of available column configurations makes Eclipse PAH columns a desirable first choice to satisfy the chromatographer's unique PAH analytical requirements.

Introduction

Some HPLC column manufacturers offer a specific column for polycyclic aromatic hydrocarbon (PAH) analysis. However, because of the large number of PAHs (over 100 compounds) and broad range of PAH matrices (such as air, water, and food), many unique HPLC methods are needed, and cannot be developed on just one or two PAH column configurations. The more column lengths and diameters available, the more rugged, optimized methods can be developed.

More importantly, particle size, an additional column dimension, greatly expands method customization, meaning even more methods can be generated and optimized for a particular PAH application. Uniform chemistry between particle sizes allows methods to be scaled up or down with predictable results. Additional column possibilities are useful for such factors as sample size, sample matrix, detector choice, speed, resolution, and solvent use.

For a specialty column (bonded phase) to be useful for a multitude of similar methods it must demonstrate reproducibility between particle sizes. This allows straightforward, predictable method transfer, minimizing method redevelopment. Ideally, the column should also have longevity and reproducibility, including between manufacturing lots.

In this work we demonstrate that Eclipse PAH columns are robust: they have long life, reproducibility, and scalability.

Experimental

Eclipse PAH ruggedness was tested on an Agilent Rapid Resolution 1200 Series LC (RRLC) system that comprised:

- G1379 degasser

- G1312B binary pump SL
  - Mobile phase A: water, B: acetonitrile. See figures for gradient conditions.
  - When using 2.1-id columns, the pump was configured in the low delay volume mode, bypassing the static mixer and pulse dampener. See reference 1 for details about using low- and standard-volume binary pump configurations.
- G1367C HiP-ALS SL autosampler
- G1316B TCC SL thermal controlled column compartment
  - Set to 25 °C. When using 2.1-id columns, the low-volume (1.6-µL) heat exchanger (G1316-8002) was used in place of the built-in 3-µL one.
- G1315C diode array detector SL
  - Set at 220, 4 nm, no reference, with a G1315-60025 flow cell (5-µL volume), response time setting of 0.5 s

Eclipse PAH Columns Available

Part Number	Description
959764-918	Eclipse PAH, 2.1 mm × 100 mm, 1.8 µm
959793-918	Eclipse PAH, 2.1 mm × 100 mm, 3.5 µm
959763-918	Eclipse PAH, 2.1 mm × 150 mm, 3.5 µm
959701-918	Eclipse PAH, 2.1 mm × 150 mm, 5 µm
959790-918	Eclipse PAH, 2.1 mm × 250 mm, 5 µm
959741-918	Eclipse PAH, 2.1 mm × 50 mm, 1.8 µm
959990-318	Eclipse PAH, 3.0 mm × 250 mm, 5 µm
959964-918	Eclipse PAH, 4.6 mm × 100 mm, 1.8 µm
959961-918	Eclipse PAH, 4.6 mm × 100 mm, 3.5 µm
959996-918	Eclipse PAH, 4.6 mm × 100 mm, 5 µm
959963-918	Eclipse PAH, 4.6 mm × 150 mm, 3.5 µm
959993-918	Eclipse PAH, 4.6 mm × 150 mm, 5 µm
959990-918	Eclipse PAH, 4.6 mm × 250 mm, 5 µm
959931-918	Eclipse PAH, 4.6 mm × 30 mm, 1.8 µm
959941-918	Eclipse PAH, 4.6 mm × 50 mm, 1.8 µm
959943-918	Eclipse PAH, 4.6 mm × 50 mm, 3.5 µm

See figures for columns used.

The PAH mixture is a certified reference material from Agilent, PN 8500-6035, diluted in acetonitrile. Elution order for all figures:

1 Toluene
2 Naphthalene
3 Acenaphthylene
4 Acenaphthene
5 Fluorene
6 Phenanthrene
7 Anthracene
8 Fluoranthene
9 Pyrene
10 Benzo(a)anthracene
11 Chrysene
12 Benzo(b)fluoranthene
13 Benzo(k)fluoranthene
14 Benzo(a)pyrene
15 Dibenzo(a,h)anthracene
16 Benzo(g,h,i)perylene
17 Indeno(1,2,3-c,d)pyrene

Results and Discussion

The standard mixture of polynuclear hydrocarbons specified in the EPA method 610 for municipal and industrial wastewater evaluated the robustness of Eclipse PAH columns. EPA method 610 calls for a 2.6 mm × 250 mm, 5 µm ODS column and a water/acetonitrile gradient. Alternative columns are allowed if certain conditions are met [2]. Interestingly, reference 2, section 1.3, states that the LC method with its specified column does not resolve all 16 PAHs.

Eclipse PAH does resolve all 16 PAHs, even in a 5-µm, 250-mm long configuration (Figure 1). Note that the critical pair, peaks 4 and 5, is well resolved ($R_s > 2$). We chose this minimum resolution of the critical pair to define a successful robust method. Mobile phase was adjusted to obtain this resolution for all Eclipse PAH column configurations; thus, the wide range of gradient delay times between low and high flow rates would not be a concern when developing the separation on a different column dimension. The analysis in Figure 1 takes about 26 minutes on the long 250-mm column. The analysis was shortened over four-fold when 1.8-µm particles in a 50-mm long column were used (Figure 2). The resolution of the critical pair remains greater than 2, but analysis time was reduced to 6.8 min.

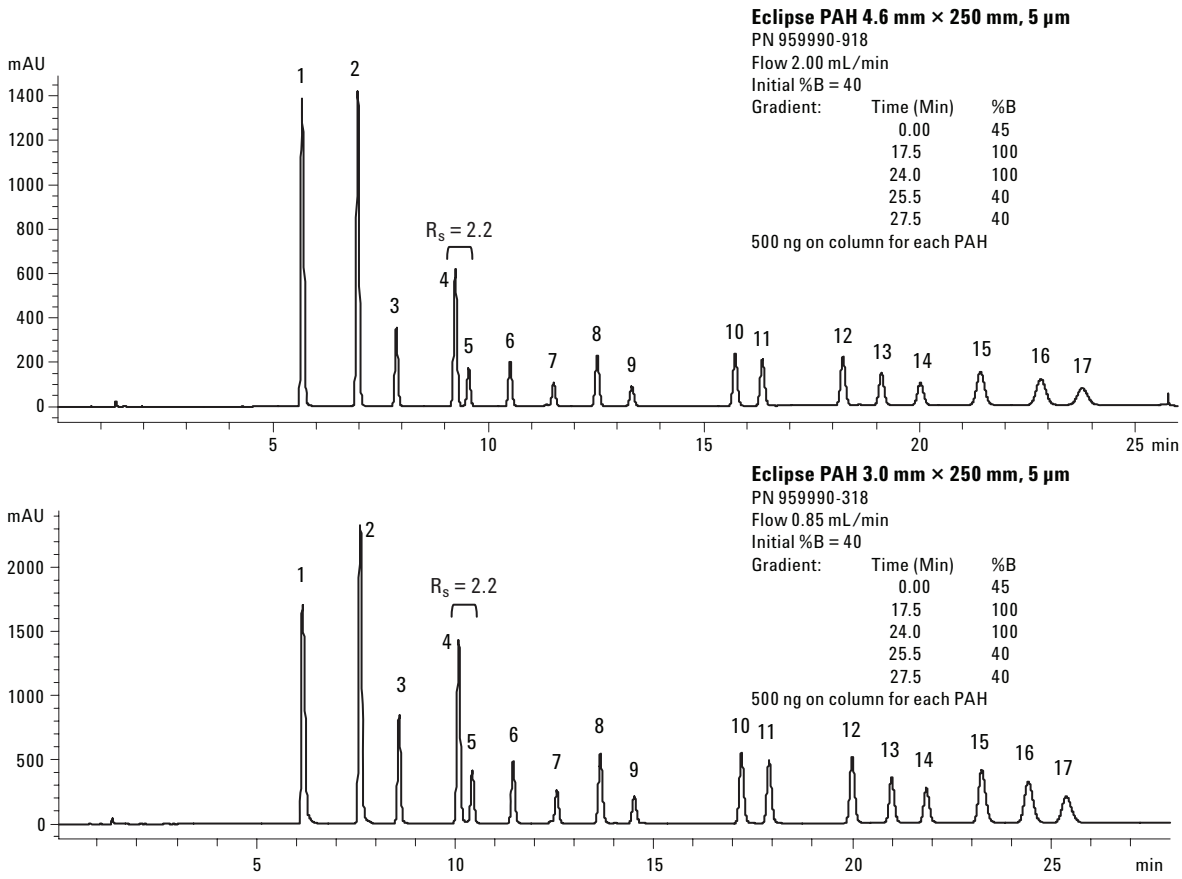

Figure 1. PAH analysis on Eclipse PAH 250-mm columns has high resolution.

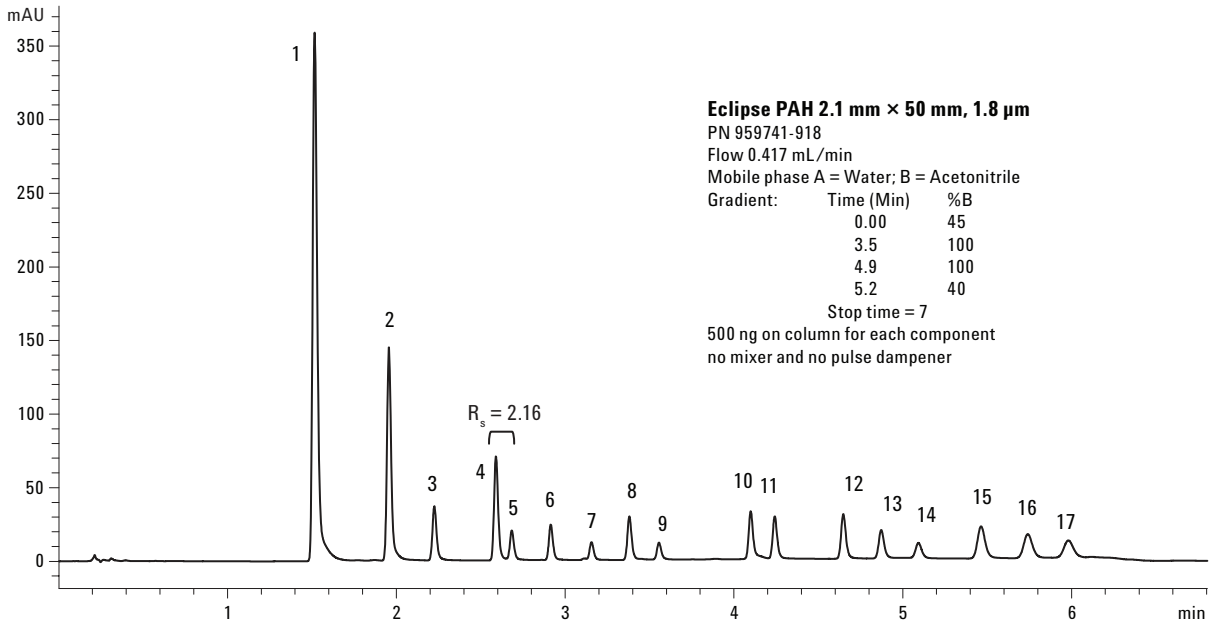

Figure 2. PAH analysis on RRHT Eclipse PAH 2.1 mm × 50 mm 1.8-µm column also has high resolution and faster analysis time.

Long Life and Reproducibility

Low solvent consumption and high throughput gained by using Rapid Resolution High Throughput (RRHT) columns such as the Eclipse PAH 2.1 x 50 mm in Figure 2 make RRHT columns ideal for column lifetime tests. We used the method in Figure 2 to test the longevity of Eclipse PAH columns. After 5,000 injections, the test was terminated with little loss in column performance. Figure 3 overlays chromatograms from the beginning, middle, and end of the life test. Selectivity, retention, and efficiency, and therefore resolution, remained relatively constant for 3,000 analyses and remained quite satisfactory for the next 2,000 injections. The table in Figure 3 lists the resolution factor values of the critical pair and a wider spaced pair and supports the method and column robustness. The test took 25 days of 24-hour operation and generated roughly 14.6 L of solvent waste. If a 4.6 x 250 mm column had been used, the test would have taken 122 days of nonstop operation and about 350 L of solvent would have been consumed.

Batch-to-Batch Reproducibility

Long column life is an important feature of Eclipse PAH; another necessity is batch-to-batch reproducibility. Besides 5- and 1.8-µm particles, Eclipse PAH is also available in 3.5 µm. Figure 4 compares two batches of 3.5-µm Eclipse PAH material made at different times. Note that the selectivity is identical between the batches, supporting the claim that manufacture of the Eclipse PAH particles is uniform. Similar results were obtained from 5- and 1.8-µm material (data not shown). Each batch of material is specifically tested with PAHs for maximum reproducibility under expected operating conditions.

Scalability Between Particle Sizes

Batch-to-batch reproducibility can be broadened to particle-size-to-particle-size reproducibility, or scalability, to fully appreciate a column's robustness. Figure 5 overlays three different particle-size columns (by definition, three distinct batches as well). Additionally, the columns comprise three lengths and two diameters. Selectivity is the same, however, for all three column configurations. This is because selectivity is related to the nature of the particle surface, not to column length or diameter. The uniform selectivity between particle sizes, or scalability, contributes to Eclipse PAH's robustness.

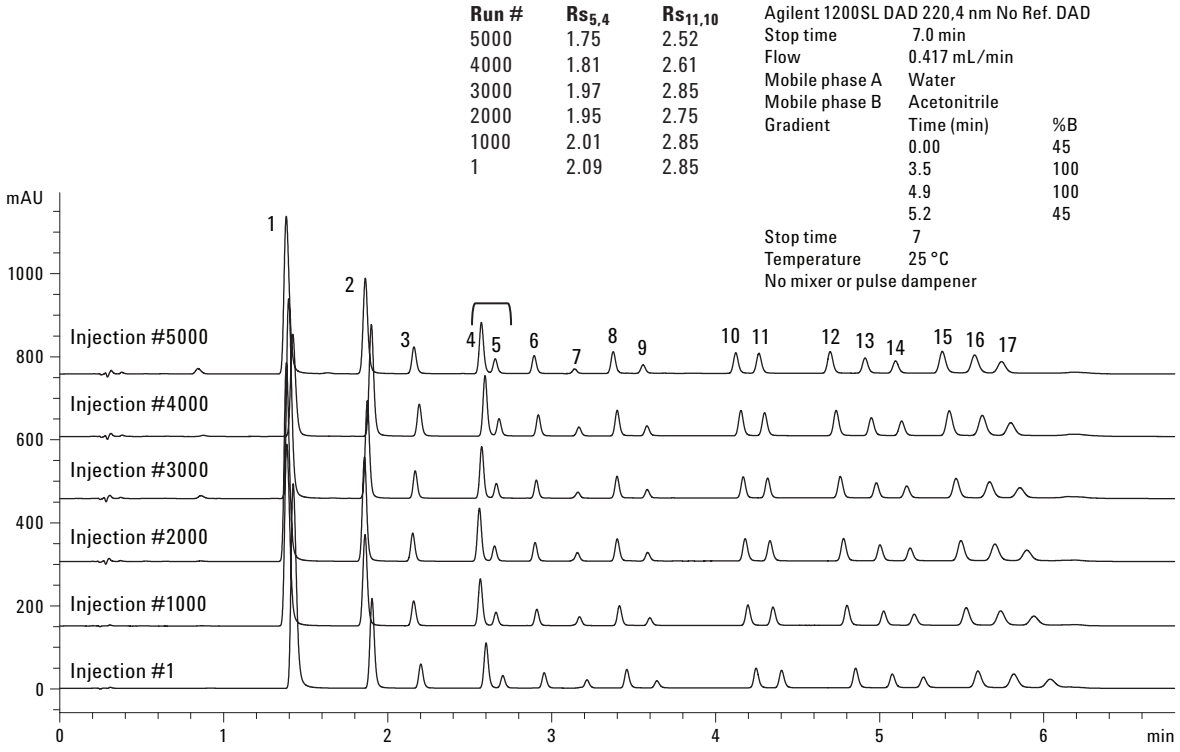

Figure 3. Life test of Eclipse PAH 2.1 x 5 mm, 1.8 µm. See the Experimental Section for peak identification.

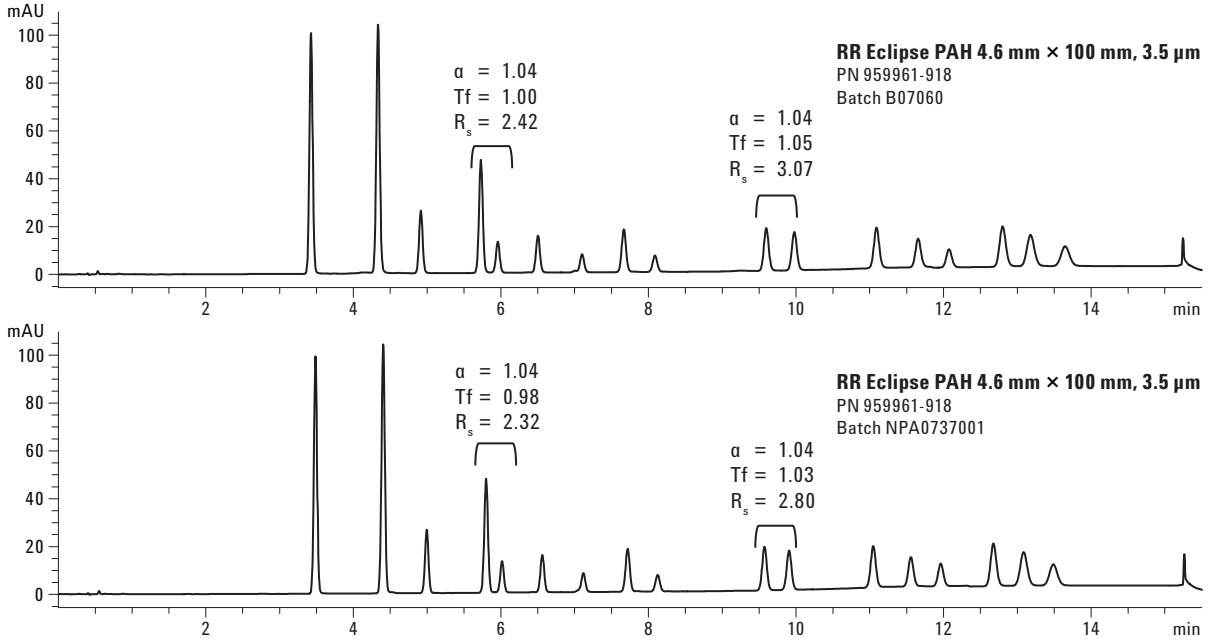

Figure 4. Batch-to-batch reproducibility of Eclipse PAH 3.5-µm material.

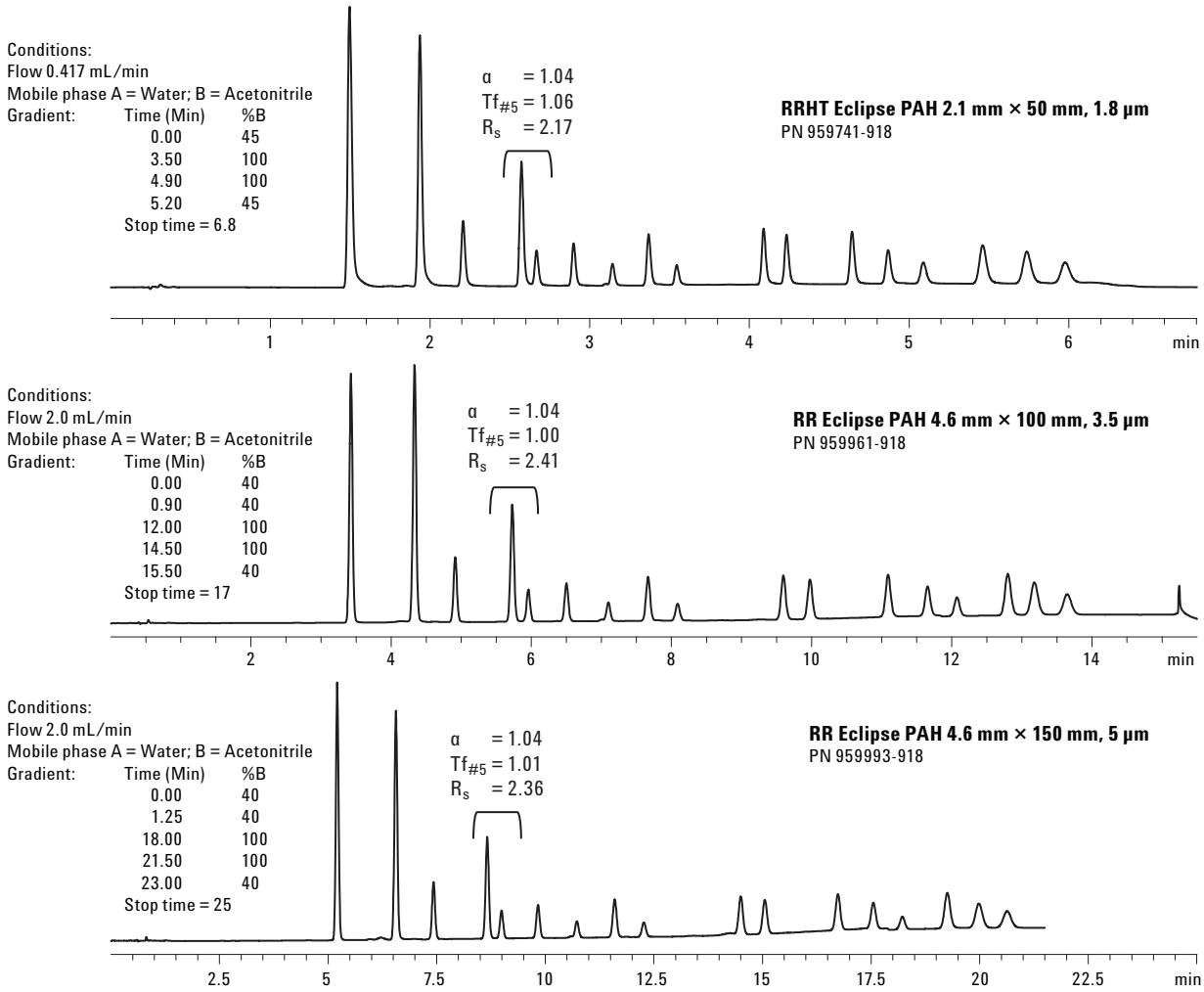

Figure 5. Scalability of Eclipse PAH columns of various particle sizes and column dimensions.

Conclusions

Eclipse PAH is a suitable rugged stationary-phase column for a wide variety of PAH analyses. Column longevity, reproducibility, and scalability were demonstrated. The large number of column configurations makes Eclipse PAH columns a first choice for method optimization. Available Eclipse PAH column dimensions allow method customization regarding sample size, matrix, detector type, analysis speed, resolution requirements, and solvent consumption.

References

1. "High-Throughput Gradient Optimization by Easily Minimizing Delay Volume," Agilent Publication 5989-6665EN (2007)
2. Appendix A to Part 136, Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater, Method 610 Polynuclear Aromatic Hydrocarbons, United States Environmental Protection Agency

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Printed in the USA
January 18, 2008
5989-7828EN

Agilent Technologies

Semivolatiles Retention Time Locked (RTL) Deconvolution Databases for Agilent GC/MSD Systems

Application

Environmental

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Abstract

The G1677AA Semivolatiles Retention Time Locked database/library can provide rapid confirmation of environmental contaminants in complex matrices when used with Deconvolution Reporting Software. Separate methods and databases are included for wastewater and drinking water, with locked retention times. Compound lists are based on U.S. EPA Method 8270 (273 compounds) and Method 525 (119 compounds). Acquisition methods for both splitless and programmable temperature vaporizing inlets are provided. Full spectra are used for identification of deconvoluted analytes, not just a few extracted ions. When used with MSD ChemStation Rev E.02 or later, quantitation of the deconvoluted data from AMDIS is possible, in addition to the normal quantitation. The G1677AA Environmental Semivolatiles retention time locking database/library is an add-on product to the base deconvolution reporting software product G1716AA.

Introduction

Agilent Deconvolution Reporting Software (DRS) is a software package that combines the information from three separate processes into one easy-to-read report: 1) MSD ChemStation identification and quantitation, 2) industry standard AMDIS deconvolution with full spectrum identification 3) NIST full spectrum Search. The primary benefit of DRS is significant time savings when interpreting results from complex matrix analyses.

Target compound identification and quantitation in environmental samples is often a tedious task and is therefore well suited to DRS. The list of target compounds varies widely, depending on geographic region, government requirements, and sample type. There is no universal list of analytes tied to specific methods that will satisfy all laboratories all of the time.

The United States Environmental Protection Agency (U.S. EPA) has published numerous GC/MSD methods for organic analytes in various matrices. U.S. EPA Method 525 is specified for drinking water and Method 8270 for wastewater, each having its own set of compounds. The compounds lists for these methods are extensive and form the basis for the deconvolution databases discussed in this note. Laboratories are not required to follow the U.S. EPA methods exactly to use the databases effectively. Compounds/spectra can easily be added by users to the databases for suitability in their own labs.

Databases/Libraries

Collections of mass spectral data are referred to as libraries or databases. DRS uses the combined general description of database/library (DBL). Retention time is a critical component of sample identification, and the compounds in these DBLs have been acquired using retention time locking (RTL).

The G1677AA Environmental Semivolatiles RTL DBL is a set of mass spectral libraries in the Agilent and NIST/AMDIS formats. There are three

separate sets of files and methods. An 8270 set includes the mass spectra and locked retention times for 243 single-component semivolatile compounds and internal standards specified by U.S. EPA Method 8270, plus 30 additional compounds of environmental interest – a total of 273 compounds. Two different 525 sets are included, one optimized for a split/splitless inlet and one optimized for a PTV inlet, designated as "long." Each 525 set includes mass spectra and locked retention times for the same 119 single-component semivolatile compounds and internal standards specified by U.S. EPA Method 525. A complete listing of the DBL compounds can be found in Appendix A.

Each DBL entry contains the following information:

- Mass spectrum acquired using Atune.u on a 5975 MSD
- Locked retention time determined on a 6890N/5975 or 7890A/5975 GC/MSD system. Compounds were injected at least once with phenanthrene-d10 locked to 9.500 ± 0.01 minutes. Phenanthrene-d10 was locked to 12.700 ± 0.01 minutes for the 525 PTV "long" method.
- Molecular formula
- Molecular weight (nominal mass)
- CAS number

Spectra were compared to those contained in the NIST05a Mass Spectral Library. Tests were performed on spiked samples containing hydrocarbon interferences and the results were compared to the list of spiked compounds.

Minimum system requirements for using the semi-VOAs DBLs

- G1716AA deconvolution reporting software, base product
- Agilent GC/MS system with E.02.00.xxx software preferred

Experimental

The instrument operating conditions used for acquiring the 8270 and 525 spectra and retention times are listed in Table 1. Splitless injection utilizing a split/splitless inlet with the column connected directly to the MSD was done. The thicker film 20-m column allows good separation of the early eluters from the solvent while providing a short run time of 17 min. An inlet temperature of 300 °C is optimum for later eluting PAHs but basic compounds, such as benzidine, have improved response factors at lower inlet temperatures, 250 to 275 °C. Retention time locking to phenanthrene-d10 at 9.500 min was done in constant-flow mode, at 0.8 mL/min. The mass range of 35 to 500 is suitable for both lists of compounds. A 6-mm large-aperture drawout lens provides better high-end linearity at the expense of some sensitivity loss. The standard 3-mm lens could be used.

Table 1. 8270 and 525 Methods – Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890N or 7890A		
Inlet	EPC split/splitless – rear location		
Mode	Splitless, 0.5 µL injected		
Inlet temperature	300 °C		
Pressure	16.9 psi initial		
Purge flow	30.0 mL/min		
Purge time	0.75 min		
Gas saver	Off		
Gas type	Helium		
Liner	Agilent helix, single-taper, p/n 5188-5397		
Oven	**240V**		
Oven ramp	°C/min	Next °C	Hold min
Initial		40	1.00
Ramp 1	25	320	4.80
Total run time	17.0 min		
Equilibration time	0.5 min		
Oven max temp.	325 °C		
Column	Agilent Technologies DB-5.625, p/n 121-5622		
Length	20.0 m		
Diameter	0.18 mm		
Film thickness	0.36 µm		
Mode	Constant flow = 0.8 mL/min		
Pressure	16.7 psi initial		
Inlet	Rear		
Outlet	MSD		
Outlet pressure	Vacuum		
RTL	System retention time locked to phenanthrene-d10 at 9.500 min		
MSD	Agilent Technologies 5975, performance turbo		
Drawout lens	6-mm large-aperture drawout lens p/n G2589-20045		
Solvent delay	2.8 min		
Tune	Atune.u		
EM voltage	Tune voltage		
Mass range	35 to 500 amu		
Threshold	0		
Sampling	2		
Quad temperature	180 °C		
Source temperature	300 °C		
Transfer line temp.	280 °C		

The instrument operating conditions used for acquiring the 525 "long" retention times are listed in Table 2. A programmable temperature vaporizing inlet (PTV) was used with a 25-µL large volume injection (LVI). PTV-LVI is popular in labs requiring lower detection limits for drinking water. Active analytes have improved performance as they

vaporize at the lowest possible temperature compared to a hot splitless injection. Phenanthrene-d10 is used as the retention time locking compound at 12.700 min running in constant-flow mode at 1.5 mL/min. The PTV parameters are not directly transferrable to the 20-m column used in Table 1, without affecting retention times. The 30-m column provides better separation for SIM analysis, allowing more ions/compound or more SIM cycles/peak than a shorter run. Users can build their own SIM-based DBLs with a requirement of 4 ions/compound for best identification with deconvolution. Alternatively, SIM/scan data acqusition could be done with the SIM data used for quantitation and the scan data used for full-spectrum deconvolution. The 45 to 450 scan range is suitable for the 525 DBL. The sampling rate of two can be changed to one to maintain an equal number of scan cycles if SIM/scan is used.

Table 2. 525 Long Method – Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890N or 7890A		
Inlet	EPC PTV – front location		
Mode	Solvent vent – 25 uL injected		
Temp ramp	°C/min	Next °C	Hold min
Initial		20	0.60
Ramp 1	600	350	1.30
Ramp 2	10	250	0.00
Cryo	On		
Cryo use temperature	100 °C		
Cryo timeout	10.00 min (On)		
Cryo fault	On		
Pressure	11.77 psi (On)		
Vent time	0.60 min		
Vent flow	100.0 mL/min		
Vent pressure	0.0 psi		
Purge flow	50.0 mL/min		
Purge time	2.50 min		
Total flow	53.9 mL/min		
Gas saver	Off		
Gas type	Helium		
PTV Liner	Agilent multi-baffle liner, no packing, p/n 5183-2037		
Oven	**120V**		
Oven ramp	°C/min	Next °C	Hold min
Initial		40	2.50
Ramp 1	50	110	0.00
Ramp 2	10	320	1.10
Total run time	26 min		
Equilibration time	0.5 min		
Oven max temperature	325 °C		
Column	Agilent Technologies HP 5 MSi, p/n 19091S-433i		
Length	30.0 m		
Diameter	0.25 mm		
Film thickness	0.25 µm		
Mode	Constant flow – 1.5 mL/min		
Pressure	11.77 psi		
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
RTL	System retention time locked to phenanthrene-d10 at 12.700 min		
Front Injector			
Sample washes	0		
Sample pumps	2		
Injection volume	25 microliters		
Syringe size	50 microliters		
PreInj. Solv A washes	0		
PreInj. Solv B washes	1		
PostInj. Solv A washes	2		
PostInj. Solv B washes	2		
Viscosity delay	1 second		
Plunger speed	Variable		
Injection speed	50 microliters/minute		
Draw speed	600 microliters/minute		
Dispense speed	6000 microliters/minute		
PreInjection dwell	0 minutes		
PostInjection dwell	0 minutes		
MSD	Agilent Technologies 5975C, performance turbo		
Drawout lens	6-mm large-aperture drawout lens p/n G2589-20045		
Solvent delay	4 min		
Low mass	45 amu		
High mass	450 amu		
Threshold	0		
Sampling	2		
Quad temperature	180 °C		
Source temperature	300 °C		
Transfer line temperature	280 °C		
Tune type	Autotune		
EM voltage	Tune voltage, 1247 V		

Results and Discussion

Retention Time Locking – or Not

Maximum productivity from DRS is realized if the GC/MSD system is retention time locked. The DRS report displays RT differences of found targets from their expected RTs, which is important for differentiating compounds with similar spectra. AMDIS parameters can be set to exclude compounds found outside their expected RT windows, which eliminates false positives. Retention time locking also eliminates the need to change SIM acquisition times, a tedious task with multiple SIM groups. Therefore, it is strongly recommended that users run with an RTL system.

Although the majority of labs run RTL, some users may choose not to do so. Two different approaches can be used in this case, each with limited success.

Approach 1 – Updating the *.cal File

The *.cal file establishes the relationship between retention times found on any given day to those

expected in the AMDIS databases (*.msl and *.cid). For the semivolatile DBL the *.cal files contain only ISTDs and surrogates. It is assumed that other analytes will track the retention time changes of these compounds. Whenever RTs change, the *.cal file RTs must be changed. This can be done by manually editing the RTs in the *.cal file using Notepad. Once all RTs have been updated, select Save, not Save As. A second choice is to have AMDIS rebuild the *.cal file. The procedure for this is in AMDIS Help.

Approach 2 – Updating the *.msl and *.cid Files

A menu item is provided in MSD data analysis, DRS > Update AMDIS Library RTs using quant database. This will update the RTs in both necessary AMDIS files using the current MSD quant database times and will save a copy of the original two AMDIS files.

Cautions: If the quant database contains an incorrect time, that time will be used. If the quant database does not contain a compound that is in the AMDIS files, AMDIS RTs will not be updated.

Using the Semivolatiles RTL DBL

The following files are installed:

8270_DRS_Demo.D	525_DRS_Demo.D	525_Long_DRS_Demo.D
8270_DRS.L	525_DRS.L	525_Long_RTL_DRS.M
8270_RTL_DRS.M	525_RTL_DRS.M	525_Long.MSL
8270.MSL	525.MSL	525_Long.CID
8270.CID	525.CID	525_Long_cal_RT.CAL
8270_cal_RT.CAL	525_cal_RT.CAL	525_Long_cal_RT.CSL
8270_cal_RT.CSL	525_cal_RT.CSL	

The typical locations into which the CD installer places the files are as follows:

Agilent MSD ChemStation datafiles *.D and Methods *.m in
C:\msdchem\MSDemo\Semivolatiles Example Data
C:\msdchem\MSDemo\Semivolatiles Example Methods

AMDIS files *.msl, *.cid, *.cal, and *.csl in
C:\NIST05\AMDIS32\LIB

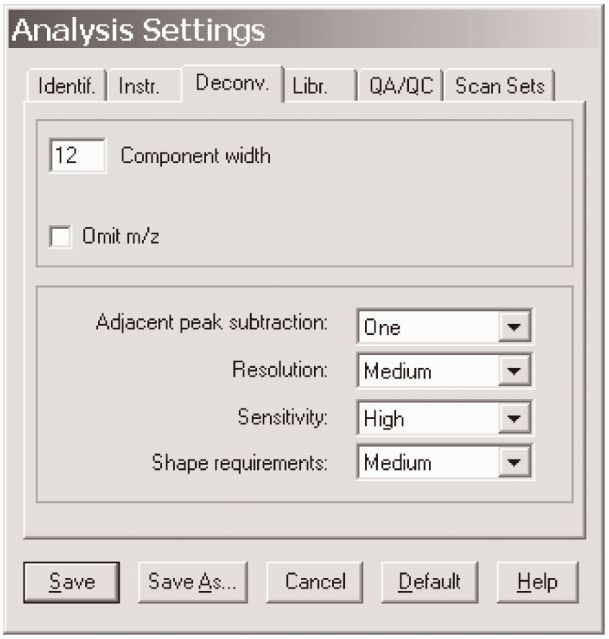
MSD ChemStation Library Files, *.L in **C:\Database**

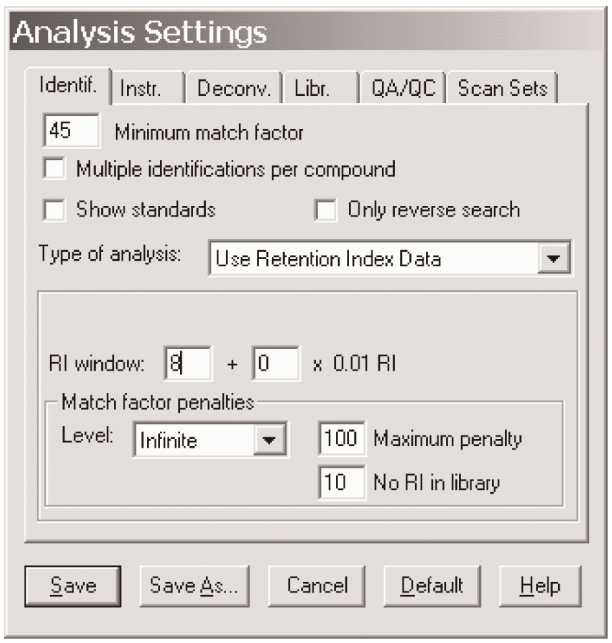
The MSD ChemStation methods contain a retention time locked quant database with single-point calibration. These methods can be used directly in data analysis, as described later. The methods also contain data acquisition parameters and retention time locking data. Users may have to resolve differences between their system and the method configuration upon loading the method in data acquisition. Additionally, new retention time locking data may have to be acquired. In most cases users will only have to relock their system if it is configured the same as the system in Table 1 or Table 2.

DRS in MSD Data Analysis

It is strongly recommended that an operator inexperienced in DRS first proceed to the General Help file section “Generating and Interpreting A Report Using DRS Manually/Interactively.” Complete all of the the Spinach A, B and C exercises for the best fundamental understanding of DRS. Then proceed as follows.

Open AMDIS as a standalone application and then select Analyze > Settings. Verify that the settings are as shown below, then select Save. If prompted to reanalyze, select No, then exit AMDIS. The settings will be permanently saved in the AMDIS initialization file onsite.ini.



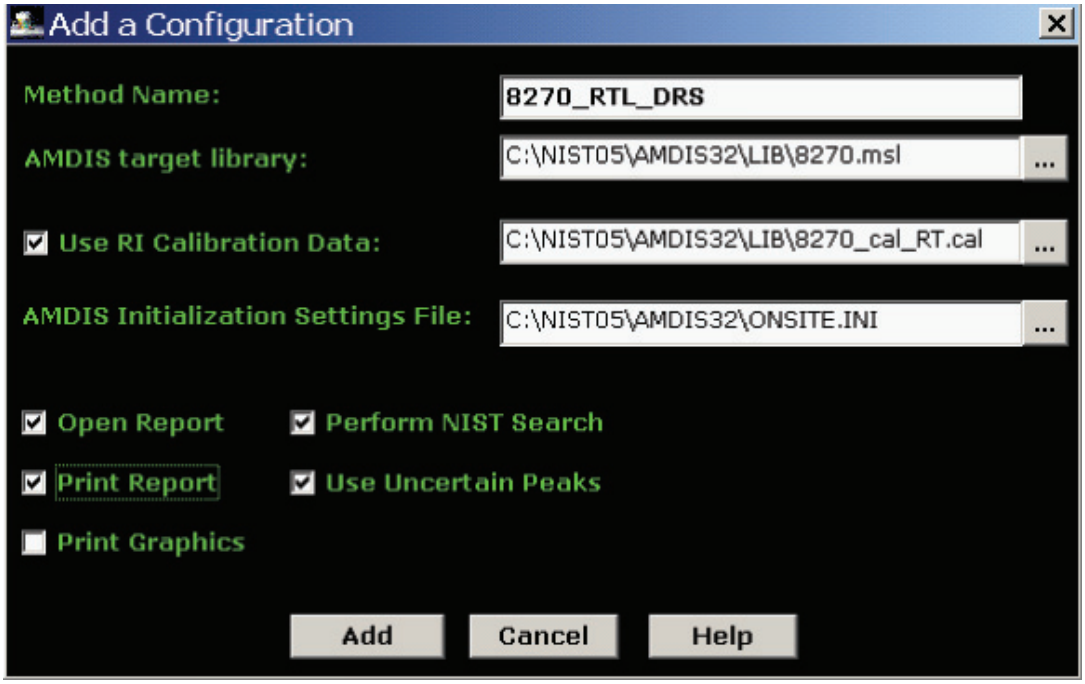


After AMDIS settings have been addressed, it will be necessary to configure a DRS method, depending on the analysis chosen. The relationship of the AMDIS and MSD ChemStation files are shown in the Table below.

MSD ChemStation method	MSD ChemStation select library	DRS configurator method	DRS configurator AMDIS target library	DRS configurator RI calibration file	DRS configurator AMDIS .ini file
8270_RTL_DRS.m	8270_DRS.L	8270_RTL_DRS	8270.msl	8270_cal_RT.cal	Onsite.ini
525_RTL_DRS.m	525_DRS.L	525_RTL_DRS	525.msl	525_cal_RT.cal	Onsite.ini
525_long_DRS.m	525_DRS.L	525_long_DRS	525_long.msl	525_long_cal_RT.cal	Onsite.ini

A DRS method must be configured for each of the applications that will be used. Let's look at one example of using the semivolatiles DBL, in this case the 8270 set.

1. Configure a NEW DRS Method using the Method Configurator as shown below.



2. Select Add then Exit > Exit and Save.
3. In the MSD ChemStation, Data Analysis View, Load Method 8270_RTL_DRS.m.
4. Load datafile 8270_DRS_Demo.D.
5. From the Data Analysis View DRS menu, select Quant + DRS Single File.

At the end of the DRS process a report similar to the one shown should be generated.

MSD Deconvolution Report
Sample Name: 5ppm 8270sm + 50GKD
Data File: C:\msdchem\1\DATA\8270_D~1.D
Date/Time: 05:00 PM Monday, Dec 17 2007

Adjacent Peak Subtraction = 1
Resolution = Medium
Sensitivity = High
Shape Requirements = Medium

The NIST library was searched for the components that were found in the AMDIS target library.

			Amount (ng)		AMDIS		NIST	
R.T.	Cas #	Compound Name	Chem station	AMDIS	Match	R.T. Diff sec.	Reverse Match	Hit Num.
2.8499	62759	N-Nitrosodimethylamine	5.56		93	–0.5	96	1
4.7302	62533	Aniline			98	4.2	96	1
5.0358	3855821	1,4-Dichlorobenzene-d4	40		99	–0.2	93	1
5.2143	106445	4-Methylphenol			66	–7.7	89	1
5.3059	98862	Acetophenone			49	–4.6		
5.3059	105055	Benzene, 1,4-diethyl-					89	1
6.2476	1146652	Naphthalene-d8	40		99	–0.1	86	1
6.2679	91203	Naphthalene	3.77		58	–0.1		
6.2679	5405798	3-Hexanone, 2,2-dimethyl-					81	1
6.787	680319	Hexamethylphosphoramid	0.28					
6.9442	91576	2-Methylnaphthalene			97	0.1	91	1
7.761	95830	4-Chloro-1,2-phenylenediamine	0.69					
7.845	5131602	4-Chloro-1,3-phenylenediamine	0.1					
7.9945	15067262	Acenaphthene-d10	40		98	–0.1	82	1
8.0256	51285	2,4-Dinitrophenol			58	0.0	68	1
8.0572	100027	4-Nitrophenol	2.44		81	–0.1	92	1
8.195	132649	Dibenzofuran	0.11					
8.3833	84662	Diethyl phthalate	0.23		78	–0.2	75	1
8.530	99558	5-nitro-o-toluidine	0.11					
8.5420	86737	Fluorene			56	0.0	80	58
8.5644	534521	4,6-Dinitro-2-methylphenol			89	–0.1	88	1
8.6164	86306	N-Nitrosodiphenylamine			45	–0.8		
8.6164	3892000	Pentadecane, 2,6,10-trimethyl-					84	1
9.2806	92671	4-Aminobiphenyl	6.1		94	–0.1	89	2
9.2829	87865	Pentachlorophenol	4.22		91	–0.1	66	11
9.4964	1517222	Phenanthrene-d10	40		97	–0.1	86	1
9.5139	120127	Anthracene			52	–3.4	70	15
9.5175	85018	Phenanthrene			68	–0.2	80	1
10.0286	84742	di-n-Butyl phthalate			74	–0.1	84	9
10.8285	92875	Benzidine	5.67		97	0.8	91	1
12.1025	91941	3,3'-Dichlorobenzidine	5.3		95	–0.4	97	1
12.1665	1719035	Chrysene-d12	40		93	–0.3	92	1
12.168	56553	Benz[a]anthracene	0.06					
12.168	218019	Chrysene	0.08					
12.168	732116	Phosmet	0.27					
13.3443	207089	Benzo[k]fluoranthene			93	–2.4	94	2
13.3443	205992	Benzo[b]fluoranthene	5.75		95	–0.2	90	2
13.381	207089	Benzo[k]fluoranthene	5.89					
13.8809	1520963	Perylene-d12	40		99	–0.6	77	1

This report is based on DRS revision A.04. Previous DRS revisions do not have AMDIS settings in the header, nor do they have a column for the amount calculated from AMDIS. The AMDIS calculated amount will be available after using QEdit in MSD ChemStation, Rev E.02 and later. Please consult the DRS A.04 Help section “Using QEdit with DRS Quantitative Data” for details.

The user can also configure DRS methods for either of the 525 sets of files, similar to that shown above for the 8270 set. Methods and demo datafiles are provided.

Conclusions

The Semivolatiles RTL DBL can provide rapid confirmation of environmental contaminants in complex matrices when used with DRS. Separate databases are provided for wastewater and drinking water, with locked retention times. Full spectra are used for identification of deconvoluted analytes. When used with MSD ChemStation Rev E.02 or later, quantitation on the deconvoluted data from AMDIS is possible, in addition to the normal quantitation. The G1677AA Environmental Semivolatiles RTL DBL is an add-on product to the base DRS product G1716AA.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

Appendix A

Lists of Compounds

Combined alphabetical listing of compounds from both the 8270_DRS.L and the 525_DRS.L, including CAS number and library entry number. Italics indicate the additional 30 compounds in the 8270 DBL. Retention time information can be found in the method quant databases, the Agilent libraries, or the AMDIS databases.

Compound name	CAS #	8270_DRS.L entry #	525_DRS.L entry #
Acenaphthene	83329	71	
Acenaphthene-d10	15067262	55	1
Acenaphthylene	208968	69	12
Acetophenone	98862	26	
2-Acetylaminofluorene	53963	185	
1-Acetyl-2-thiourea	591082	271	
Alachlor	15972608		54
Aldrin	309002	137	61
Ametryn	834128		55
2-Aminoanthraquinone	117793	267	
Aminoazobenzene	60093	253	
4-Aminobiphenyl	92671	111	
3-Amino-9-ethylcarbazole	132321	264	
Anilazine	101053	141	
Aniline	62533	12	
o-Anisidine	90040	240	
Anthracene	120127	124	43
Aramite	140578	155	
Atraton	1610179		31
Atrazine	1912249		35
Azinphos-methyl	86500	199	
Azobenzene (conv: 1,2-diphenylhydrazine)	103333	93	
Barban	101279	160	
Benz[a]anthracene	56553	193	103
Benzidine	92875	149	
Benzo[a]pyrene	50328	216	114
Benzo[b]fluoranthene	205992	212	112
Benzo[ghi]perylene	191242	223	119
Benzo[k]fluoranthene	207089	213	113
Benzoic acid	65850	37	
p-Benzoquinone	106514	238	
Benzyl alcohol	100516	18	
alpha-BHC (alpha-HCH)	319846	104	28
beta-BHC (beta-HCH)	319857	109	38
delta-BHC (delta-HCH)	319868	123	47
gamma-BHC (lindane)	58899	115	39
Bis(2-chloroethoxy)methane	111911	39	
Bis(2-chloroethyl) ether	111444	13	
Bis(2-chloroisopropyl) ether	108601	21	
Bis(2-ethylhexyl)phthalate	117817	191	108
Bromacil	314409		58
4-Bromophenyl phenyl ether	101553	103	
Bromoxynil	1689845	97	
Butachlor	23184669		79
Butifos	78488		85
Butyl benzyl phthalate	85687	173	95
Butylate	2008415		8
Captafol	2425061	186	
Captan	133062	142	
Carbaryl	63252	129	
Carbazole	86748	126	
Carbofuran	1563662	110	
Carbophenothion	786196	176	
Carboxin	5234684		88
Chlordane (NOS)	57749	252	
alpha-Chlordane	5103719		80
gamma-Chlordane	5103742		73
Chlorfenvinphos	470906	145	
4-Chloroaniline	106478	44	
Chlorobenzilate	510156	164	91
2-Chlorobiphenyl	2051607	73	16
5-Chloro-2-methylaniline	95794	256	
4-Chloro-3-methylphenol	59507	51	
3-(Chloromethyl)pyridine	3099318	276	
1-Chloronaphthalene	90131	270	
2-Chloronaphthalene	91587	62	
Chloroneb	2675776		17
2-Chlorophenol	95578	15	
4-Chloro-1,2-phenylenediamine	95830	260	
4-Chloro-1,3-phenylenediamine	5131602	259	
4-Chlorophenyl phenyl ether	7005723	84	

Compound name	CAS #	8270_DRS.L entry #	525_DRS.L entry #
Chlorothalonil	1897456		49
Chlorpropham	101213		26
Chlorpyrifos	2921882		63
Chrysene	218019	194	105
Chrysene-d12	1719035	153	99
Coumaphos	56724	208	
p-Cresidine	120718	237	
Crotoxyphos	7700176	146	
Cyanazine	21725462		64
Cycloate	1134232		25
2-Cyclohexyl-4,6-dinitro-phenol	131895	266	
Dacthal (DCPA)	1861321		66
4,4'-DDD	72548	167	93
4,4'-DDE	72559	154	86
4,4'-DDT	50293	179	98
Demeton-O	298033	251	
Demeton-S	126750	107	
Diallate	2303164	100	
2,4-Diaminotoluene	95807	268	
Diazinon	333415		44
Dibenz[a,h]anthracene	53703	222	118
Dibenz(a,j)acridine	224420	250	
Dibenzofuran	132649	77	
Dibenzo(a,e)pyrene	192654	249	
Dibrom (naled)	300765	94	
1,2-Dibromo-3-chloropropane	96128	275	
Dichlone	117806	116	
1,2-Dichlorobenzene	95501	19	
1,3-Dichlorobenzene	541731	16	
1,4-Dichlorobenzene	106467	17	
1,4-Dichlorobenzene-d4	3855821	1	
3,3'-Dichlorobenzidine	91941	192	
2,3-Dichlorobiphenyl	16605917	105	29
2,4-Dichlorophenol	120832	41	
2,6-Dichlorophenol	87650	45	
Dichlorvos	62737	48	4
Dicrotophos	141662	248	
Dieldrin	60571	161	87
Di(2-ethylhexyl)adipate	103231		101
Diethyl phthalate	84662	82	22
Diethylstilbestrol	56531	181	
Diethyl sulfate	64675	247	
Dimethoate	60515	108	
3,3'-Dimethoxybenzidine	119904	265	
p-(Dimethylamino)azobenzene	60117	163	
7,12-Dimethylbenz[a]anthracene	57976	211	
3,3'-Dimethylbenzidine	119937	174	
a,a-Dimethylphenethylamine	122098	40	
1,3-Dimethyl-2-nitrobenzene-ss	81209		3
2,4-Dimethylphenol	105679	36	
Dimethyl phthalate	131113	66	11
Di-n-butyl phthalate	84742	131	59
1,2-Dinitrobenzene	528290	274	
1,3-Dinitrobenzene	99650	67	
1,4-Dinitrobenzene	100254	273	
4,6-Dinitro-2-methylphenol	534521	89	
2,4-Dinitrophenol	51285	72	
2,4-Dinitrotoluene	121142	76	19
2,6-Dinitrotoluene	606202	68	15
Dinocap I	39300453	190	
Di-n-octyl phthalate	117840	207	
Dinoseb	88857	119	
Diphenamid	957517	239	68
Diphenylamine	122394	91	
5,5-Diphenylhydantoin	57410	257	
1,2-Diphenylhydrazine	122667	272	
Disulfoton	298044	120	45
Disulfoton sulfone	2497065		75
Disulfoton sulfoxide	2497076		6
Endosulfan I	959988	150	78
Endosulfan II	33213659	169	92
Endosulfan sulfate	1031078	182	96
Endrin	72208	166	90
Endrin aldehyde	7421934	172	94
Endrin ketone	53494705	189	
EPN	2104645	197	
EPTC	759944		7
Ethion	563122	168	
Ethoprophos	13194484		24
Ethyl carbamate	51796	246	
Ethyl methanesulfonate	62500	9	
Etridiazole	2593159		13
Famphur	52857	171	
Fenamiphos	22224926		82
Fenarimol	60168889		109
Fensulfothion	115902	159	
Fenthion	55389	139	
Fluchloralin	33245395	125	
Fluoranthene	206440	152	
2-Fluorobiphenyl	321608	60	
Fluorene	86737	86	21
Fluridone	59756604		116

Compound name	CAS #	8270_DRS.L entry #	525_DRS.L entry #
2-Fluorophenol	367124	7	
Heptachlor	76448	130	53
Heptachlor epoxide -isomer B	1024573	143	70
2,2',3,3',4,4',5-Heptachlorobiphenyl	35065306	203	
2,2',3,3',4,4',6-Heptachlorobiphenyl	52663715		104
2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065293	195	
2,2',3,4,4',5',6-Heptachlorobiphenyl	52663691	187	
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663680	184	
1,2,3,4,6,7,8-Heptachlorodibenzo-furan	67562394	215	
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	35822469	217	
Hexachlorobenzene	118741	106	30
2,2',3,4,4',5'-Hexachlorobiphenyl	35065282	180	
2,2',3,4,5,5'-Hexachlorobiphenyl	52712046	175	
2,2',3,5,5',6-Hexachlorobiphenyl	52663635	162	
2,2',4,4',5,5'-Hexachlorobiphenyl	35065271	170	
2,2',4,4',5,6'-Hexachlorobiphenyl	60145224		89
Hexachlorobutadiene	87683	47	
Hexachlorocyclopentadiene	77474	56	5
1,2,3,4,7,8-Hexachlorodibenzofuran	70648269	205	
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	39227286	209	
Hexachloroethane	67721	29	
Hexachlorophene	70304	214	
Hexachloropropene	1888717	46	
Hexamethylphosphoramid	680319	245	
Hexazinone	51235042		100
Hydroquinone	123319	244	
Indeno[1,2,3-cd]pyrene	193395	221	117
Isodrin	465736	140	
Isophorone	78591	34	2
Isosafrole	120581	61	
Kepone	143500	177	
Leptophos	21609905	201	
Malathion	121755	132	
Maleic anhydride	108316	243	
Merphos	150505		72
Mestranol	72333	242	
Methapyrilene	91805	138	
Methoxychlor	72435	188	106
3-Methylcholanthrene	56495	218	
4,4'-Methylenebis (2-chloroaniline)	101144	263	
4,4'-Methylenebis (N,N-dimethylaniline)	101611	262	
Methyl methanesulfonate	66273	6	
2-Methylnaphthalene	91576	53	
Methyl paraoxon	950356		46
Methyl parathion	298000	128	
2-Methylphenol	95487	20	
3-Methylphenol	108394	23	
4-Methylphenol	106445	22	
Metolachlor	51218452		62
Metribuzin	21087649		51
Mevinphos	7786347	63	9
Mexacarbate	315184	241	
MGK 264 - a	113484		67
MGK 264 - b	113484		69
Mirex	2385855	204	
Molinate	2212671		20
Monocrotophos	6923224	98	
Naphthalene	91203	43	
Naphthalene-d8	1146652	30	
1,4-Naphthoquinone	130154	65	
1-Naphthylamine	134327	80	
2-Naphthylamine	91598	78	
Napropamide	15299997		83
Nicotine	54115	58	
5-Nitroacenaphthene	602879	255	
2-Nitroaniline	88744	64	
3-Nitroaniline	99092	70	
4-Nitroaniline	100016	87	
5-Nitro-o-anisidine	99592	254	
Nitrobenzene	98953	32	
Nitrobenzene-d5	4165600	31	
4-Nitrobiphenyl	92933	258	
Nitrofen	1836755	165	
2-Nitrophenol	88755	35	
4-Nitrophenol	100027	74	
4-Nitroquinoline-1-oxide	56575	136	
5-Nitro-o-toluidine	99558	85	
N-Nitrosodiethylamine	55185	8	
N-Nitrosodimethylamine	62759	2	
N-Nitrosodi-n-butylamine	924163	49	
N-Nitrosodi-n-propylamine	621647	25	
N-Nitrosodiphenylamine	86306	90	
N-Nitrosomethylethylamine	10595956	4	
N-Nitrosomorpholine	59892	27	
N-Nitrosopiperidine	100754	33	
N-Nitrosopyrrolidine	930552	24	
trans-Nonachlor	39765805		81
Norflurazon	27314132		97
2,2',3,3',4,4',5,5',6-Nonachlorobi-phenyl	40186729	210	
2,2',3,3',4,5',6,6'-Octachlorobi-phenyl	40186718		107

Compound name	CAS #	8270_DRS.L entry #	525_DRS.L entry #
Octachlorodibenzofuran	39001020	220	
Octachlorodibenzo-p-dioxin	3268879	219	
Octamethyl pyrophosphoramide	152169	239	
4,4'-Oxydianiline	101804	261	
Parathion (ethyl)	56382	134	
Pebulate	1114712		14
Pentachlorobenzene	608935	75	
2,2',3,4,5'-Pentachlorobiphenyl	38380028	156	
2,2',3',4,6'-Pentachlorobiphenyl	60233252		71
2,2',4,5,5'-Pentachlorobiphenyl	37680732	148	
2,3,3',4',6-Pentachlorobiphenyl	38380039	158	
1,2,3,7,8-Pentachlorodibenzofuran	57117416	198	
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	40321764	202	
Pentachloroethane	76017	14	
Pentachloronitrobenzene	82688	113	
Pentachlorophenol	87865	112	37
cis-Permethrin	54774457		110
trans-Permethrin	51877748		111
Perylene-d12	1520963	206	115
Phenacetin	62442	101	
Phenanthrene	85018	122	42
Phenanthrene-d10	1517222	88	32
Phenobarbital	50066	236	
Phenol	108952	11	
Phenol-d5	4165622	10	
p-Phenylenediamine	106503	50	
Phorate	298022	102	
Phosalone	2310170	200	
Phosmet	732116	196	
Phosphamidon I	13171216	118	
Phthalic anhydride	85449	235	
2-Picoline (2-Methylpyridine)	109068	5	
Piperonyl sulfoxide	120627	234	
Prometon	1610180		34
Prometryn	7287196		56
Propyzamide (Pronamide)	23950585	114	41
Propachlor	1918167		23
Propazine	139402		36
Propylthiouracil	51525	233	
Pyrene	129000	147	76
Pyrene-d10-ss	1718521		74
Pyridine	110861	3	
Resorcinol	108463	232	
Safrole	94597	52	
Simazine	122349		33
Simetryn	1014706		52
Stirofos (Tetrachlorvinphos)	22248799		77
Strychnine	57249	231	
Sulfallate	95067	230	
Sulfotepp	3689245	95	
Tebuthiuron	34014181		18
Terbacil	5902512		48
Terbufos	13071799	121	40
Terbutryne	886500		57
Terphenyl-d14	1718510	157	
1,2,4,5-Tetrachlorobenzene	95943	54	
2,2',3,5'-Tetrachlorobiphenyl	41464395	135	
2,2',4,4'-Tetrachlorobiphenyl	2437798		60
2,2',5,5'-Tetrachlorobiphenyl	35693993	133	
2,3',4,4'-Tetrachlorobiphenyl	32598100	144	
2,3,7,8-Tetrachlorodibenzofuran	51207319	178	
2,3,7,8-Tetrachlorodibenzo-p-dioxin	1746016	183	
2,3,4,6-Tetrachlorophenol	58902	79	
Tetrachlorvinphos	961115	151	
Tetraethylpyrophosphate (TEPP)	107493	81	
Thiophenol (Benzenethiol)	108985	229	
Thionazin	297972	83	
Toluene diisocyanate	584849	228	
o-Toluidine	95534	28	
Toxaphene	58002190	227	
Triadimefon	43121433		65
2,4,6-Tribromophenol	118796	96	
1,2,4-Trichlorobenzene	120821	42	
2,2',5-Trichlorobiphenyl	37680652	117	
2,4,5-Trichlorobiphenyl	15862074		50
2,4',5-Trichlorobiphenyl	16606023	127	
2,4,5-Trichlorophenol	95954	59	
2,4,6-Trichlorophenol	88062	57	
Tricyclazole	41814782		84
O,O,O-Triethyl phosphorothioate	126681	38	
Trifluralin	1582098	92	27
2,4,5-Trimethylaniline	137177	269	
Trimethyl phosphate	512561	226	
1,3,5-Trinitrobenzene	99354	99	
Triphenylphosphate-ss	115866		102
Tris(2,3-dibromopropyl) phosphate	126727	224	
Tri-p-tolylphosphate	78320	225	
Vernolate	1929777		10

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Printed in the USA
February 8, 2008
5989-7875EN

Agilent Technologies

Femtogram GC/MSD Detection Limits for Environmental Semivolatiles Using a Triple-Axis Detector

Application

Environmental

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Abstract

The analysis of semivolatiles at very low levels presents challenges due to analyte activity, background contamination, and instrument sensitivity. Method requirements vary worldwide, with the least sensitive specifying 1-µL injections and full-scan data acquisition. The lowest detection limits can be achieved using a programmable temperature vaporizing (PTV) inlet, trace ion detection (TID), and a triple-axis detector (TAD) with the MSD operating in SIM mode.

Introduction

Low-level semivolatiles analysis is used to concurrently measure a mixture of acids, bases, neutrals, and pesticides in drinking water or source water. Most laboratories analyze for > 100 compounds, with a chromatographic run time of 25 to 40 minutes. Sample extraction is accomplished using liquid-solid extraction (LSE) with C_{18} disks or cartridges. Liquid-liquid extraction with a solvent such as dichloromethane is an alternative technique. Extract injection is typically 1 µL hot splitless with the MSD operating in full-scan mode, as specified in some commonly used methods such as USEPA Method 525.2 [1].

Sensitivity is an area where laboratories are seeking improved performance; it can be affected by sample preparation, extract volume injected, instrument tuning, signal acquisition, and overall system activity. Sensitivity is also a confusing term, with all of the following used interchangeably: maximum sensitivity, minimum sensitivity, best sensitivity, lowest detection limit, instrument detection limit (IDL), and method detection limit.

Previous publications have focused on activity/linearity, speed, productivity, and large-volume injection [2–5]. Sensitivity is a factor in all of these, and many times is a trade-off.

This application addresses the parameters that affect the IDL, that is, the "sensitivity" of the GC/MSD system. There are statistical ways to calculate the IDL, but these may not answer the questions, "How much can I actually see?" or "What is the lowest amount that will produce a peak I can integrate?"

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized. For the best sensitivity, parameters should be chosen that transfer the maximum amount of analyte onto the column. Furthermore, the entire system must be inert, as sensitivity is almost always lost on active analytes first.

Many analysts associate the use of PTV only with large-volume injection (LVI) in solvent vent mode [4]. LVI will allow lower levels of calibration, but

Table1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 7890A or 6890N		
Inlet	EPC PTV		
Mode	Splitless		
Temperature ramp	°C/min	Next °C	Hold min
Initial		20	0.05
Ramp 1	600	350	0.90
Ramp 2	10	250	0.00
Cryo	On		
Cryo use temperature	100 °C		
Cryo timeout	10.00 min (On)		
Cryo fault	On		
Pressure	11.40 psi (On)		
Purge flow	30.0 mL/min		
Purge time	1.50 min		
Total flow	34.4 mL/min		
Gas saver	Off		
Gas type	Helium		
PTV Liner	Agilent multi-baffle liner, no packing, p/n 5183-2037		
Oven	120V		
Oven ramp	°C/min	Next °C	Hold min
Initial		40	2.50
Ramp 1	50	110	0.00
Ramp 2	10	320	1.10
Total run time	26 min		
Equilibration time	0.5 min		
Oven max temperature	325 °C		
Column	Agilent Technologies HP 5 MSi, p/n 19091S-433i		
Length	30.0 m		
Diameter	0.25 mm		
Film thickness	0.25 µm		
Mode	Constant flow		
Pressure	11.40 psi		
Nominal initial flow	1.4 mL/min		
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
RTL	System retention time locked to phenanthrene-d10 at 12.700 min		

Front Injector	
Sample washes	1
Sample pumps	2
Injection volume	2.0 µL
Syringe size	10 µL
PreInj Solv A washes	0
PreInj Solv B washes	1
PostInj Solv A washes	3
PostInj Solv B washes	2
Viscosity delay	0 seconds
Plunger speed	Fast
PreInjection dwell	0 minutes
PostInjection dwell	0 minutes
MSD	Agilent Technologies 5975C, Triple-Axis Detector
Drawout lens	3 mm standard aperture drawout lens
Solvent delay	4 min
Low mass	45 amu
High mass	450 amu
Threshold	0
Sampling	2
Quad temp	180 °C
Source temp	300 °C
Transfer line temp	280 °C
Tune type	Autotune
EMV mode	Gain factor = 1

MSD-SIM

AutoSIM was used to pick ions, groups and switching times

Number of groups	25
Compounds/group	Varied 1 to 22
Ions/group	Varied 2 to 45
Dwell time, msec	Varied 5 to 50
Cycles/peak	Minimum 10

Calibration Standards

Ultra Scientific, North Kingstown, RI. p/n DWK-5252. Four mixtures, co-diluted in dichloromethane, resulting in 108 compounds at 7 concentration levels: 10, 4, 1, 0.4, 0.1, 0.04, and 0.01 ppm. Each level spiked with 3 Internal Standards at 2 ppm and 4 surrogate standards at 2 ppm. Each level then diluted 1:100 in dichloromethane, resulting in 7 concentration levels: 100, 40, 10, 4, 1, 0.4, and 0.1 ppb (pg/uL) with IS/SS at 2 ppb.

method development is necessary to optimize recovery of compounds while eliminating the solvent. LVI also injects more matrix and may not improve Signal-to-Noise (S/N) due to chemical noise. The PTV has other operating modes; "cold" splitless mode was used here. Splitless injection into a cold inlet instead of a typical hot splitless inlet offers these advantages:

1. Solvent expansion is minimized; analytes do not travel outside the liner and contact metal surfaces, thereby minimizing degradation.
2. Analytes vaporize at the lowest temperature, also minimizing degradation.
3. Volatile solvent is transferred onto the column first; analyte peak shape is improved for injections of 2 to 5 µL.

Figure 1 shows the PTV temperature and flow programs together with the oven program. The PTV is held at 20 °C, a temperature below the boiling point of the solvent dichloromethane, 39.8 °C, during the fast injection period, 0.05 min. At the end of the injection period, the PTV is rapidly heated to 350 °C, transferring analyes onto the column. At the end of the splitless time, 1.5 min, the inlet is purged at 30 mL/min. The PTV is allowed to cool during the run.

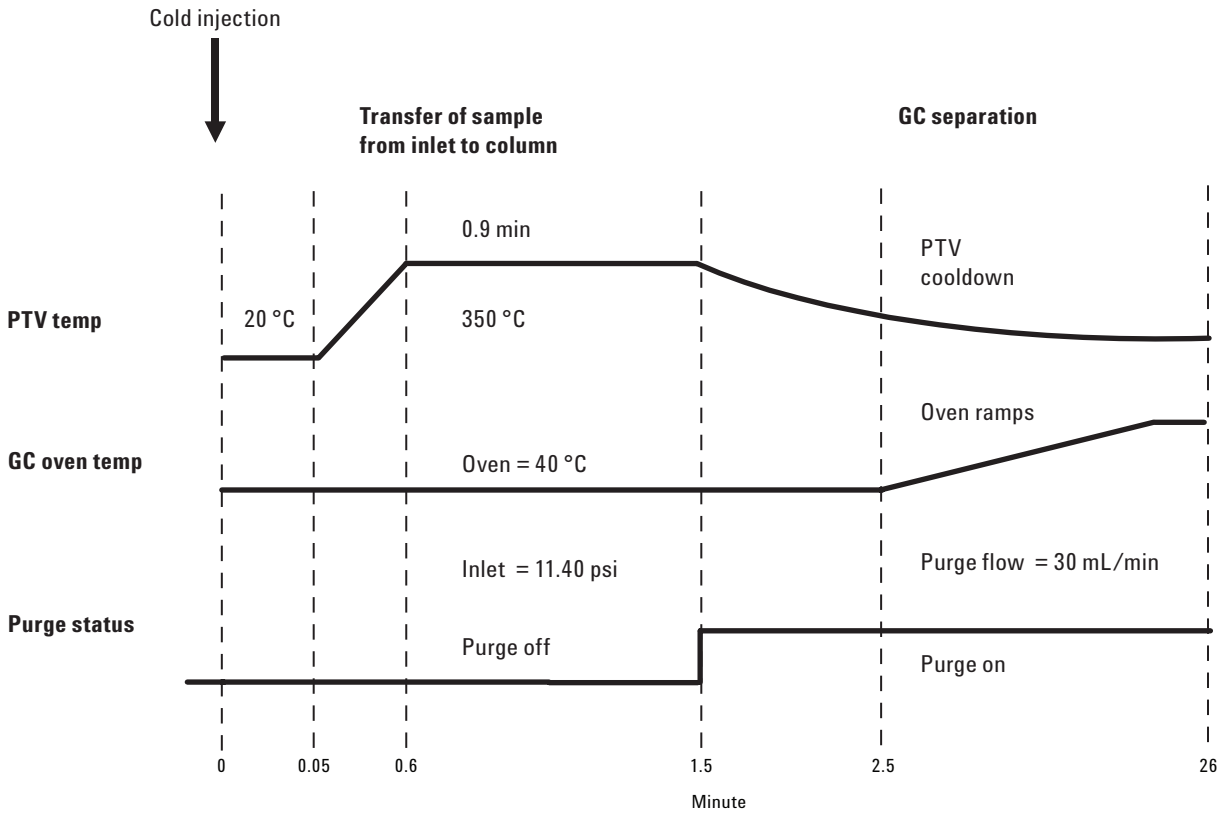

Figure 1. PTV cold splitless temperature and flow programs.

The PTV program ramp can be adjusted and multiple ramps are possible. The PTV inlet liner (p/n 5183-2037) is multi-baffled and deactivated. It does not contain glass wool, which could contribute to active compound degradation. This liner has sufficient capacity to accommodate a 2- to 5-µL injection volume at fast speed. A 2-µL injection was used for all data presented here.

The oven program relationship to the PTV parameters is shown in Figure 1. The oven starts at 40 °C and is held there during the injection cycle and splitless transfer of analytes onto the column. The oven then programs rapidly to 110 °C, followed by a slower ramp for compound separation. There is an extra 1 min of oven hold time at 40 °C, which is between 1.5 and 2.5 min. This maintains the retention time locked (RTL) times for analytes while providing room for the injection to be scaled up to LVI, if desired. The 240V oven was used, but a 120V oven can also achieve the ramp rates found in Table 1.

The HP-5MSi column is designed for inertness and is well suited to this method. This is the latest version of the most popular column in environmental laboratories, the HP-5MS. The column was run in constant-flow mode at 1.4 mL/min to maintain peak shape and sensitivity.

The system was RTLocked to phenanthrene-d10 at 12.700 min. The primary benefit of RTL for this analysis is maintaining constant switching times for SIM groups. After clipping the column, a rerun and analysis of the locking standard is all that is needed to restore shifted peak times. Quantitation database and integration events times also do not have to be changed. Additional RTL applications detailing the numerous benefits of RTL are available at www.agilent.com/chem. It is almost impossible to use a method with this many SIM groups without RTL, in a productive laboratory.

The standard 3-mm drawout lens was used for best sensitivity. Previous work has shown improved linearity across a wide calibration range using the optional 6-mm lens [1]. Using the 6-mm lens will show a typical loss of 2 to 5x in the IDL.

The 5975C MSD was equipped with a Triple-Axis Detector (TAD) [6]. The TAD presents several advantages to the user, one of which is, “Although signal is enhanced, neutral noise is substantially reduced through the off-axis design.” This increase in S/N for clean samples with minimal chemical noise can help reach a lower IDL. Trace ion detection (TID) was switched on during all data acquisition [7]. TID is a filtering routine to minimize noise and is selectable in the software.

Scan parameters are listed and data were collected in either scan mode or in SIM mode. None of the runs was made in synchronous SIM/scan mode. A sampling rate of 2 was used, as it is typical of most methods on a 250-µm id column. This sampling rate, with a 45 to 450 mass range, resulted in at least 10 scans across each peak.

AutoSIM setup was used in combination with the scan quantitation database to pick ions, groups, and switching times. The SIM acquisition table from AutoSIM was used directly with only two modifications. Tebuthiuron (ion 156) and tricyclazole (ion 189) are known for poor peak shape. Their ions were manually added to the groups across which the peaks eluted. A target ion plus one qualifier ion were used for all internal (ISTDs) and surrogate standards (SSs). A target ion plus two qualifier ions were used for all other analytes, if they were present in sufficient abundance in the spectra. A minimum of 10 SIM data points were acquired across each peak.

A source temperature of 300 °C was used instead of the typical 230 to 250 °C range. This higher temperature has been used to minimize peak tailing, and therefore improve sensitivity for PAHs [5].

The compound list was taken from USEPA 525 and is typical of the analytes that laboratories worldwide are interested in analyzing at low levels. The USEPA 8270 list was not used, as it is targeted at higher concentrations of compounds in waste samples that contain high levels of matrix and are not comparable here. The best way to improve sensitivity for solids and waste samples is through extract cleanup. The standards were prepared in dichloromethane only for the single component analytes, except disulfoton sulfoxide and disulfoton sulfone, which were not included in the commercially available mixture. Standards were not prepared for multicomponent toxaphene or the Aroclors.

A typical calibration range for low-level semivolatiles is 0.1 to 10 ppm as defined in USEPA 525. Standards were made from 0.01 to 10 ppm, containing 2 ppm of ISTDs and SSs. A dilution of 1:100 of each of these yields a range of 0.1 to 100 ppb, with ISTDs and SSs at 20 ppb, for a lower working range. Atrazine and alachlor are present in two of the stock mixes, so their concentrations are twice that of other analytes. Pentachlorophenol is present at four times the other analyte concentrations, as described in USEPA 525.

Results

The standard solutions from 0.1 to 100 ppb were run in both SIM and scan modes. Data from the 0.1-ppb scan injections showed insufficient response or were too noisy to reproducibly integrate. The SIM data at 0.1 ppb were significantly improved compared to the scan data and could be routinely used. A listing of selected analytes with S/N measured from 1.0 ppb scan runs (2 pg) are shown in Table 2, together with data from 0.1- (0.2-pg) and 1.0-ppb SIM runs. Each value is an average of three acquisitions on one system, using peak-to-peak noise.

Table 2. Signal-to-Noise for Selected Analytes, SIM and Scan Modes

Compound	Ion	pg → RT	0.2 SIM S/N	2.0 SIM S/N	2.0 Scan S/N
Hexachlorocyclopentadiene	237	7.960	6.3	77	7.5
Trifluralin	264	11.608	4.4	49	7.7
Simazine	201	12.274	1.0	16	2.4
Atrazine	200	12.385	3.1	30	13
Pentachlorophenol	266	12.492	2.4	20	3.7
Chlorothalonil	266	13.146	2.6	26	2.9
Aldrin	66	14.661	1.6	15	1.9
Heptachlor epoxide	353	15.429	6.2	49	3.4
4,4′-DDE	246	16.557	7.0	72	17
Carboxin	143	16.696	2.4	22	4.0
Endrin	263	17.003	2.3	22	4.1
4,4′-DDD	235	17.323	7.5	76	7.5
4,4′-DDT	235	18.000	5.9	60	5.9

There is excellent agreement between the SIM S/N values at the two levels for most compounds. This shows that the responses are real and that the entire system is inert. There is a slight loss of simazine and minimal interference for pentachlorophenol and heptachlor epoxide at the lowest level, 0.2 pg. At the 200 femtogram level, this is no surprise.

The scan S/N at 2.0 pg is lower than SIM, as expected, by 3- to 15-fold. The gains in S/N moving from scan to SIM are related to the dwell time versus the original sampling rate.

Extracted Ion Currents (EICs) from the 1.0-ppb level for both SIM and scan are shown in Figures 2a to 2d. It can clearly be seen that either the SIM or scan signals could be used for quantitation based on S/N and peak shape. Of particular note is the response and very good peak shape for pentachlorophenol, even at an 8-pg full scan.

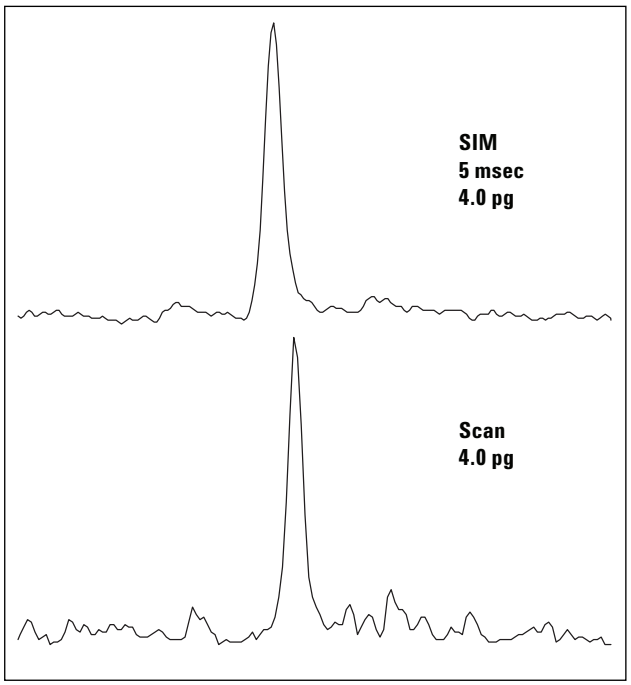

Figure 2a. Atrazine – Extracted Ion 200, RT 12.350 min.

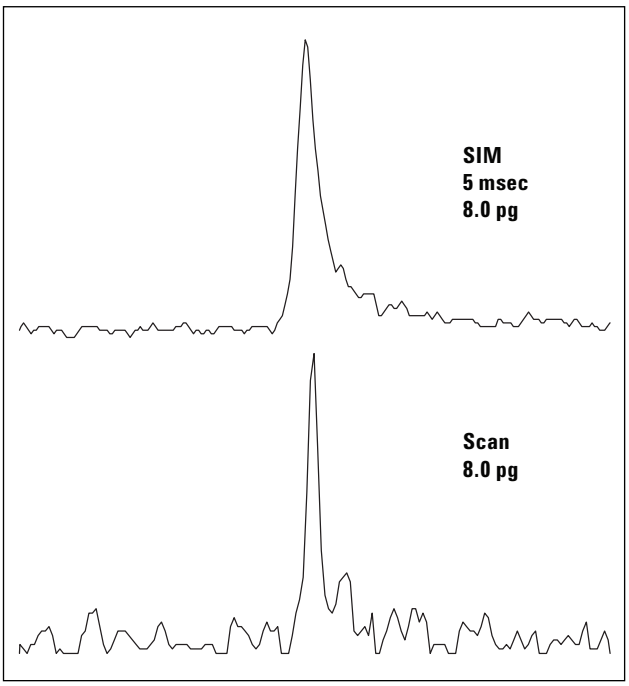

Figure 2b. Pentachlorophenol – Extracted Ion 266, RT 12.445 min.

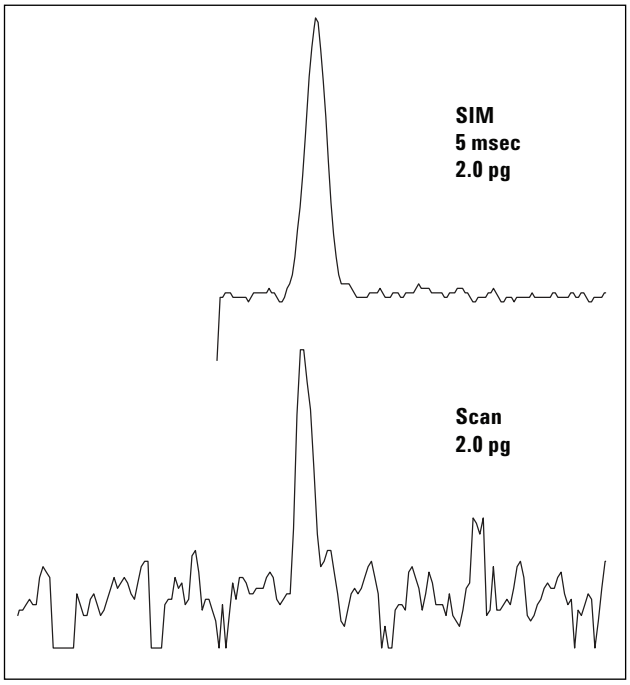

Figure 2c. Aldrin – Extracted Ion 66, RT 14.616 min.

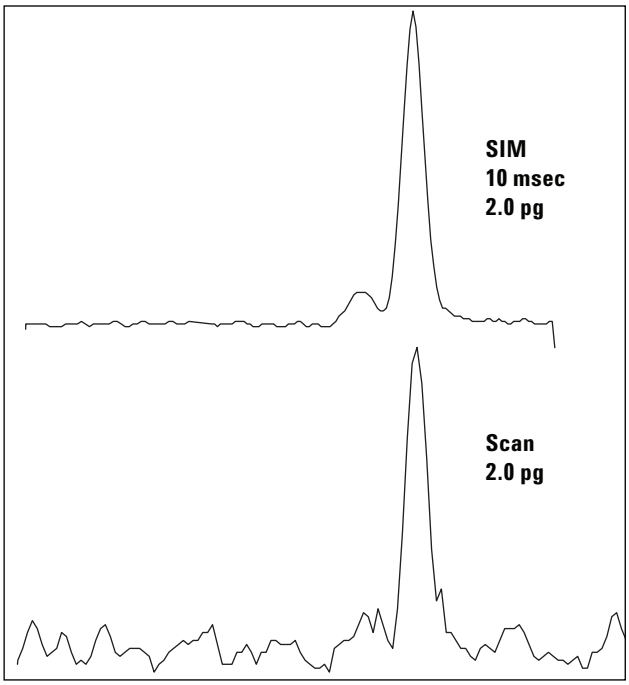

Figure 2d. 4,4′-DDT – Extracted Ion 235, RT 18.00 min.

Although linearity is not the focus of this application, it is a measure of inertness, reproducibility, and sensitivity. Linearity can be determined by the percent relative standard deviation (%RSD) of the relative response factor (RRF) for each compound across the calibration range. The %RSD and the RRF calculations are done automatically by the GC/MSD ChemStation software in conjunction with Excel. There is no correct %RSD, as it is method dependent. The %RSDs of the RRFs for selected compounds are shown in Table 3.

Table 3. Linearity of Selected Analytes

Calibration range pg →	0.2–200 SIM	2–200 Scan
Compound	**%RSD**	**%RSD**
Dichlorvos	1.9	7.0
Mevinphos	10.1	7.0
2,3-Dichlorobiphenyl	5.3	3.0
Atrazine	14.2	14.5
Pentachlorophenol	6.3	33.0
Anthracene	2.2	3.0
Chlorothalonil	7.6	25.0
Heptachlor epoxide	6.6	13.0
4,4′-DDE	4.5	9.0
4,4′-DDD	7.4	8.0
4,4′-DDT	4.0	5.9

At first glance some of the %RSD values appear high, such as pentachlorophenol (PCP) and chlorothalonil. These are calibrated, however, from 2 to 200 pg in scan mode, which is 50-fold lower than USEPA 525 mandates. The SIM data are calibrated from 0.2 to 200 pg, which is 500-fold lower and a 10-fold wider range. This demonstrates both inertness and detectability at the femtogram level.

As an additional overall measure of system linearity, the average of all %RSDs was calculated at 8% for SIM data and 13% for scan data. Not all compounds were calibrated to the 0.1-ppb level, as they did not have a signal that could be reliably measured. The phthalates, easily detected at low levels, were excluded from these averages due to common laboratory contamination.

EICs at the 200-femtogram level, from SIM, are shown for six different compounds in Figures 3. All are easily seen and measured against noise. As an analyst's measure of sensitivity, the question from the introduction was "How much can I actually see?" The answer: very low picogram levels for most environmental semivolatiles in scan mode. The IDL using SIM is even lower, in the femtogram range.

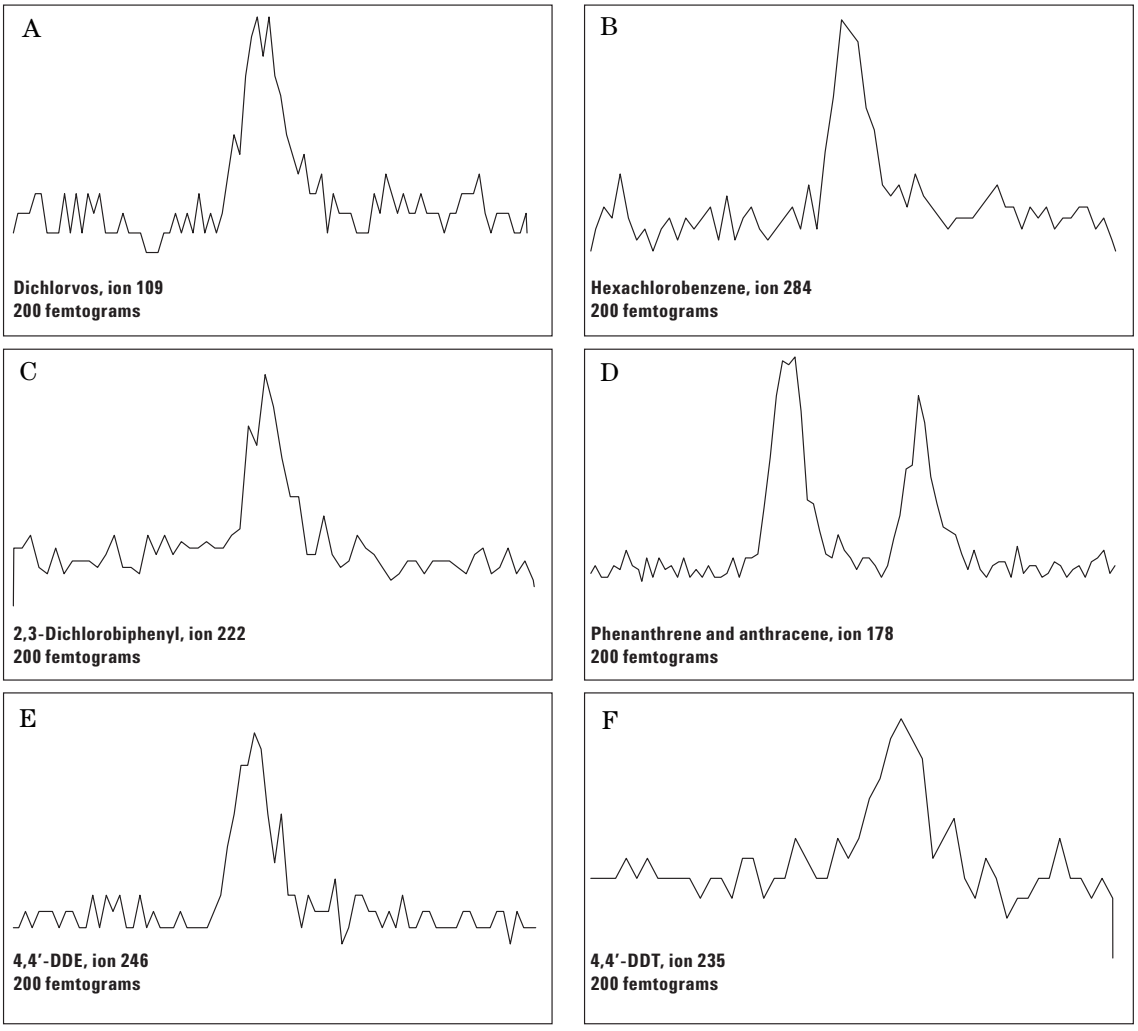

Figure 3. EICs at the 200 femtogram level.

Conclusions

Traditional semivolatiles methods can be altered to achieve better instrument detection limits. There have been advancements in hardware, such as the Triple-Axis Detector (TAD), that improve sensitivity. Signal handling using Trace Ion Detection (TID) provides better S/N through lower noise. The PTV, used in "cold" splitless mode, maximizes the amount of sample on the column, while vaporizing analytes at the lowest possible temperature. Coupled with an inert column and source, the PTV provides an easy way to improve sensitivity. Methods that require only a target ion and a few qualifier ions for identification can often be changed to SIM from scan, improving S/N by 3- to 50-fold. Combining all of these hardware, software, and operating parameters can result in femtogram instrument detection limits (IDLs) and sensitivity you can use.

References

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Printed in the USA
April 14, 2008
5989-8342EN

Agilent Technologies

Semivolatile Analysis Using an Inertness Performance Tested Agilent J&W DB-5ms Ultra Inert Column

Application

Environmental

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Abstract

Agilent Technologies Inc. has implemented new testing procedures to more effectively evaluate GC column inertness performance. The new testing procedure uses deliberately aggressive probes to thoroughly investigate column inertness quality. The value of using probes such as 1-propionic acid, 4-picoline, and trimethyl phosphate to establish a column inertness baseline is discussed. This baseline inertness profile is then extended to a real-world application example with challenging analytes in the semivolatile sample set. Inertness performance with analytes such as 2,4-dinitrophenol, benzoic acid, and benzidine clearly shows the advantage of using the Agilent J&W DB-5ms Ultra Inert columns for semivolatile analysis.

Introduction

Semivolatile analyses using methods similar to USEPA method 8270 [1] are important in environmental laboratories worldwide. A number of very active analytes presents significant challenges for analysts, equipment providers, and column manufactures in terms of inertness. Acidic compounds such as benzoic acid or 2,4-dinitrophenol and strong bases such as pyridine or benzidine are examples of active species found in the semivolatile sample set. These chemically charged species are particularly susceptible to adsorption onto active surfaces in the sample flow path, including the column itself. Both system and column inertness are critical for effective analysis of these active chemical species.

For many years Grob's mix [2] has been the standard mix to evaluate capillary GCs and columns. This mix consists of a series of alkanes, a substituted phenol (acidic component), an amine (basic component), an alcohol, and a diol. Virtually all capillary column manufactures have used Grob's or a very similar test mix to evaluate column performance historically. These mixtures work well to evaluate column efficiency, system suitability against solute discrimination during injection, and potential solute absorption in the chromatographic flow path. Inertness evaluation based on single acidic and basic species in these mixes, though valuable, falls short of the rigorous requirements for inertness that applications on modern capillary GC columns require [3–4]. Modern GC applications demand a more comprehensive approach to properly investigate column inertness performance.

Experimental

Baseline inertness testing of columns was on an Agilent 6890N GC equipped with a 7683B autosampler and an FID. Semivolatile application-specific chromatograms were generated using an Agilent 6890N GC/5975B MSD equipped with a 7683B autosampler.

Tables 1 and 2 list the chromatographic conditions used on each of the chromatographic systems. Table 3 lists flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions 6890N/FID System

GC:	Agilent 6890N
Sampler :	Agilent 7683B, 0.5-µL syringe (Agilent p/n 5188-5246), 0.02-µL split injection, 1 ng each component on column
Carrier:	Hydrogen constant pressure 38 cm/s
Inlet:	Split/splitless; 250 °C, 1.4 mL/min column flow, split flow 900 mL/min, gas-saver flow 75 mL/min. on at 2.0 min
Inlet liner:	Deactivated single taper w/glass wool (Agilent p/n 5183-4647)
Column:	Agilent J&W DB-5ms Ultra Inert, 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven:	65 °C isothermal
Detection:	FID at 325 °C, 450 mL/min air, 40 mL/min hydrogen, 45 mL/min nitrogen makeup

Table 2. Chromatographic Conditions 6890N/5975B MSD System

GC:	Agilent 6890N/5975B MSD
Sampler :	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5181-5246), 1.0-µL splitless injection, 5 ng each component on column
Carrier:	Helium constant flow 30 cm/s
Inlet:	Split/splitless; 260 °C, 53.7 mL/min total flow, purge flow 50 mL/min on at 0.5 min, gas-saver flow 80 mL/min on at 3.0 min
Inlet liner:	Deactivated single taper w/glass wool (Agilent p/n 5183-4647)
Column:	Agilent J&W DB-5 ms Ultra Inert, 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven:	40 °C (1 min) to 100 °C (15 °C/min), 10 °C to 210 °C (1 min), 5 °C/min. to 310 °C (8 min)
Detection:	MSD source at 300 °C, quadrupole at 180 °C, transfer line at 290 °C, scan range 50–550 AMU

The flow path supplies used in these experiments are listed in Table 3.

Table 3. Flow Path Supplies

Vials:	Amber screw cap (Agilent p/n 5182-0716)
Vial caps:	Blue screw cap (Agilent p/n 5282-0723)
Vial inserts:	100-µL glass/polymer feet (Agilent p/n 5181-1270)
Syringe:	5 µL (Agilent p/n 5181-1273)
Septum:	Advanced Green (Agilent p/n 5183-4759)
Inlet liners:	Deactivated single taper w/glass wool (Agilent p/n 5183-4647) for FID Deactivated single taper direct connect (Agilent p/n G1544-80730) for MSD
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
20x magnifier:	20x magnifier loupe (Agilent p/n 430-1020)

Sample Preparation

Test probes for baseline inertness evaluation were purchased from Sigma Alrich (Milwaukee, WI 53201, USA). Dichloroethane used was Burdick and Jackson spectral grade purchased thorough VWR International (West Chester, PA 19380, USA). semivolatile standard (USEPA 8270) solutions were obtained either from Ultra Scientific (North Kingstown, RI 02852, USA) or AccuStandard (New Haven, CT 06513, USA).

Solutions were prepared using dichloroethane solvent and class A volumetric pipettes and flasks.

Results and Discussion

Baseline Inertness Profile for the Ultra Inert Columns

One means of quickly evaluating the suitability of a chromatographic system and the column component of that system is the deliberate injection of challenging analyte mixes on the system. Good sample recoveries and peak shapes quickly show that the injection system is functioning properly and establish a baseline inertness profile for the column. The baseline inertness profile then serves as a predictor for successful analysis of chemically active species like those in the semivolatile sample set. The use of more demanding test mixes to certify column inertness performance is the approach taken for every column offered in the Ultra Inert series of capillary GC columns.

This application illustrates the implementation of more rigorous testing procedures to certify GC capillary column inertness. The baseline test mix selected for inertness contains 1-propionic acid, 4-picoline, trimethyl phosphate, and 1-heptanol. Key column evaluation criteria include efficiency of n-decane elution at a k' of 5, probe peak shapes, and peak height ratios of 4-picoline and trimethyl phosphate relative to closely eluting alkanes. The peak height ratio of active analytes, such as 4-picoline and trimethyl phosphate, relative to less active alkanes indicate the degree of surface activity for the reactive analyte. A higher ratio indicates better inertness. Testing with these aggressive probes provides more probative tools for evaluating inertness with problematic acidic and basic species. This testing procedure raises the bar for column inertness QC testing and sets a new industry standard for consistent column performance.

Figure 1 shows a baseline inertness chromatogram for an Ultra Inert DB-5ms column. Please note the peak shapes for trimethyl phosphate. This compound exhibits minor peak tailing in this example chromatogram and, for this analyte, represents very good peak shape. The observable peak tailing for this analyte is what makes it an excellent tool for evaluating column inertness. On a lesser column this peak may not be seen at all.

Semivolatile Challenging Analytes

The evaluation of column performance went beyond the new baseline testing for inertness and looked at an abbreviated list of compounds specific to the USEPA Method 8270 sample set. The semivolatiles mix [5] contained N-nitrosodimethylamine, aniline, benzoic acid, 2,4-dinitrophenol, 4-nitrophenol, 2-methyl-4,6-dinitrophenol, pentachlorophenol, 4-aminobiphenyl, benzidine, 3,3'-dichlorobenzidine, benzo [b] fluoroanthene, benzo [k] fluoroanthene as well as recommended internal standards. These species were selected to range in polarity from basic to acidic species and from very early eluting nitrosamine to late eluting polynuclear aromatic hydrocarbons (PAHs). Figure 2 is a total ion chromatogram of the challenging analyte mix with a 5-ng on-column loading of each component.

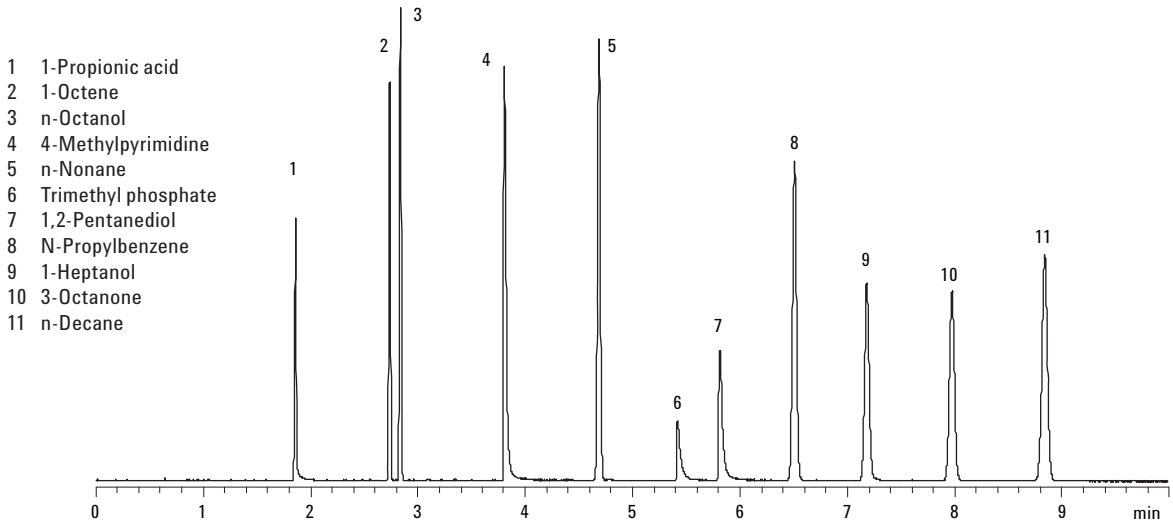

Figure 1. **Baseline inertness test chromatogram,** 1 ng/component load on the Agilent J&W DB-5ms Ultra Inert column (Agilent p/n 122-5532UI), chromatographic conditions as in Table 1, flow path supplies as in Table 3.

1 N-nitrosodimethylamine
2 Aniline
3 1,4-Dichlorobenzene-D4
4 Benzoic acid
5 Naphthalene-D8
6 Acenapthene-D10
7 2,4-Dinitrophenol
8 4-Nitrophenol
9 2-Methyl-4,6-dinitrophenol
10 Pentachlorophenol
11 4-Aminobiphenyl
12 Phenanthrene-D10
13 Benzidine
14 Chrysene-D12
15 3,3′-Dichlorobenzidine
16 Benzo [b] fluoroanthene
17 Benzo [k] fluoroanthene
18 Perylene-D12

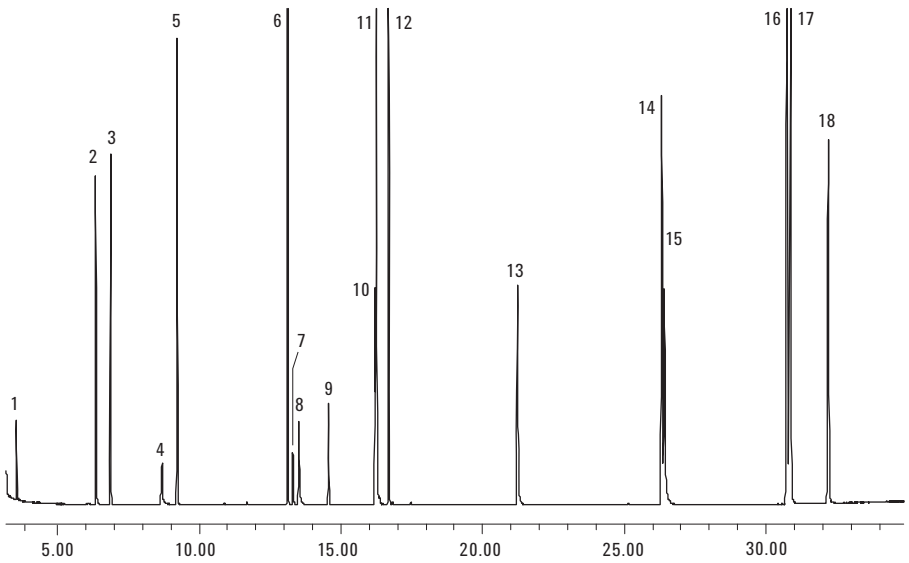

Figure 2. Abbreviated semivolatile test chromatogram, 5 ng/component load on the Agilent J&W DB-5ms Ultra Inert column (Agilent p/n 122-5532UI), chromatographic conditions as in Table 2, flow path supplies as in Table 3.

One key assessment criterion for USEPA 8270 system suitability is the response factor for 2,4-dinitrophenol and its most closely eluting internal standard acenaphthene-d10. The minimum acceptable average response factor (over the entire concentration range) is 0.050 and the typical range is between 0.1 to 0.2. This response tends to decrease at lower concentrations and as the chromatographic system or the standard starts to deteriorate. In Figure 2, response factors for 2,4-dinitrophenol were greater than 0.1, and for 4-nitrophenol, they were greater than 0.2, each at a concentration of 5 µg/mL. These values are indicative of excellent column performance even at low standard concentration.

The recovery of benzidine is another key indicator of inertness performance for semivolatile analysis. This particular base is subject to thermal breakdown in the inlet and to oxidation from standing in solution. Injection temperatures above 260 °C caused benzidine recoveries to drop dramatically. It was necessary to balance benzidine recoveries with the elution of heavier PAHs when setting injection port temperatures. An injection port temperature setting of 260 °C gave good recoveries for benzidine and was still hot enough for higher molecular weight PAHs to volatilize.

Semivolatile Large Mix

Figure 3 shows a 5-ng on-column loading of a broader range of semivolatile analytes. This large mixture was prepared by combing AccuStandard® semivolatile mixes 1, 2, 3, 4a, 4b, 5, and 6 all at a nominal concentration of 5 µg/mL. In total, 93 semivolatile compounds were included in this mix, ranging in boiling points from very low-boiling N-nitrosodimethylamine to high-boiling benzo (g,h,i) perylene. In addition, a wide diversity of analyte polarities was represented in this mix. The highlighted area in Figure 3 shows the elution and peak shape of highly basic benzidine and its response relative to the nearest eluting peak, flouranthene. Even in this large mix, benzidine gave good relative response and peak shape.

1 N-nitrosodimethylamine
2 2- Methyl pyridine
3 Benzidene
4 Fluoranthene
5 Benzo (g,h,i) perylene

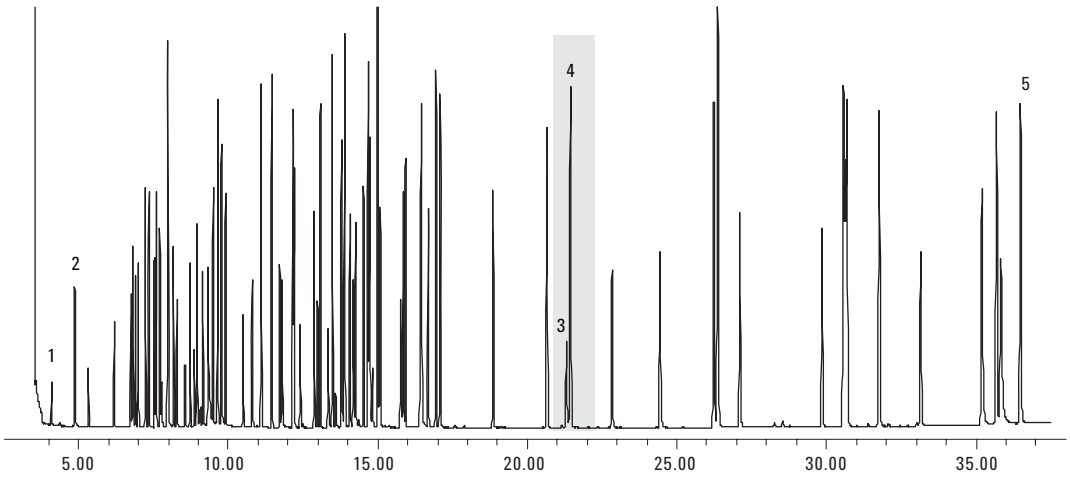

Figure 3. **Semivolatile (large mix) test chromatogram,** 5 ng/component load on the Agilent J&W Ultra Inert DB-5ms column (Agilent p/n 122-5532UI), chromatographic conditions as in Table 2, flow path supplies as in Table 3. Several peaks of interest are labeled to indicate early- and late-eluting species. Benzidine (peak 3) and fluoranthene (peak 4) peaks are shown in the highlighted section.

Conclusions

Rigorous column inertness testing with aggressive probes ensures consistent and reliable column inertness performance for active analytes. Challenging probes such as 1-propionic acid, 4-picoline, and trimethyl phosphate are better predictive indicators of column behavior toward active analytes than traditional Grob style mixes used by many column manufacturers. Inertness testing with these aggressive probes produces columns with well-defined baselines for inertness performance.

Columns with well-defined inertness baselines provide a reliable platform for the analyst to begin analysis of semivolatiles. The Ultra Inert DB-5ms column used in this series of experiments demonstrates excellent inertness performance for some of the most difficult analytes in the semivolatile sample set, including N-nitrosodimethylamine, 2,4-dinitrophenol, 4-nitrophenol, and benzidine. The good recoveries and peak shapes observed for these difficult species, even with a 5-ng on-column loading, are indicative of successful semivolatile analyses on these new Ultra Inert DB-5ms columns.

References

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Printed in the USA
May 19, 2008
5989-8616EN

Agilent Technologies

EPA Method 1694: Agilent's 6410A LC/MS/MS Solution for Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS

Application Note

Environmental

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Abstract

An analytical methodology for screening and confirming the presence of 65 pharmaceuticals in water samples was developed using the Agilent G6410A Triple Quadrupole mass spectrometer (QQQ). The method was developed following the guidelines in EPA Method 1694. Four distinct chromatographic gradients and LC conditions were used according to the polarity and extraction of the different pharmaceuticals. Positive and negative ion electrospray were used with two multi-reaction monitoring (MRM) transitions (a quantifier and a qualifier ion for each compound), which adds extra confirmation in this methodology compared with the EPA method. Linearity of response of three orders of magnitude was demonstrated ($r^2 > 0.99$) for all the pharmaceuticals studied. The analytical performance of the method was evaluated for one wastewater sample collected from Boulder Creek, Colorado; positive identifications for carbamazepine and diphenhydramine were found for this sample using the methodology developed in this work.

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Introduction

The analytical challenge of measuring emerging contaminants in the environment has been a major research focus of scientists for the last 20 years. Pharmaceuticals and personal care products (PPCPs) are an important group of contaminants that have been targeted, especially in the last decade. In the area of PPCPs there are several methods addressing the analysis of these analytes, including EPA Method 1694 [1], which was recently published (December 2007). This EPA protocol uses solid-phase extraction (SPE) for water sample preparation [1]. The extracts are then analyzed directly by a tandem mass spectrometer using a single transition for each compound. This application note describes the Agilent solution to this method, which is demonstrated with the Agilent model 6410A LC/MS QQQ. The Agilent initial implementation for EPA Method 1694 consists of 65 analytes (of 75 total analytes) and 17 labeled internal standards (of 20 total), which are a mixture of PPCPs that are analyzed each by a single MRM transition. (Note that the other compounds and internal standards could not be obtained at this time.) The method also uses Agilent C-18 and Hydrophilic Interaction Chromatography (HILIC) columns for all analytes. To provide additional confirmation, a second MRM transition was added for 60 of the 65 analytes analyzed. This gives an even greater assurance of correct identification than prescribed by the EPA. Table 1 shows the list of pharmaceuticals studied here.

Table 1. Analytes Studied in This Work

List of Group 1 Compounds EPA 1694: 46 Analytes

Acetaminophen	Codeine	Flumequine	Penicillin V	Sulfanilamide
Ampicillin	Cotinine	Fluoxetine	Roxithromycin	Thiabendazole
Azithromycin	Dehydronifedipine	Lincomycin	Sarafloxacin	Trimethoprim
Caffeine	Digoxigenin	Lomefloxacin	Sulfachloropyridazine	Tylosin
Carbadox	Diltiazem	Miconazole	Sulfadiazine	Virginiamycin
Carbamazepine	1,7-Dimethylxanthine	Norfloxacin	Sulfadimethoxine	Digoxin*
Cefotaxime	Diphenhydramine	Ofloxacin	Sulfamerazine	
Ciprofloxacin	Enrofloxacin	Oxacillin	Sulfamethazine	
Clarithromycin	Erythromycin	Oxolinic acid	Sulfamethizole	
Cloxacillin	Erythromycin anhydrate	Penicillin G	Sulfamethoxazole	

*Compound formed intractable Na adduct with current conditions.

List of Group 2, 3, and 4 Compounds: EPA 1694: 19 Analytes

Anhydrotetracycline (2)	Doxycycline (2)	Minocycline (2)	Triclocarban (3) Triclosan (3) Warfarin (3)
Chlorotetracycline (2)	4-Epianhydrotetracycline (2)	Tetracycline(2) Meclocycline (2)	Albuterol (4) Cimetidine (4) Metformin (4)
Demeclocycline(2)	4-Epitetracycline(2)	Gemfibrozil (3) Ibuprofen (3) Naproxen (3)	Ranitidine (4)

List of Labeled Internal Standards

$^{13}C_2$-^{15}N-Acetaminophen	$^{13}C_2$-Erythromycin	$^{13}C_6$-Sulfamethazine	$^{13}C_3$-Trimethoprim
$^{13}C_3$-Atrazine	Fluoxetine-d_6	$^{13}C_6$-Sulfamethoxazole	Warfarin-d_5
$^{13}C_3$-Caffeine	Gemfibrozil-d_6	$^{13}C_6$-2,4,5-Tricloro-phenoxyacetic acid	Carbamazepine-d_{10} (Extra compound, not EPA list)
$^{13}C_3$-^{15}N-Ciprofloxacin	$^{13}C_3$-Ibuprofen	$^{13}C_6$-Triclocarban	
Cotinine-d_3	^{13}C-Naproxen-d_3	$^{13}C_{12}$-Triclosan	

Experimental

Sample Preparation

Pharmaceutical analytical standards were purchased from Sigma, (St. Louis, MO). All stable isotope labeled compounds used as internal standards were obtained from Cambridge Isotope Laboratories (Andover, MA). Individual pharmaceutical stock solutions (approximately 1,000 µg/mL) were prepared in pure acetonitrile or methanol, depending on the solubility of each individual compound, and stored at –18 °C. From these solutions, working standard solutions were prepared by dilution with acetonitrile and water.

Water samples were collected from the wastewater treatment plant at the Boulder Creek outfall (Boulder, CO) and extracted as per the EPA method. Agilent has introduced a polymeric SPE sorbent with hydrophilic/lipophilic properties that may also be appropriate for this application. "Blank" wastewater extracts were used to prepare the matrix-matched standards for validation purposes. The wastewater extracts were spiked with the mix of pharmaceuticals at different concentrations (ranging from 0.1 to 500 ng/mL or ppb) and subsequently analyzed by LC/MS/MS.

LC/MS/MS Instrumentation

The analytes were subdivided in groups (according to EPA protocol for sample extraction) and LC conditions for the chromatographic separation of each group are as follows.

LC Conditions for Group 1-acidic extraction, positive electrospray ionization (ESI+) instrument conditions

Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm, 3.5 µ (p/n 959793-902)
Column temperature	25 °C
Mobile phase	10% ACN and 90% H_2O with 0.1% HCOOH
Flow rate	0.2–0.3 mL/min
Gradient	t_0 = 10% ACN, 0.2 mL/min t_5 = 10% ACN, 0.2 mL/min t_6 = 10% ACN, 0.3 mL/min t_{24} = 60% ACN, 0.3 mL/min t_{30} = 100% ACN
Injection volumes	15 µL

LC conditions for Group 2-acidic extraction, positive electrospray ionization (ESI+) instrument conditions

Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm, 3.5 µ (p/n 959793-902)
Column temperature	25 °C
Mobile phase	10% ACN and 90% H_2O with 0.1% HCOOH
Flow rate	0.2 mL/min
Gradient	t_0 = 10% ACN t_{10} = 10% ACN t_{30} = 100% ACN
Injection volumes	15 µL

LC conditions for Group 3-acidic extraction, negative electrospray ionization (ESI–) instrument conditions

Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm, 3.5 µ (p/n 959793-902)
Column temperature	25 °C
Mobile phase	40% MeOH and 60% H_2O with 5 mM ammonium acetate, pH 5.5
Flow rate	0.2 mL/min
Gradient	$t_{0.5}$ = 40% MeOH t_7 = 100% MeOH
Injection volumes	15 µL

LC conditions for Group 4-acidic extraction, positive electrospray ionization (ESI+) instrument conditions

Column	Agilent ZORBAX HILIC Plus 2.1 × 100 mm, 3.5 µm (p/n 959793-901 custom order until November 1, 2008)
Column temperature	25 °C
Mobile phase	98% ACN and 2% H_2O with 10 mM ammonium acetate, pH 6.7
Flow rate	0.25 mL/min
Gradient	t_0 = 98% ACN t_5 = 70% ACN t_{12} = 70% ACN
Injection volumes	15 µL

The mass spectrometer conditions were general to all groups and are as follows.

MS Conditions

Mode	Positive and negative (depending on group) ESI using the Agilent G6410A Triple Quadrupole mass spectrometer
Nebulizer	40 psig
Drying gas flow	9 L/min
V capillary	4000 V
Drying gas temperature	300 °C
Fragmentor voltage	70–130 V
Collision energy	5–35 V
MRM	2 transitions for every compound as shown in Table 1
Dwell time	10 msec

Results and Discussion

Optimization of LC/MS/MS Conditions

The initial study consisted of two parts. First was to optimize the fragmentor voltage for each of the pharmaceuticals studied in order to produce the largest signal for the precursor ion. Typically the protonated molecule was used for the precursor ion. Each compound was analyzed separately using an automated procedure (MassHunter Optimizer software, Agilent Technologies, Santa Clara, CA) to check the fragmentor at each voltage. The data was then selected for optimal fragmentor signal and each compound was optimized again to determine automatically the collision energies for both the quantifying and qualifying ions. Optimal collision energies varied between 5 and 35 V. The MRM transitions and optimized energies used for this study are shown in Tables 2A to 2D.

Table 2A. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 1 (The labeled standards are bold.)

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Acetaminophen	90	152 → 110 152 → 65	15 35
$^{13}C_2$-^{15}N-Acetaminophen	**90**	**155 → 111** **155 → 93**	**15** **25**
Ampicillin	70	350 → 160 350 → 106	10 15
$^{13}C_3$-Atrazine	**120**	**219 → 177** **219 → 98**	**15** **25**
Azithromycin	130	749.5 → 591.4 749.5 → 158	30 35
Caffeine	110	195 → 138 195 → 110	15 25
$^{13}C_3$-Caffeine	**110**	**198 → 140** **198 → 112**	**15** **25**
Carbadox	80	263 → 231 263 → 130	5 35
Carbamazepine	110	237 → 194 237 → 179	15 35
Carbamazepine-d_{10}	**110**	**247 → 204** **247 → 202**	**15** **35**
Cefotaxime	90	456 → 396 456 → 324	5 5
Ciprofloxacin	110	332 → 314 332 → 231	20 35
$^{13}C_3$-^{15}N-Ciprofloxacin	**110**	**336 → 318** **336 → 235**	**15** **35**

Table 2A. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 1 (The labeled standards are bold.) continued

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Clarithromycin	110	748.5 → 158 748.5 → 590	25 15
Cloxacillin	90	436 → 160 436 → 277	15 15
Codeine	130	300 → 215 300 → 165	25 35
Cotinine	90	177 → 98 177 → 80	25 25
Cotinine-d_3	**90**	**180 → 80** **180 → 101**	**25** **25**
Dehydronifedipine	130	345 → 284 345 → 268	25 25
Digoxigenin	90	391 → 355 391 → 337	15 15
Digoxin	No response, Na adduct		
Diltiazem	130	415 → 178 415 → 150	25 25
1,7-Dimethylxanthine	90	181 → 124 181 → 99	15 15
Diphenhydramine	70	256 → 167 256 → 152	15 35
Enrofloxacin	130	360 → 316 360 → 342	15 15
Erythromycin	90	734.5 → 158 734.5 → 576	35 15
$^{13}C_2$-Erythromycin	**90**	**736.5 → 160** **736.5 → 578**	**25** **15**
Erythromycin anhydrate	90	716.5 → 158 716.5 → 116	25 25
Flumequine	90	262 → 174 262 → 244	35 15
Fluoxetine	90	310 → 148	5
Fluoxetine-d_6	**90**	**316 → 154**	**5**
Lincomycin	110	407 → 126 407 → 359	25 15
Lomefloxacin	130	352 → 308 352 → 265	15 25
Miconazole	90	415 → 159 415 → 69	35 25
Norfloxacin	70	320 → 302 320 → 276	15 15
Ofloxacin	110	362 → 318 362 → 261	15 25

Table 2A. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 1 (The labeled standards are bold.) continued

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Oxacillin	70	402 → 160 402 → 243	15 5
Oxolinic acid	90	262 → 244 262 → 216	15 25
Penicillin G	90	335 → 160 335 → 176	5 5
Penicillin V	70	351 → 160 351 → 114	5 25
Roxithromycin	130	837.5 → 679 837.5 → 158	15 35
Sarafloxacin	130	386 → 299 386 → 368	25 25
Sulfachloropyridazine	90	285 → 156 285 → 92	10 25
Sulfadiazine	110	251 → 156 251 → 92	15 25
Sulfadimethoxine	80	311 → 156 311 → 92	20 35
Sulfamerazine	110	265 → 156 265 → 92	15 25
Sulfamethazine	90	279 → 156 279 → 186	15 15
$^{13}C_6$-Sulfamethazine	**90**	**285 → 186** **285 → 162**	**25** **25**
Sulfamethizole	80	271 → 156 271 → 92	10 25
Sulfamethoxazole	110	254 → 156 254 → 92	15 25
$^{13}C_6$-Sulfamethoxazole	**110**	**260 → 162** **260 → 98**	**15** **25**
Sulfanilamide	70	173 → 156 173 → 92	5 15
Thiabendazole	130	202 → 175 202 → 131	25 35
$^{13}C_6$-2,4,5-Trichlorophenoxyacetic acid	**110**	**259 → 201** **259 → 165**	**5** **25**
Trimethoprim	110	291 → 230 291 → 261	25 25
$^{13}C_3$-Trimethoprim	**110**	**294 → 233** **294 → 264**	**25** **25**
Tylosin	110	916.5 → 174 916.5 → 772	35 35
Virginiamycin	110	526 → 508 526 → 355	5 15

Table 2B. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 2

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Anhydrotetracycline	90	427 → 410 427 → 154	15 25
Chlorotetracycline	110	479 → 462 479 → 197	15 35
Demeclocycline	130	465 → 430 465 → 448	25 15
Doxycycline	110	445 → 428 445 → 154	15 25
4-Epianhydrotetracycline (EATC)	90	427 → 410 427 → 105	15 35
4-Epitetracycline (ETC)	110	445 → 410 445 → 427	15 5
Minocycline	90	458 → 441	15
Tetracycline (TC)	110	445 → 410 445 → 427	15 5

Table 2C. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 3

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Gemfibrozil	100	249 → 121	5
Gemfibrozil-d_6	**100**	**255 → 121**	**5**
Ibuprofen	75	205 → 161	5
$^{13}C_3$-Ibuprofen	**75**	**208 → 163**	**5**
Naproxen	75	229 → 169 229 → 170	25 5
^{13}C-Naproxen-d_3	**75**	**233 → 169** **233 → 170**	**25** **5**
Triclocarban	100	313 → 160 313 → 126	10 25
$^{13}C_6$-Triclocarban	**90**	**319 → 160** **319 → 132**	**5** **25**
Triclosan	75	287 → 35	5
$^{13}C_{12}$-Triclosan	**75**	**299 → 35**	**5**
Warfarin	125	307 → 117 307 → 161	35 15
Warfarin-d_5	**90**	**312 → 161** **312 → 255**	**15** **25**

Table 2D. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 4

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Albuterol (Salbutamol)	90	240 → 148	15
		240 → 166	5
Cimetidine	100	253 → 159	10
		253 → 95	25
Metformin	80	130 → 60	10
		130 → 71	25
Ranitidine	110	315 → 176	15
		315 → 130	25

Chromatographic separation was done independently for each group and a dwell time of 10 msec was used for every MRM transition. Figures 1A to 1D show the chromatograms corresponding to 100 ppb standard on column for all the pharmaceuticals studied. Extracted ion chromatograms are overlaid for each one of the target analytes according to their respective protonated molecule and product-ion MRM transitions.

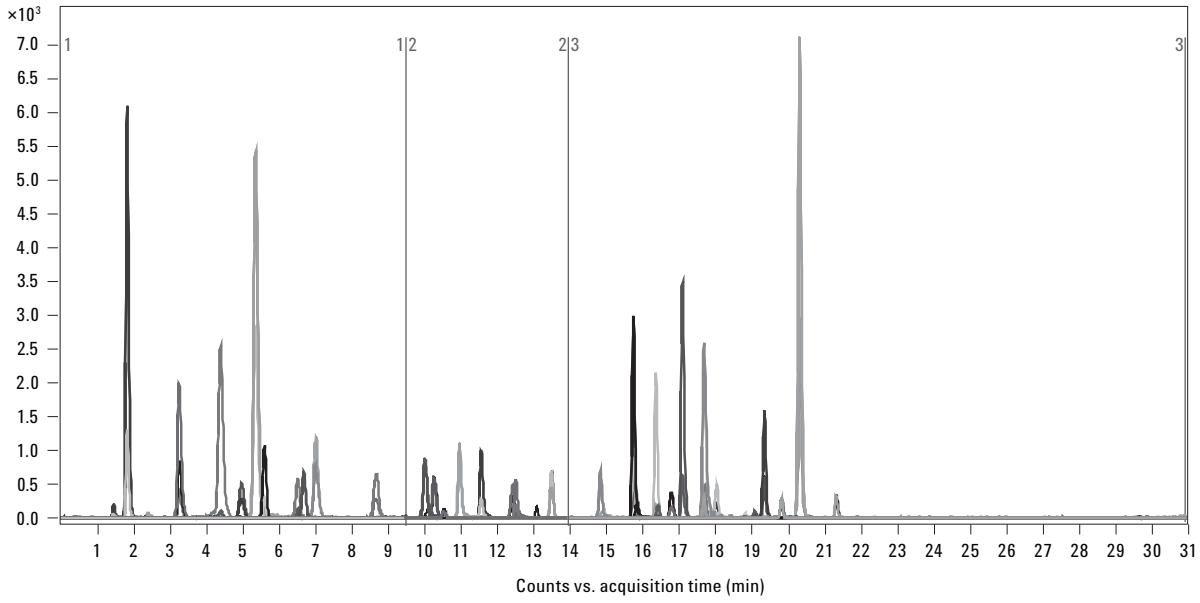

Figure 1A. MRM extracted chromatogram for pharmaceuticals in Group 1. Three time segments were used in this chromatographic separation.

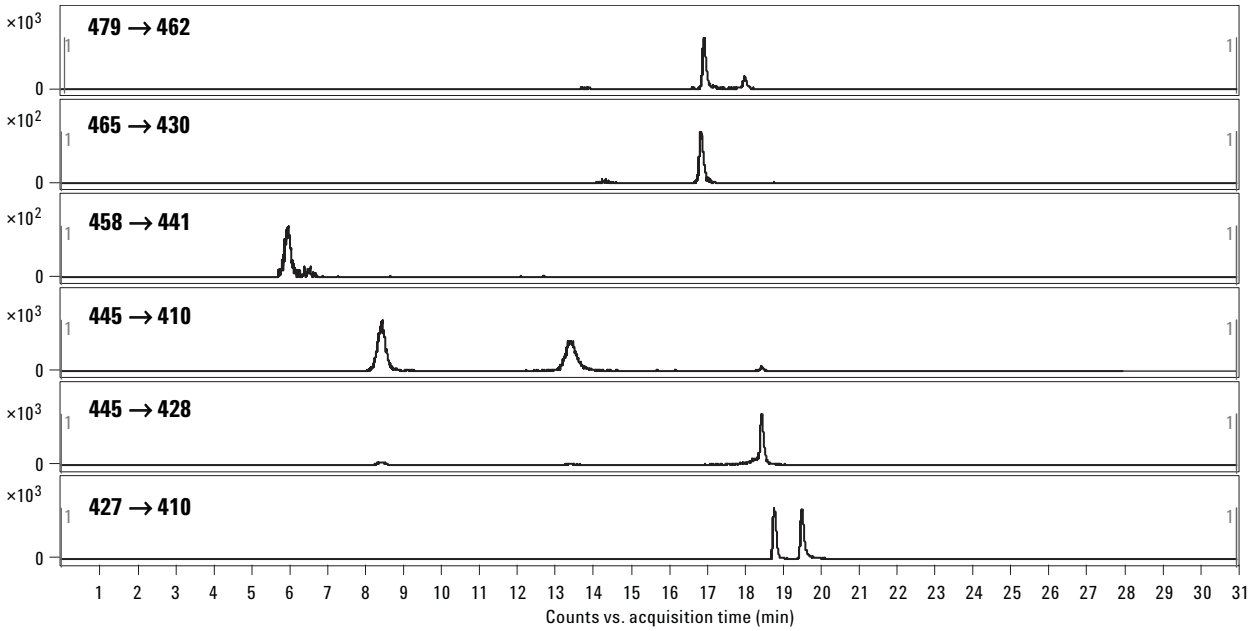

Figure 1B. MRM extracted chromatogram for pharmaceuticals in Group 2. Only one transition shown. See Table 2B for compound identification.

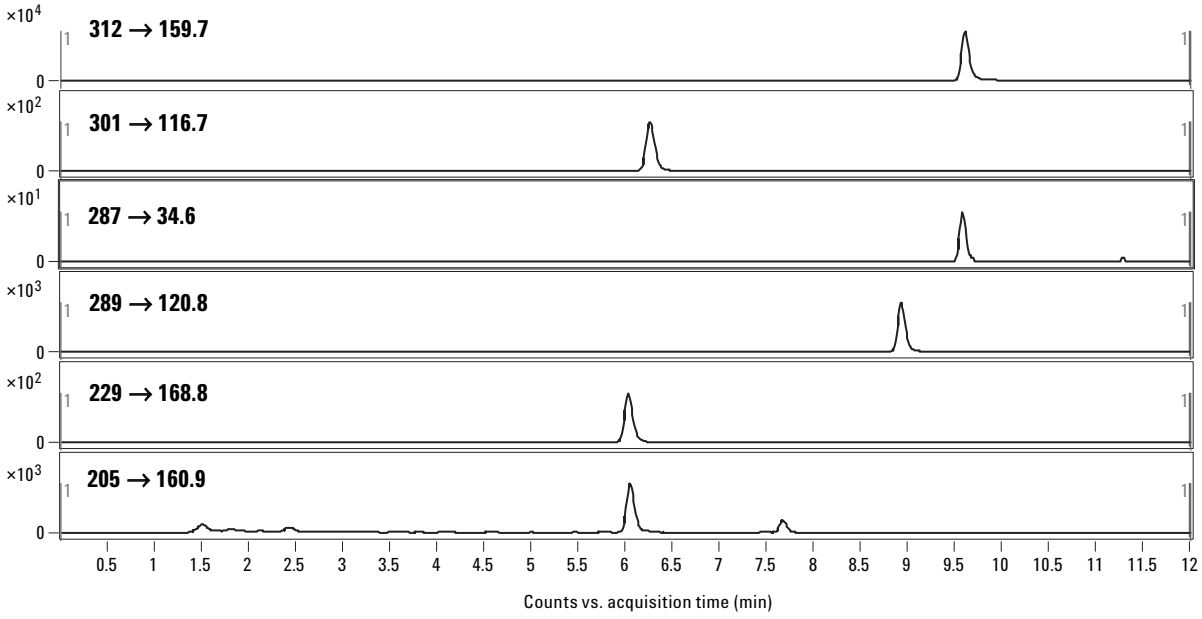

Figure 1C. MRM extracted chromatogram for pharmaceuticals in Group 3. Only one transition shown. See Table 2C for compound identification.

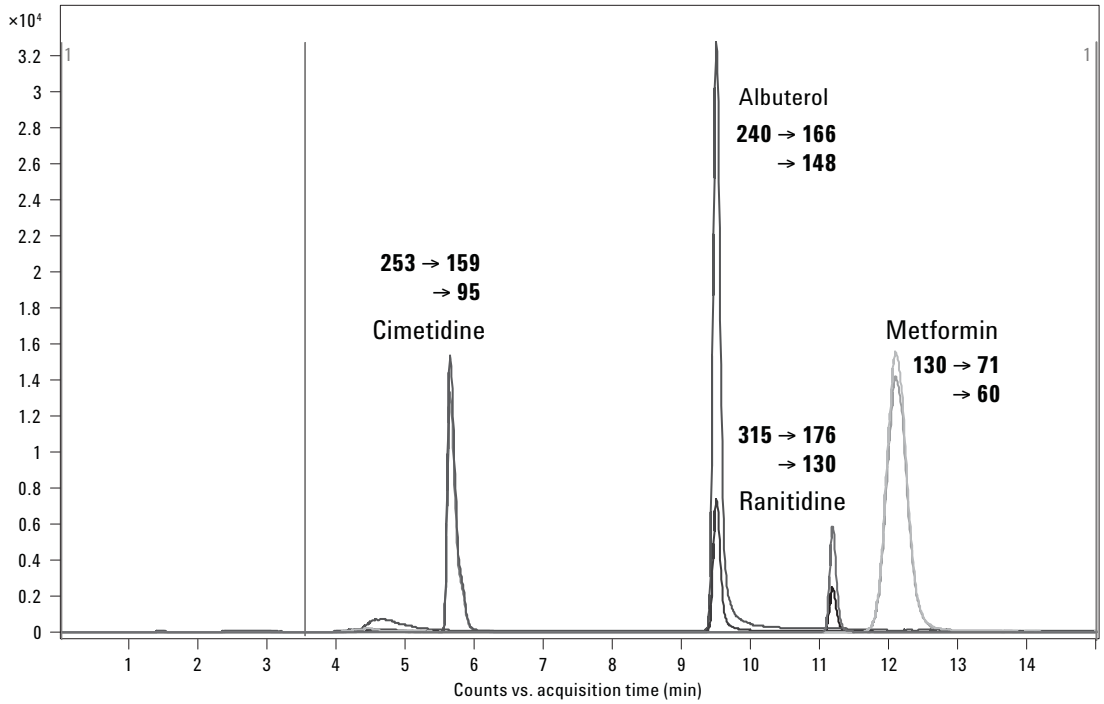

Figure 1D. MRM extracted chromatogram for pharmaceuticals in Group 4.

Application to Wastewater Samples

To confirm the suitability of the method for analysis of real samples, matrix-matched standards were analyzed in a wastewater matrix from an effluent site, at eight concentrations (0.1, 0.5, 1, 5, 10, 50, 100, and 500 ng/mL or ppb concentrations). Figure 2 shows an example standard curve for acetaminophen in the wastewater matrix. In general, all compounds gave linear results with excellent sensitivity over three orders of magnitude, with r^2 values of 0.99 or greater.

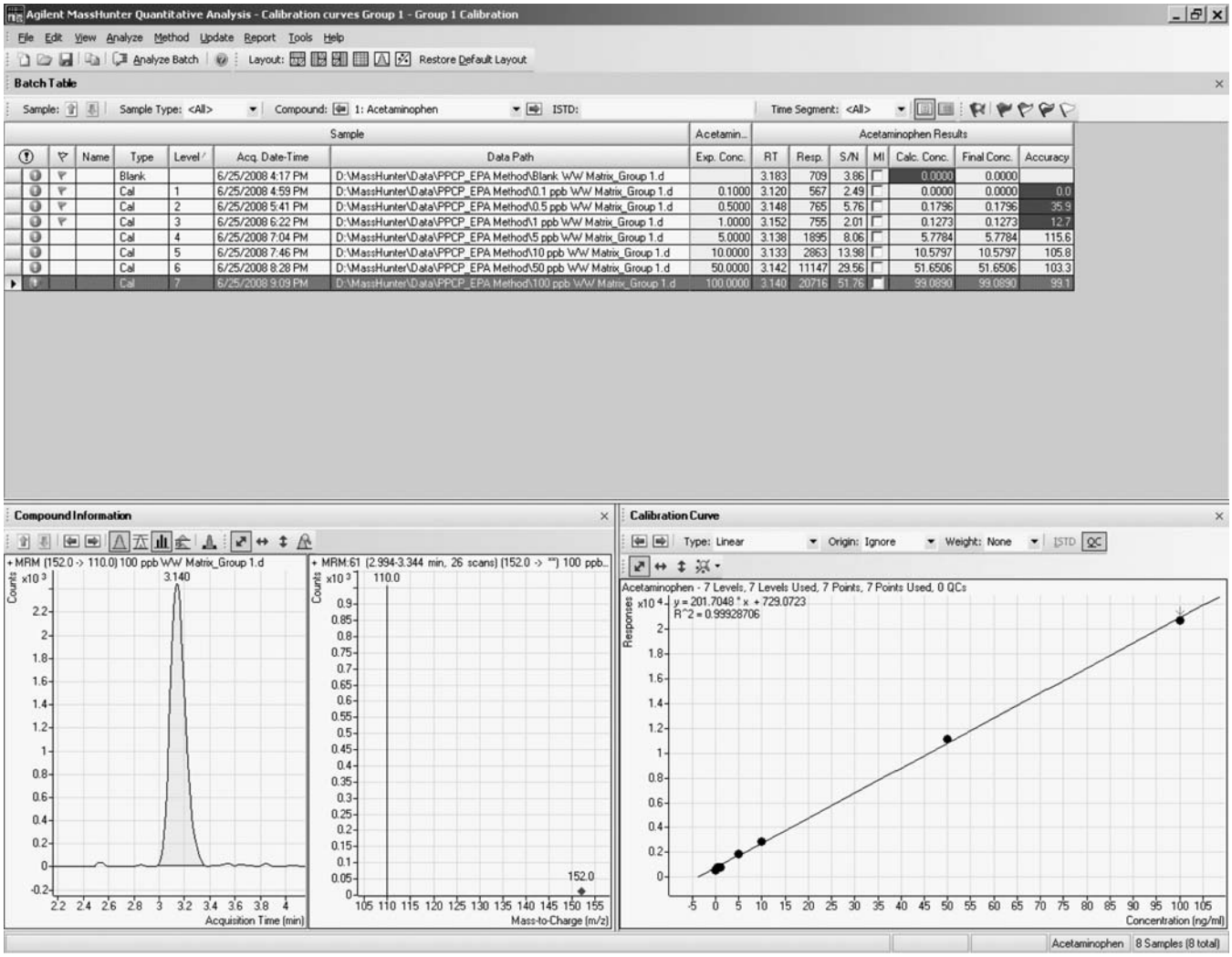

Figure 2. Calibration curve for acetaminophen in a wastewater matrix using a seven-point curve from 0.1 to 100 ng/mL (ppb) using a linear fit with no origin treatment.

Finally, a "blank" wastewater sample was analyzed and the presence of two pharmaceuticals, carbamazepine and diphenhydramine, could be confirmed with two MRM transitions. Figure 3 shows the ion ratios qualifying for these two compounds in a wastewater extract. As shown in Figure 3 in the two ion profiles, both pharmaceuticals were easily identified in this complex matrix due to the selectivity of the MRM transitions and instrument sensitivity.

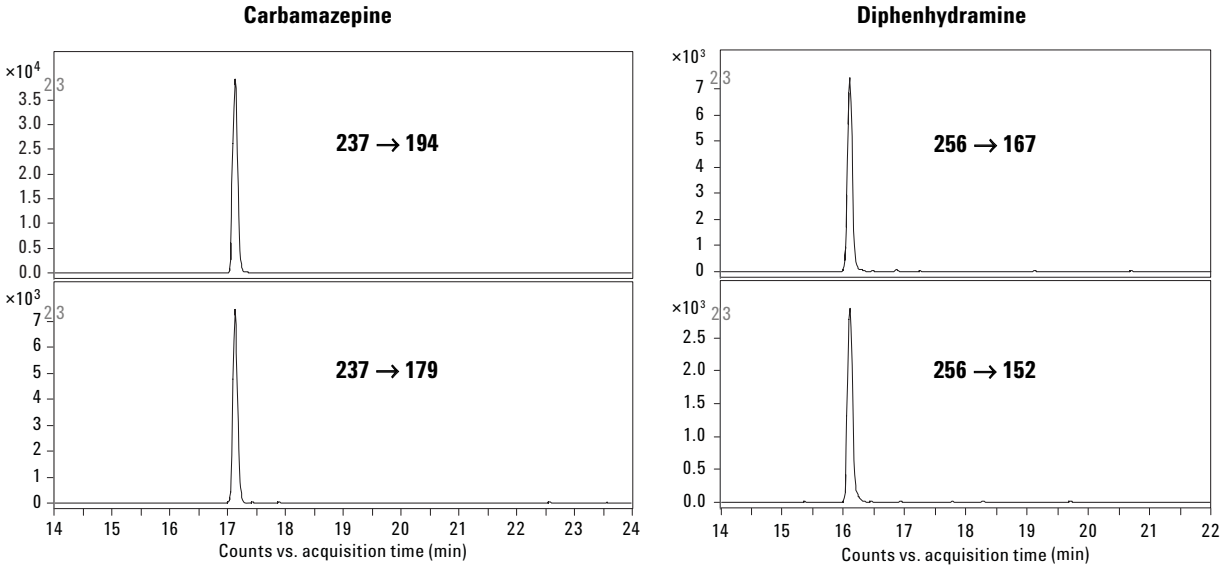

Figure 3. MRM chromatograms of a wastewater sample for carbamazepine and diphenhydramine using two transitions.

Conclusions

The results of this study show that the Agilent 6410A Triple Quadrupole is a robust, sensitive, and reliable instrument for the study of pharmaceuticals in water samples, using high throughput methods. The Agilent 6410A Triple Quadrupole has been shown to be a successful instrument for the implementation of EPA Method 1694.

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Printed in the USA
September 19, 2008
5989-9665EN

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Semivolatile Organics Analysis Using an Agilent J&W HP-5ms Ultra Inert Capillary GC Column

Application Note

Environmental

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Abstract

Trace-level semivolatile organics analyses using methods such as USEPA Method 8270 are important tools for assessing environmental contaminants worldwide. The wide-ranging chemical diversity of target semivolatiles can prove chromatographically challenging. This application note demonstrates the benefits of using an Agilent J&W HP-5ms Ultra Inert Capillary GC column with electron impact single quadrupole scanning mass spectrometry for trace-level semivolatiles analysis.

Agilent Technologies has implemented new testing procedures to more effectively evaluate GC column inertness performance. These new testing procedures employ deliberately aggressive probes to thoroughly investigate column inertness and quality. These aggressive probes, including 1-propionic acid, 4-picoline, and trimethyl phosphate, are used to verify each column's inertness performance.

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Introduction

USEPA Method 8270 [1] is a commonly used method for detecting semivolatile organic compounds in environmental samples by GC/MS. This method encompasses several classes of analytes, including amines, alcohols, polycyclic aromatic hydrocarbons, and phenols. The acidic and basic nature of many of the analytes makes minimizing any column or instrument activity critical to good chromatography and reliable results.

Minimizing activity in the GC column is essential in maximizing an analyte's response. Nitrophenols are among the most active compounds in semivolatiles series. 2,4-Dinitrophenol in particular is notorious for showing low response through adsorption onto active sites in the flow path during analysis. At low concentrations, the response factor (RF) for 2,4-dinitrophenol can fall below the minimum average RF of 0.050 required by USEPA 8270 due to interaction between the analyte and sample flow path. Capillary GC column activity as a potential source of result uncertainty has been effectively eliminated with the Ultra Inert series of columns.

A custom standard containing an abbreviated list of analytes specific to USEPA Method 8270 was analyzed to evaluate column performance. This semivolatiles "short mix" contained n-nitrosodimethylamine, aniline, benzoic acid, 2,4-dinitrophenol, 4-nitrophenol, 2-methyl-4,6-dinitrophenol, pentachlorophenol, 4-aminobiphenyl, benzidine, 3,3'dichlorobenzidine, benzo[b]fluoranthene, and benzo[k]fluoranthene, along with the recommended internal standards. These target analytes were chosen based on their chemical activity, as well as their poor chromatographic behavior. The short mix is particularly useful for rapid evaluation of system performance for semivolatiles analysis. Challenging analytes from early-eluting nitrosoamines through late-eluting PAHs are represented in this mix and chromatographic performance can be assessed quickly.

A second "large mix" standard containing a broader selection of semivolatiles was also evaluated to show the Ultra Inert's performance when analyzing a more complex sample. This standard contained a variety of acidic, basic, and neutral groups, which ranged from very low-boiling components to high-boiling polycyclic aromatic hydrocarbons.

Experimental

An Agilent 6890N GC/5975B MSD equipped with a 7683B autosampler was used for this series of experiments. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions for EPA Method 8270 Calibration Standards

GC:	Agilent 6890N/5975B MSD
Sampler:	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5181-1273) 1.0 µL splitless injection
Carrier:	Helium 30 cm/s, constant flow
Inlet:	Splitless; 260 °C, purge flow 50 mL/min at 0.5 min
	Gas saver 80 mL/min at 3 min
Inlet liner:	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column:	Agilent HP-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm (Agilent p/n 19091S-433UI)
Oven:	40 °C (1 min) to 100 °C (15 °C/min), 10 °C/min to 210 °C (1 min), 5 °C/min to 310 °C, hold 8 min
Detection:	MSD source at 300 °C, quadrupole at 180 °C, transfer line at 290 °C, scan range 45 to 450 amu

Table 2. Flow Path Supplies

Vials:	Amber screw top glass vials (Agilent p/n 5183-2072)
Vial caps:	Blue screw caps (Agilent p/n 5182-0723)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe:	5 µL (Agilent p/n 5181-1273)
Septum:	Advanced Green (Agilent p/n 5183-4759)
Inlet liners:	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
20x magnifier:	20x magnifier loupe (Agilent p/n 430-1020)

Sample Preparation

A 12-component custom semivolatiles mix was purchased from Ultra Scientific (Kingston, RI) and used to prepare a seven-level calibration standard set. The stock semivolatiles solution as delivered had a nominal concentration of 2,000 µg/mL. An internal standard mix as recommended by USEPA Method 8270 was purchased from AccuStandard (New Haven, CT). The internal/surrogate solution as delivered had a

nominal concentration of 4,000 µg/mL. The calibration standards were prepared with component and internal standard concentrations of 80, 40, 20, 10, 5, 2, and 1 µg/mL. All solutions were prepared in dichloromethane using class A volumetric pipettes and flasks. The dichloromethane used was Burdick and Jackson spectral grade purchased thorough VWR International (West Chester, PA). Dichloromethane was used as a reagent blank and syringe wash solvent.

The EPA 8270 Calibration Level 2 standard set was purchased from AccuStandard containing 83 semivolatile components and internal standards. The large mix calibration standard was prepared at an analyte concentration of 5 µg/mL.

Results and Discussion

Baseline Inertness Profile for Ultra Inert Columns

The basic approach for inertness verification for the Agilent J&W Ultra Inert series of capillary GC columns is testing with aggressive active probes at low concentration and low temperature. This is a rigorous approach that establishes consistent baseline inertness profiles for each column in the Agilent J&W Ultra Inert GC column series. The baseline inertness profile then serves as a predictor for successful analysis of chemically active species that tend to adsorb onto active sites, particularly at trace level like the semivolatiles in this application example. A more detailed description of the test mix and additional application examples can be found in references 2 through 7.

Semivolatiles Analysis (USEPA 8270)

In this application note a seven-level semivolatile calibration curve set was evaluated over the concentration range of 1 to 80 µg/mL on an Agilent J&W Ultra Inert HP-5ms 30 m × 0.25 mm × 0.25 µm (p/n 19091S-433UI). An example chromatogram of a 1-µL injection of the 1 µg/mL short mix calibration standard is shown in Figure 1. Scanning mode was used exclusively for this analysis.

Pentachlorophenol and benzidine are two components that are used to verify inlet and column inertness. Excessive peak tailing of these components would indicate column activity. Analysis of the short mix standard yielded sharp, symmetrical peak shapes for the problematic analytes as shown in Figure 2. Good separation was obtained in the analysis of the 5-ng on-column 8270 large mix standard for each of the semivolatiles, which is shown in Figure 3.

Semivolatile analysis by USEPA Method 8270 requires a minimum average RF of 0.050 for a system performance check compound such as 2,4-dinitrophenol. 2,4-Dinitrophenol is a highly active analyte that has proven to be one of the most challenging compounds, often yielding lower than expected

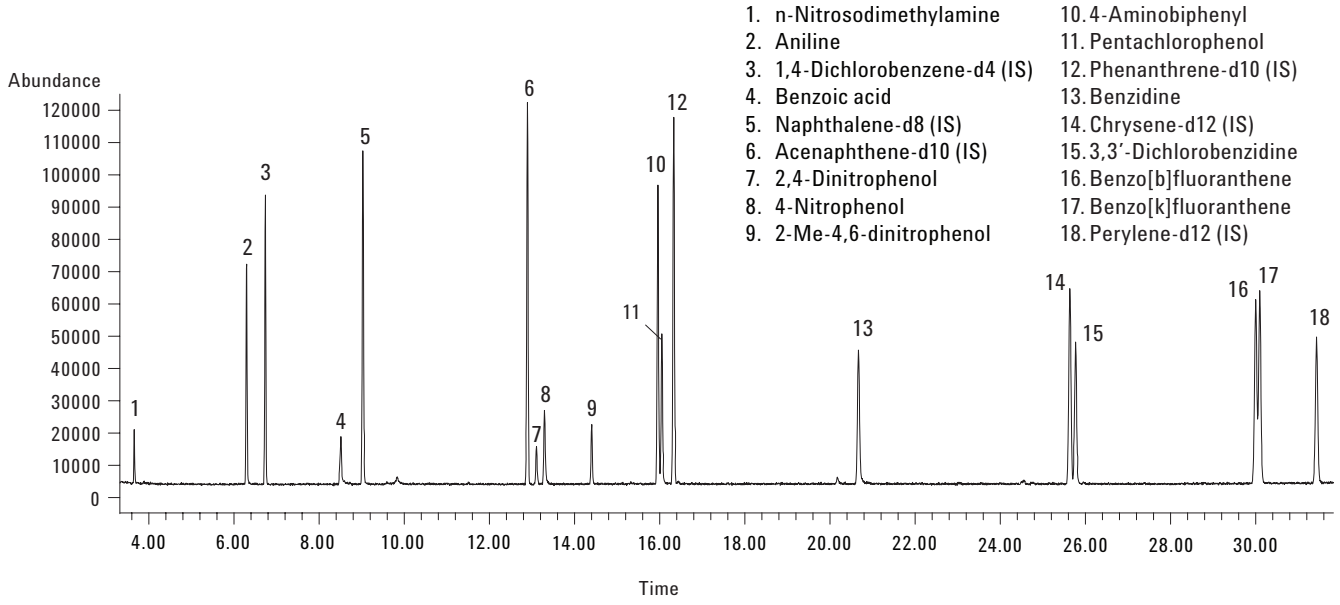

Figure 1. Total ion chromatogram (SCAN mode) of the 1-ng on-column EPA8270 short mix standard solution loading on an Agilent J&W HP-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 19091S-433UI). Chromatographic conditions are listed in Table 1.

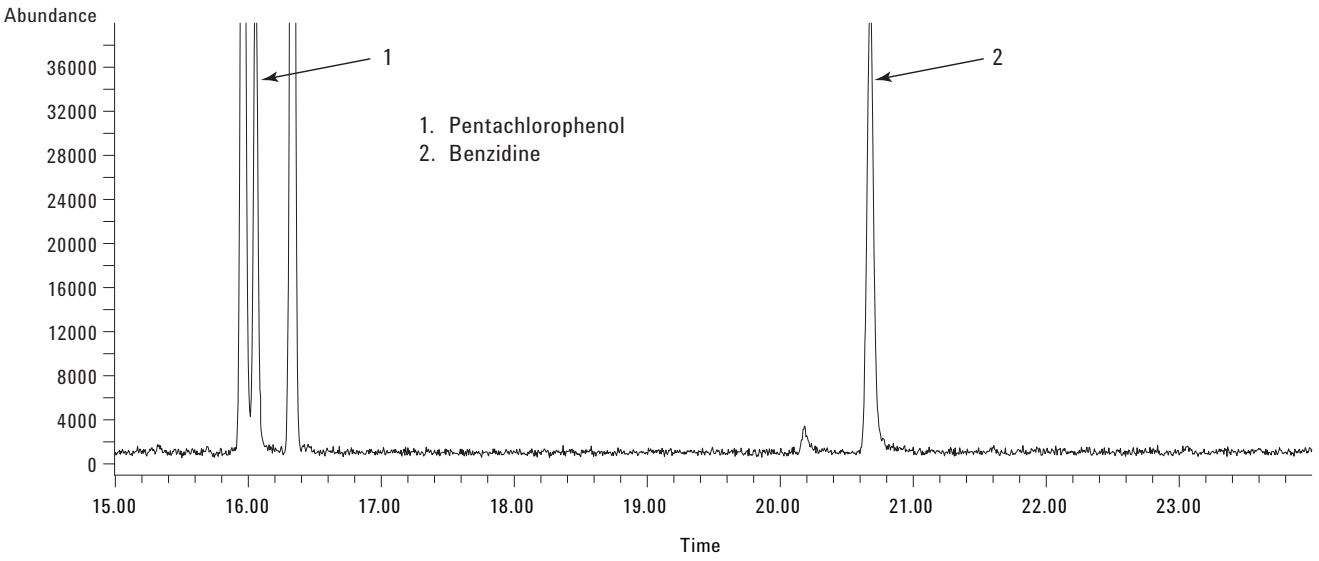

Figure 2. Enlarged section of the total ion chromatogram for a 1-µL injection of 1.0 µg/mL EPA 8270 short mix standard. The peaks of interest noted in the figure are two semivolatiles that are prone to peak tailing. Chromatographic conditions are listed in Table 1.

1. n-Nitrosodimethylamine
2. Pyridine
3. 2-Fluorophenol
4. Phenol-d5
5. Phenol
6. Aniline
7. Bis(2-chloroethyl)ether
8. 2-Chlorophenol
9. 1,3-Dichlorobenzene
10. 1,4-Dichlorobenzene-D4
11. 1,4-Dichlorobenzene
12. Benzyl alcohol
13. 1,2-Dichlorobenzene
14. o-Cresol
15. Bis(2-chloroisopropyl)ether
16. p-Cresol
17. n-Nitroso-di-n-propylamine
18. Hexachloroethane
19. Nitrobenzene-d5
20. Nitrobenzene
21. Isophorone
22. 2-Nitrophenol
23. 2,4-Dimethylphenol
24. Benzoic acid
25. Bis(2-chloroethoxy)methane
26. 2,4-Dichlorophenol
27. 1,2,4-Trichlorobenzene
28. Naphthalene-d8
29. Naphthalene
30. 4-Chloroaniline
31. Hexachlorobutadiene
32. 4-Chloro-3-methylphenol
33. 2-Methylnaphthalene
34. Hexchlorocyclopentadiene
35. 2,4,6-Trichlorophenol
36. 2,4,5-Trichlorophenol
37. 2-Fluorobiphenyl
38. 2-Chloronaphthalene
39. 2-Nitroaniline
40. Dimethyl phthalate
41. 2,6-Dinitrotoluene
42. Acenaphthylene
43. 3-Nitroaniline
44. Acenaphthene-d10
45. Acenaphthene
46. 2,4-Dinitrophenol
47. 4-Nitrophenol
48. Dibenzofuran
49. 2,4-Dinitrotoluene
50. Diethyl phthalate
51. Fluorene
52. 4-Chlorophenyl phenyl ether
53. 4-Nitroaniline
54. 2-Me-4,6-dinitrophenol
55. n-Nitrosodiphenylamine
56. Azobenzene
57. 2,4,6-Tribromophenol
58. 4-Bromophenyl phenyl ether
59. Hexachlorobenzene
60. Pentachlorophenol
61. Phenanthrene-d10
62. Phenanthrene
63. Anthracene
64. Carbazole
65. Dibutylphthalate
66. Fluoranthene
67. Benzidine
68. Pyrene
69. p-Terphenyl-d14
70. Benzyl butyl phthalate
71. 3,3-Dichlorobenzidine
72. Benzo[a]anthracene
73. Chrysene-D12
74. Chrysene
75. Bis(2-ethylhexyl)phthalate
76. Di-n-octyl phthalate
77. Benzo[b]fluoranthene
78. Benzo[k]fluoranthene
79. Benzo[a]pyrene
80. Perylene-d12
81. Indeno[1,2,3,cd]pyrene
82. Dibenzo[a,h]anthracene
83. Benzo[g,h,i]perylene

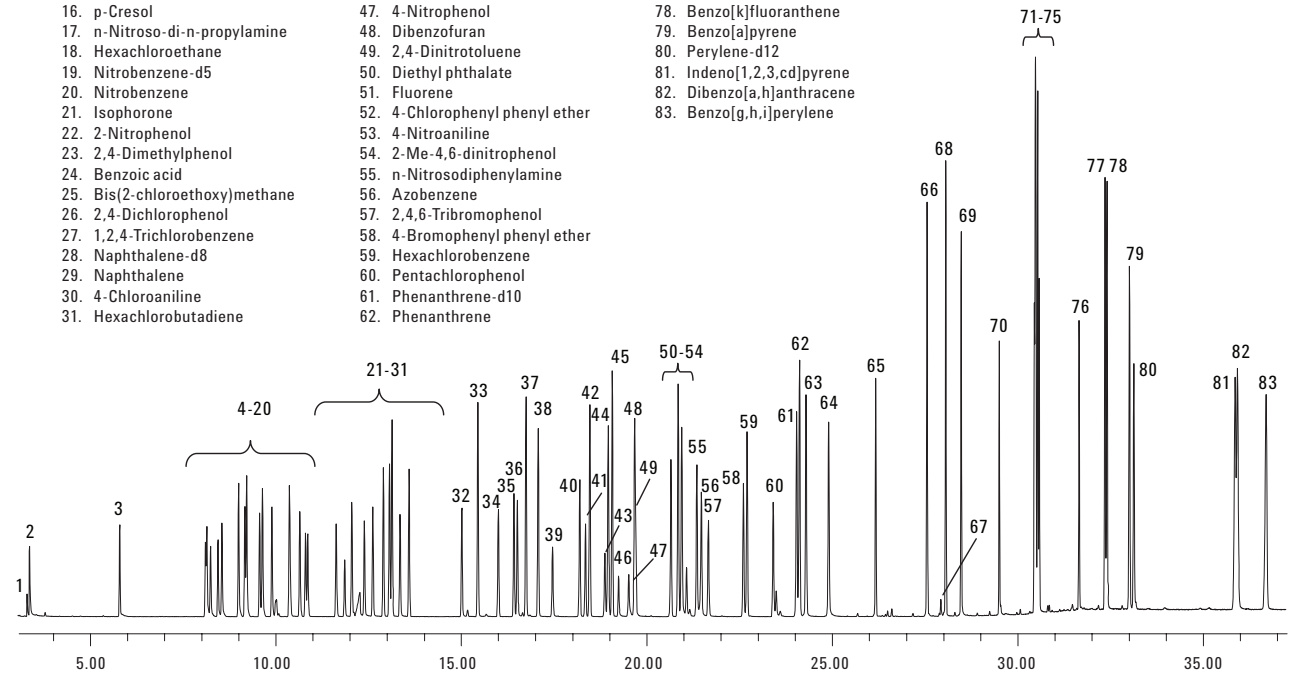

Figure 3. Total ion chromatogram (SCAN mode) of 5-ng on-column loading of EPA 8270 calibration (large mix) standard solution on an Agilent J&W HP-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 19091S-433UI). Chromatographic conditions are listed in Table 1.

response factors at lower concentrations. In the analysis of the short mix calibration standard, the response for 2,4-dinitrophenol was greater than 0.1 at the 1-ng level. The average response was 0.15 over the concentration range studied. An example chromatogram for the signal-to-noise ratio for a 1-ng on-column loading of 2,4-dinitrophenol is shown in Figure 4. The signal-to-noise ratio for this difficult analyte was greater than 16 to 1. This demonstrates the excellent performance of the HP-5ms Ultra Inert GC column.

Linearity was excellent across the range studied, giving R^2 values of 0.990 or greater for even the more difficult phenols. Figure 5 indicates the correlation coefficients for several of the more active analytes.

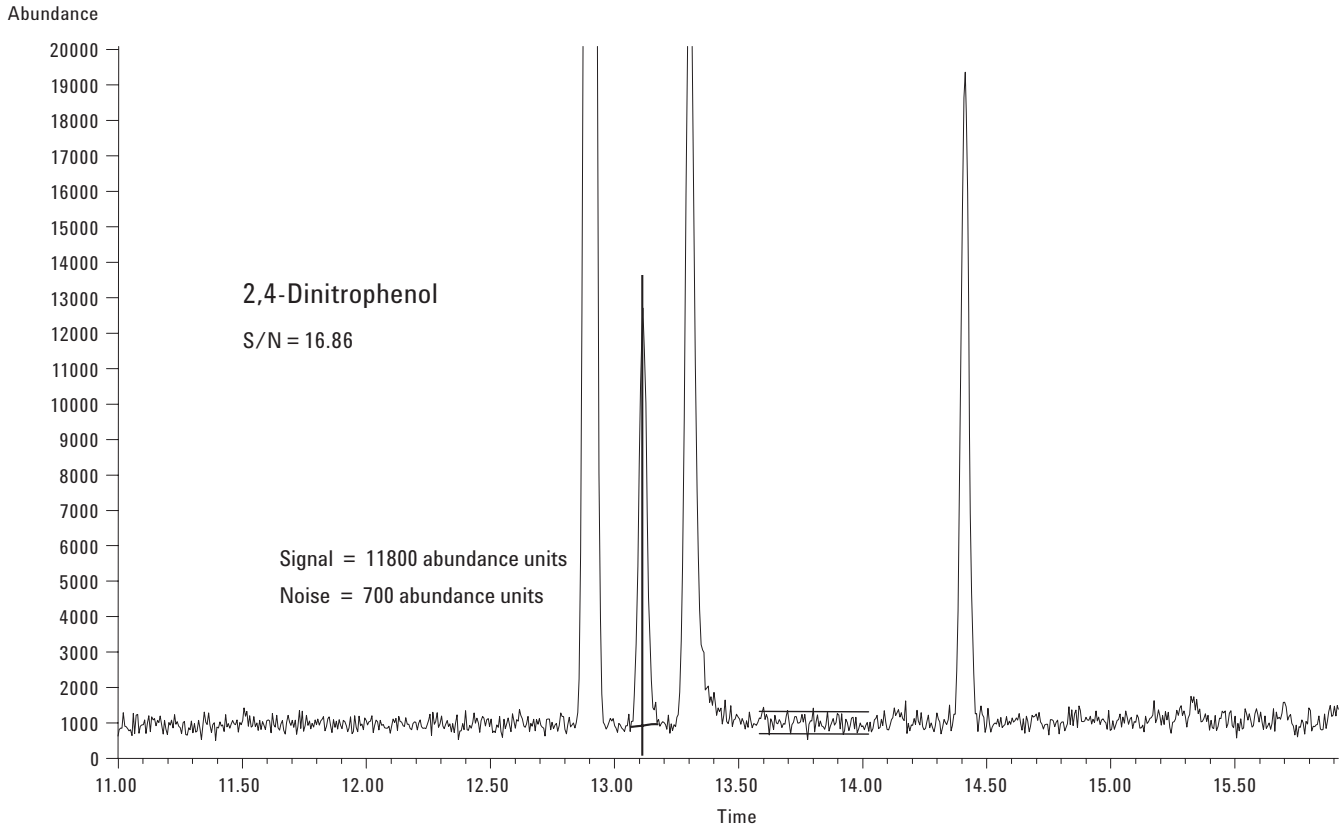

Figure 4 . Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 1 µg/mL EPA Method 8270 short mix standard on an Agilent J&W HP-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 19091S-433UI). The peak in the figure is 2,4-dinitrophenol, one of the more demanding semivolatiles. This injection represents an on-column loading of 1 ng per component. Chromatographic conditions are listed in Table 1.

	R^2
n-Nitrosodimethylamine	0.995
2,4-Dinitrophenol	0.990
4-Nitrophenol	0.995
Pentachlorophenol	0.995
Benzidine	0.995

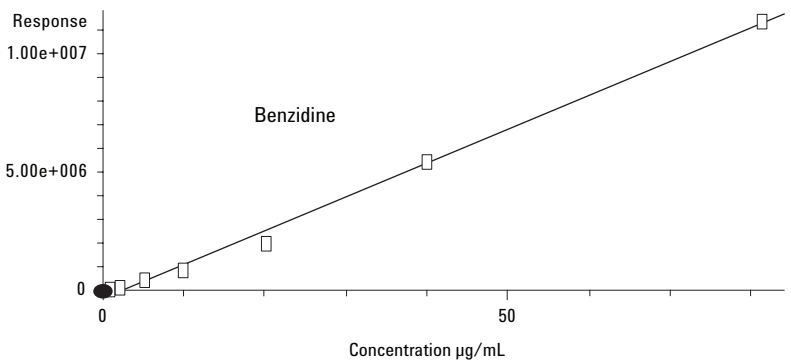

Figure 5. Correlation coefficients for some of the more challenging analytes in the EPA Method 8270 short mix standard over the 1 to 80 µg/mL range of this study and an example linear regression plot for benzidine.

Conclusions

This application successfully demonstrates the use of an Agilent J&W HP-5ms Ultra Inert capillary GC column for low-level semivolatile organics. Linearity was excellent for all semivolatiles studied, yielding 0.99 or greater R^2 values down to a 1-ng column loading of each component. One of the reasons for excellent linearity and high R^2 values is the highly inert surface of the column. The lack of chemically active sites makes these columns an excellent choice for semivolatiles analyses.

This study was done using SCAN mode on an Agilent 6890N/5975B GC/MSD equipped with an inert electron impact source. The signal-to-noise ratio for a 1-ng on-column loading of 2,4-dinitrophenol was greater than 16 to 1 with this system. This result clearly shows the power of using an Agilent J&W HP-5ms Ultra Inert column for low-level semivolatile organics analysis. Lower limits of quantification are expected when using one of Agilent's latest GC/MS offerings, such as the 7890A/5975C GC/MSD Triple-Axis Detector coupled with an Agilent J&W HP-5ms Ultra Inert GC capillary column.

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Published in the USA
January 20, 2009
5990-3416EN

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Determination of Sub-ppb Level of Phthalates in Water by Auto-SPME and GC-MS

Application

Environmental

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Abstract

A solid-phase microextraction (SPME) method for the analysis of phthalates in water samples was developed on the CTC CombiPAL autosampler GC-MS platform. In this method, the sample preparation process was automated by using a CombiPAL autosampler, including the SPME fiber precondition, adsorption, and desorption, which improve the precision of the SPME method. The extraction temperature, extraction time, and salt-out effect are also studied. The optimized condition was applied to the analysis of real samples. The detection limits of the phthalates in this method are at the sub-ppb level.

Introduction

Phthalic acid esters (phthalates, PAEs) are key additives in many plastics to keep the plastics soft at room temperature. Because phthalates are not chemically but only physically bound to the plastic structure, the phthalates can leach from the plastic products. Due to their widespread use, relatively large amounts of these compounds are released into the environment. In recent years, considerable attention has been paid to human exposure to phthalates because of their suspected carcinogenic and estrogenic properties.

Liquid-liquid extraction (LLE) techniques have been widely used to isolate of PAEs from aqueous samples. These procedures are typically time-consuming, labor-intensive, and use a large amount of solvent. Solid-phase microextraction (SPME) is a fast, sensitive, solventless, and economical sample preparation method for gas chromatography analysis. The main advantages of SPME compared to solvent extraction are the reduction in solvent use, the combination of extraction and analysis into one step, and the ability to examine smaller sample sizes. It can also provide high sensitivity and can be used for polar and nonpolar analytes in a wide range of matrices with direct injection to both the gas chromatograph (GC) and the liquid chromatograph (LC).

Extraction of analytes from aqueous samples can be performed either by direct immersion of the fiber into the liquid phase or by headspace sampling. Adsorbed analytes are then thermally desorbed in the injection port of a GC and analyzed using an appropriate column and detector.

The CombiPAL provides a fully automated SPME sample preparation process. All movements of the SPME fiber from precondition, adsorption, and desorption are software controlled for optimum precision. Prior and during extraction, the samples can be shaken and heated. This approach dramatically reduces sample preparation time for semi-volatile compounds. Variable vial penetration depth allows compound extraction to be performed in liquid phase or in the headspace. After the compounds are thermally desorbed in the hot GC injector, the fiber may be regenerated in a heated and purged cleaning station.

In this application, an automated SPME sample preparation process is demonstrated by using CombiPAL combined with GC-MS to determine plasticizers in a water sample.

Experimental

CombiPAL

Pre-incubation time:	60 s
Incubation temperature:	40 °C
Pre-inc. agitator speed:	500 rpm
Agitator on time:	5 s
Agitator off time:	2 s
Vial penetration:	25 mm
Extraction time:	1200 s
Desorb to:	GC Inj1
Injection penetration:	54 mm
Desorption time:	120 s
Post fiber condition time:	300 s

SPME

SPME fiber is from Supelco company (595 North Harrison Road Bellefonte, PA, USA), the fiber type is polydimethylsiloxane/divinylbenzene (PDMS/DVB) and the coating thickness is 65 µm.

6890 GC

Inlet temperature:	270 °C
Gas type:	Helium
Oven condition:	50 °C Ramps 10.00 °C /min to 260 °C (3.00 min)
Column:	DB-5ms 30 m × 250 mm, 0.25 µm
Mode:	Constant flow
Flow rate:	1.3 mL/min

5975 MS

Acquisition mode:	Synchronous SIM/scan
Mass range:	40–300
Sample:	3
Dwell time:	30 ms
MS source:	230 °C
MS quad:	150 °C

For other parameters, see Table 1.

The PAEs standards (shown in Table 1) were bought from Guo Yao Group (Shanghai, China). The PAEs were dissolved in methanol at a concentration of 1,000 ng/mL and diluted by MiliQ water to the tested concentration.

Results and Discussion

Because PAEs are semivolatile compounds, immersion extraction mode was selected, and the sample volume was 18 mL.

Lots of unrelated peaks emerged in GC chromatograms when the extraction temperature was over 40 °C, which would shorten the lifetime of fiber, so a compromise has to be made between the lifetime of the extraction phase and the rate of equilibrium. We chose 40 °C for all extractions in the following experiments.

The effect of extraction time versus amount extracted at 40 °C was studied. The extraction efficiency for different compounds was proportional to extraction time. Figure 1 shows the profile of extraction time versus response. As seen in Figure 1, when the extraction time was over 20 minutes, the responses changed slightly, which means that the extraction of most compounds reached equilibrium at this point. In this experiment, 20 minutes was selected as the extraction time.

Salting-out effects by adding NaCl in the sample were also studied. The results showed that the extraction efficiency of DEP, DMP, and DBP was improved when salt was added, and that of DCHP, DEHP, and DPP (see compound names in Table 1) was decreased as shown in Figure 2. In this experiment, 20% (W/V) salt concentration was chosen. Figure 3 shows the SIM chromatogram of PAEs at the optimized condition. The chromatogram shows that improvements can be made to shorten the analysis time by adjusting the oven program.

Table 1. Compound Information

Compound name	Abbreviation	Retention time (min)	SIM ions
Phthalic acid, bis-n-pentyl ester	DPP	10.179	135, 149, 163, 177
Phthalic acid, bis-isononyl ester	DEHP	11.862	93, 105, 149, 177
Di-cyclohexyl phthalate	DCHP	15.749	93, 104, 149, 167
Diethyl phthalate	DEP	17.517	93, 105, 149, 177
Dimethyl phthalate	DMP	20.666	104, 135, 163, 194
Dibutyl phthalate	DBP	20.836	93, 149, 104, 205

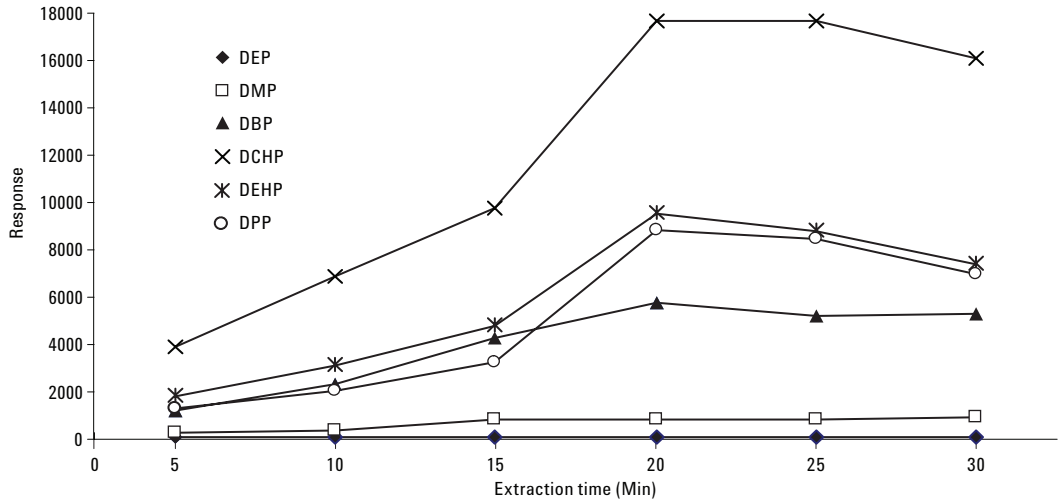

Figure 1. The profile of extraction time versus response (at 40 °C).

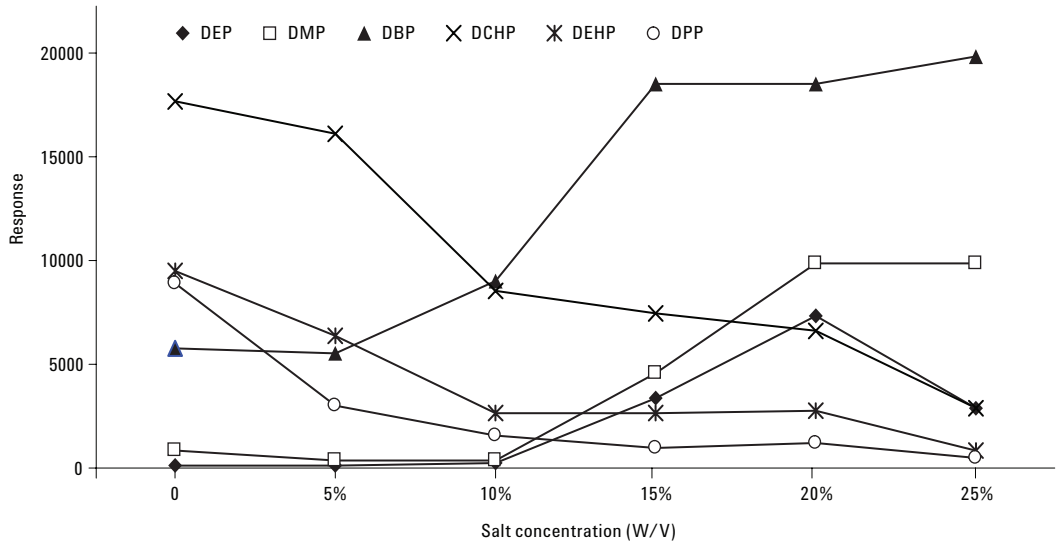

Figure 2. The effect of salt concentration on extraction.

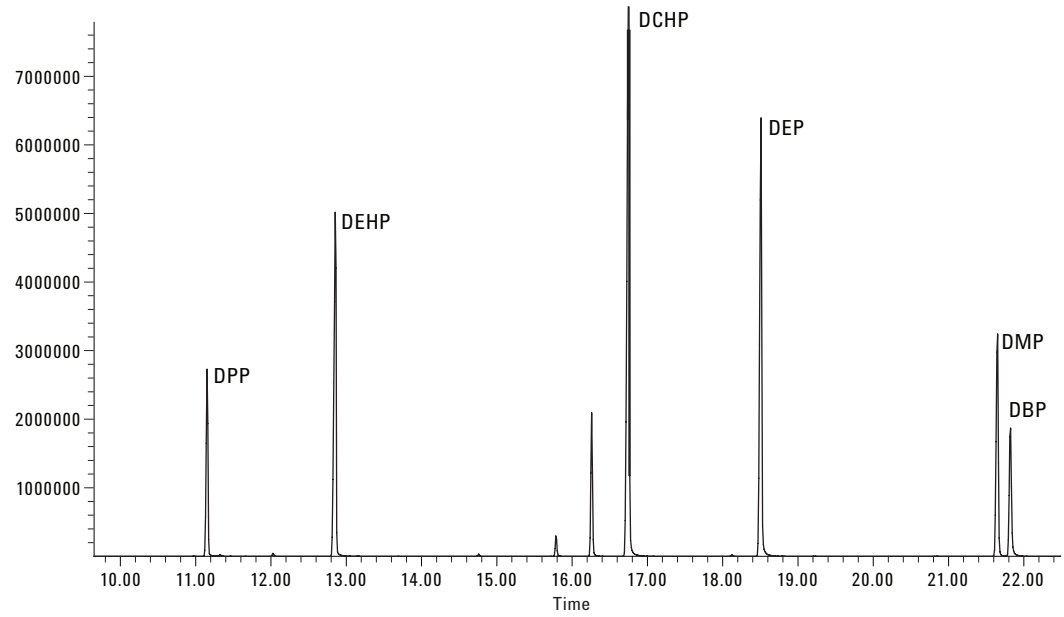

Figure 3. SIM of PAEs at the optimized extraction condition.

The linearity of the analytes was determined by calibration solutions with the concentration range from 0.5 ppb to 1 ppm at the optimized extraction condition. Table 2 shows the concentration ranges and correlating coefficients. The precision of the analysis, represented as relative standard deviations (RSDs) at 1 ppb, is also shown in Table 2. The RSDs for the organic esters are less than 10% except that of DPP; the detection limit is calculated at S/N of 3.

To demonstrate the performance of the optimized SPME method, tap water, potable water, and purified water from a water dispenser were analyzed for the phthalates' presence. Table 3 shows the phthalates detected in these three samples.

Conclusions

The CombiPAL autosampler with SPME is used for the analysis of PAEs in water. The precondition, extraction, adsorption, and desorption of SPME are automated and precisely controlled, which improves the precision of SPME method. Because the analytes concentrate into the coating of SPME, trace-level contaminates can be detected by using SPME. In this application, the detection limits for PAEs are down to sub-ppb level.

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Table 2. Method Validation Results

	Linear range (ng/mL)	Correlation coefficients (r^2)	RSD(%) N=7 (1 ppb)	Detection limits (ng/mL)
DPP	1–1000	0.996	12	0.34
DEHP	1–1000	0.996	8.6	0.29
DCHP	0.5–1000	0.989	8.9	0.08
DEP	1–1000	0.999	7.8	0.29
DMP	1–1000	0.998	7.1	0.38
DBP	1–1000	0.970	5.6	0.23

Table 3. Sample Analysis Results (quantitation unit = ng/mL)

	Tap water	Potable water	Purified water
DPP	n.d.[1]	n.d.	n.d.
DEHP	n.d.	n.d.	n.d.
DCHP	40.5	n.d.	n.d.
DEP	n.d.	78.9	n.d.
DMP	n.d.	23.6	n.d.
DBP	61.3	45.7	25.0

[1] None detected

Printed in the USA
January 14, 2008
5989-7726EN

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Analysis of Semivolatiles Using High Efficiency Capillary GC Columns

Application Brief

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Introduction

U.S. EPA Method 8270 is broadly applicable for analysis of semivolatiles using capillary gas chromatography with mass spectral detection. EPA 8270 is widely used in both contract analytical and government environmental laboratories. The method is capable of concurrently measuring a mixture of 70 to 100 acidic, basic, and neutral species. Shifting these important analyses from 0.25-mm id to 0.18-mm id or high efficiency GC columns is a viable means of obtaining faster results and improving laboratory productivity.

In this example, 77 compounds of interest and six internal standards are resolved on a 0.18-mm id high efficiency GC column using 7 minutes of analysis time. The same compounds and internal standards were also resolved using a 0.25-mm id column where 25 minutes of analysis time was required. Analysis speed using the high efficiency column was 8 minutes faster, resulting in a 32% reduction in analysis time.

Experimental

Method translation software available from Agilent Technologies translates chromatographic parameters from an existing method to the new column format with a few simple keystrokes [1].

Column dimensions, flow, and temperature parameters from an existing method are entered into a table along with the desired new column dimensions. The software then generates flow and temperature setpoints for the new translated method. Often these new setpoints yield a successfully translated method with the same separation and elution order with no additional method development. In this example, one-to-one phase-ratio correspondence was maintained between the 0.25-mm and 0.18-mm id column formats, enhancing the reliability of the software's predicted conditions. Keeping the phase ratio constant helps maintain peak elution order on the new column.

Instrument conditions are described in Table 1, and Figures 1 and 2.

Highlights

- 0.18-mm id, also known as high-efficiency GC columns, deliver faster results for U.S. EPA 8270 analyses.
- 32% reduction in analysis time when translating 0.25-mm id column method to the 0.18-mm id format.
- Resolution of 77 peaks of interest is maintained for the faster 0.18-mm id separation.
- DB-5.625 column: Agilent DB-5.625 column in 0.18-mm id provides faster sample analysis without loss of resolution.

Table 1. Experimental Conditions

Column:	Figure 1. 30 m x 0.25 mm x 0.50 µm DB-5.625 column, Agilent Technologies part number 122-5632 Figure 2. DB-5.625 20 m x 0.18 mm x 0.36 µm column, Agilent Technologies part number 121-5622
Carrier:	He constant-flow mode 1.1 mL/min
Oven:	40 °C for 1.00 min, 25 °C/min to 320 °C 4.80 min hold
Injection:	Splitless 0.5 µL injected at 300 °C, QuickSwap pressure 5.0 psi during acquisition, 80.0 psi during backflush with inlet set to 1.0 psi during backflush
Detector:	Agilent Technologies 5975C Performance Turbo MSD equipped with a 6-mm large-aperture draw-out lens, Agilent Technologies part number G2589-20045

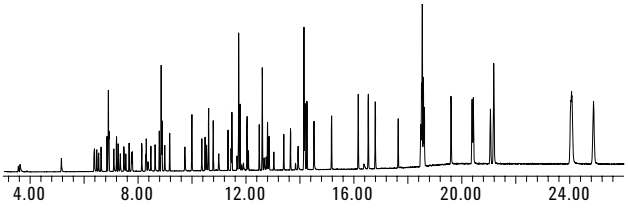

Figure 1. Figure 1. U. S. EPA Method 8270, 5 ng/mL System Performance Check Compounds Chromatogram using a 30-m x 0.25-mm x 0.50-µm DB-5.625 column, Agilent Technologies part number 122-5632. Please refer to Table 1 for instrument conditions.

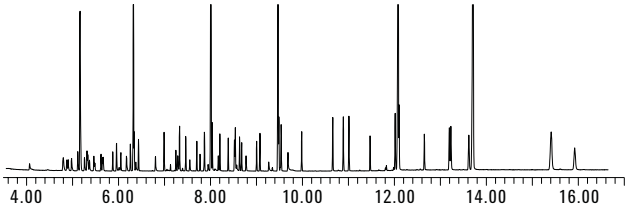

Figure 2. U.S. EPA Method 8270, 5 ng/mL System Performance Check Compounds Chromatogram using a 20-m x 0.18-mm x 0.36-µm DB-5.625 column Agilent Technologies part number 121-5622. Please refer to Table 1 for instrument conditions.

Discussion of Results

Figures 1 and 2 depict the resolution of 77 compounds of interest along with six internal standards first on a 30 m x 0.25 mm x 0.5 µm (Agilent part number 122-5632) standard-bore capillary column (Figure 1) and second on a 20 m x 0.18 mm x 0.36 µm (Agilent part number 121-5622) high efficiency column (Figure 2). Peak resolution and quantification are comparable, and in both cases meet EPA 8270 criteria for System Performance Check Compounds (SPCCs) and Continuous Calibration Compounds (CCCs) over a calibration range from 1 to 200 ppm; 5 ppm SPCC chromatograms were selected for visualization purposes.

Significant improvement in analysis time was achieved by shifting the column used from a 0.25-mm id standard-bore capillary to a 0.18-mm id high efficiency GC column example; the 0.25-mm id column required 25 minutes of run time, and the 0.18-mm id column required 17 minutes. In this semi-volatile analysis example, 25 minutes of run time were required for the 0.25-mm id column, and 17 minutes were required on the 0.18-mm id column. Moving the analysis to a 0.18-mm id column yielded 8 minutes in time savings or 32% faster sample analysis.

Typical run time for EPA 8270 analysis using 0.25-mm id or standard-bore capillary columns is 25 minutes, excluding post-analysis bakeout and system cooldown time often required for dirty samples. When bakeout and subsequent system cooldown periods are accounted for, the overall cycle time climbs to 57 minutes. As shown above, a time saving of 8 minutes was achieved by using a high efficiency column. Further improvements in the cycle time for EPA 8270 analysis are achieved through the use of several advanced features on the Agilent 7890A GC. A QuickSwap device installed in a 7890A can be used to backflush heavy material matrix contaminants back out of the inlet, dramatically reducing matrix bakeout time [2]. Faster cooldown and thermal isolation features available on the 7890A GC also reduce system cycle times for dirty samples. The combination of a high efficiency column and the unique features of the 7890A reduce sample analysis time from 57 minutes to 24.3, a 32.7-minute time saving per sample run.

Conclusions

High efficiency GC columns provide a straightforward way to obtain faster results for EPA 8270 analysis without compromising resolution.

References

1. Method translation software: free download of method translation software available at http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm.
2. Mike Szelewski, “Significant Cycle Time Reduction Using the Agilent 7890A/6975C GC/MSD for EPA Method 8270,” Agilent Technologies publication 5989-6026EN

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Printed in the USA
November 30, 2007
5989-7500EN

Agilent Technologies

Separation of Explosives in EPA 8330: Column Choices Optimize Speed, Resolution, and Solvent Use

Application

Environmental

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Abstract

ZORBAX Extend-C18 columns separate the explosive compounds in EPA method 8330, and the variety of column configurations available allows customized HPLC methods based on resolution, speed, and even solvent usage. For example, a fast method for the explosive-materials standard (EPA 8330) uses 1.8-µm, short length columns. The method was then customized using two other Extend-C18 column configurations. Each column highlights a combination of resolution, speed, and/or solvent savings. The advantage is being able to choose which combination of resolution, speed, and solvent usage is needed by simple column substitution.

Introduction

The ZORBAX Rapid Resolution High Throughput (RRHT, 1.8 µm) LC column line has over 120 column choices, including 11 bonded phases and silica, three column diameters, and six lengths. In addition, there are another 150+ Rapid Resolution (3.5 µm) column choices, allowing customization of HPLC methods to meet the analyst's tailored objectives. Many ZORBAX column choices are available because the stationary phase chemistry (both silica support and bonded phase) between 5-, 3.5- and 1.8-µm particles is uniform.

EPA 8330 explosives residues are typically analyzed by a 4.6 mm × 250 mm, 5 µm C18 column [1] but can be improved by newer technology: smaller 1.8-µm or 3.5-µm ZORBAX particles and Extend-C18 bonded phase. Many different Extend-C18 columns can be chosen (the combination of column length, diameter, and particle size) to provide a satisfactory separation, and each separation exemplifies a newer column technology's benefit and supports the end user's choice of speed, resolution, and solvent usage.

High-efficiency 1.8-µm particles in 100-mm length columns reduce analysis time and have about the same efficiency compared to 5-µm particles in 250-mm columns. Therefore, they are helpful by saving time in method development or generating more data in a limited amount of time. But these columns will generate a higher back pressure that some people may not desire. It is still possible to obtain the same resolution but using a longer 3.5-µm column. The end result is an analysis time still shorter than that achieved with a 250-mm, 5-µm column.

Experimental

The Agilent 1200 Rapid Resolution LC (RRLC) system:

- G1312B binary pump SL with mobile phase A: 5 mM ammonium formate in water, B: methanol
- G1376C automatic liquid sampler (ALS) SL
- G1316B Thermally Controlled Column (TCC) Compartment SL using the low-volume heat exchanger kit (PN G1316-80003)
- G1365C multiwavelength detector (MWD) at 254 nm, with a G1315-60024 micro flow cell (3-mm path, 2-µL volume), response time setting of 0.5 s

ZORBAX columns:

- Rapid Resolution High Throughput (RRHT) Extend-C18, 4.6 mm × 100 mm, 1.8 µm, PN 728975-902
- Rapid Resolution (RR) Extend-C18, 4.6 mm × 100 mm, 3.5 µm, PN 764953-902
- Solvent Saver Plus Extend-C18, 3.0 mm × 100 mm, 3.5 µm, PN 764953-302

The sample is a 1:1 mix of EPA 8330 Mix A (cat. no. 47283) and EPA 8830 Mix B (cat. no. 47284) from Sigma-Aldrich (Bellefonte, PA), diluted in methanol:water.

Results and Discussion

Selectivity, or the relative band spacing between two peaks, is different among C18 columns. In many cases the difference is small, so adjusting mobile phase organic strength can fine tune the retention to achieve comparable resolution between one C18 column and an alternative C18 column. Temperature may also influence selectivity, and small adjustments in temperature can fine tune the resolution.

For complex mixtures, fine tuning organic strength and temperature could be used to improve resolution and ultimately make a method more robust. Determining the combination of temperature, % organic, and what column (stationary phase) is best is frequently discovered by experimentation. This is time consuming at the very least and often daunting. Fortunately, research narrows the testing.

Consider an explosive residue standard of 14 nitroaromatic and nitramine compounds. Trace residues of these explosives were analyzed by time-of-flight LCMS by Kinghorn et al. using an Extend-C18, 4.6 mm × 250 mm, 5-µm column and a methanol/water gradient at a temperature of 40 °C [2]. Additionally, EPA method 8330 describes an HPLC method for the 14 compounds using an isocratic methanol/water mobile phase and a C18 column. Temperature is not specified, but the method states, *"If column temperature control is not employed, special care must be taken to ensure that temperature shifts do not cause peak misidentification."* [1]

In both methods a lack of selectivity required a TOF detector or additional analysis by an orthogonal stationary phase to confirm peak identity.

We separated the 14 compounds with enough resolution to make the MS detector or secondary analysis by a different stationary phase redundant.

The above methods narrowed our method-development starting conditions to:

- Extend-C18 (from successful Kinghorn method)
- Isocratic mobile phase A: 5 mM ammonium formate, B: Methanol (so new method is similar to EPA 8330). The ammonium formate was selected based on recommendations from a pre-existing method. The difference between water and 5 mM ammonium formate was not investigated.
- 40 °C controlled temperature (to ensure constant selectivity)
- RRHT column configuration 4.6 mm × 100 mm, 1.8 µm (for rapid analyses with efficiency comparable to the 4.6 mm × 250 mm, 5-µm columns used in the Kinghorn and EPA methods)

The methanol composition of the mobile phase was lowered incrementally from 50 to 25% until all 14 were reasonably resolved. A critical pair (peaks 6 and 7) persisted as partially resolved. Further decreasing organic strength would result in excessive retention of peaks 12, 13, and 14. Temperature was then optimized. A one-degree temperature increase (41°C) provided enough selectivity to resolve the critical pair. Figure 1 demonstrates temperature's selectivity effect on these compounds.

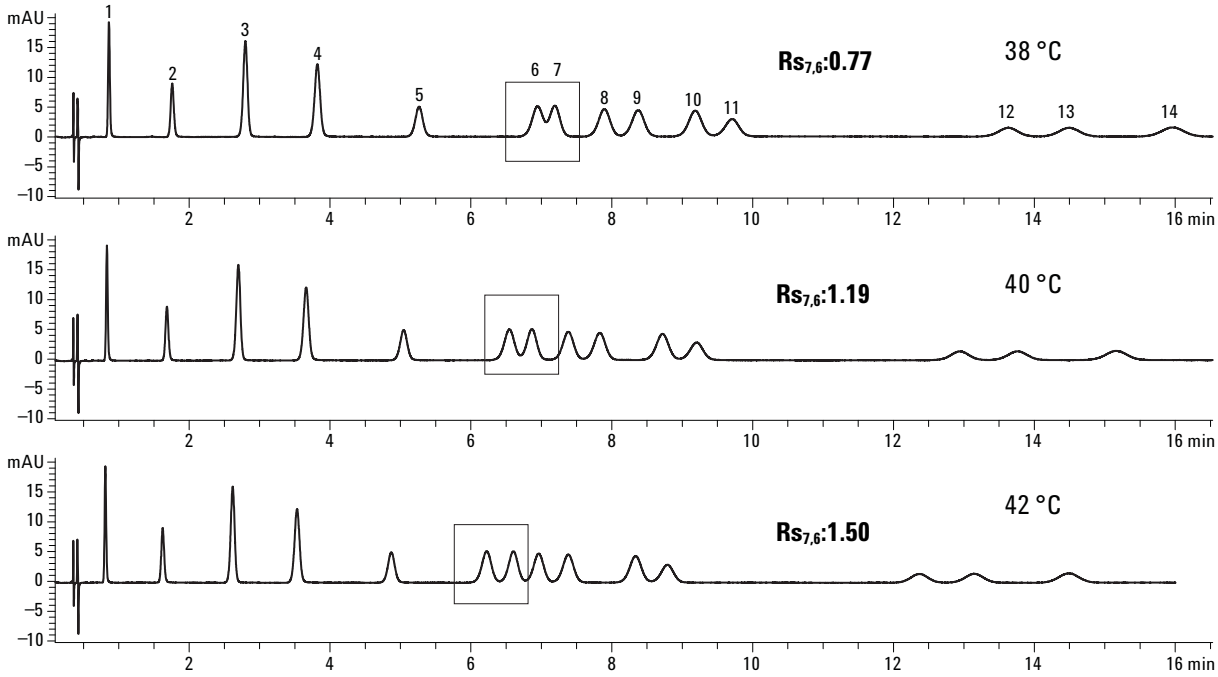
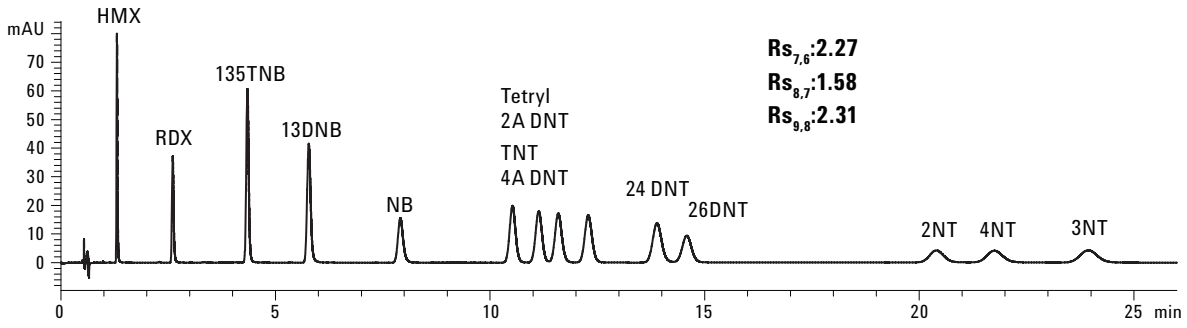


Figure 1. Temperature optimizes critical pair resolution.

Extend-C18 provides ample selectivity for the 14 nitroaromatics and nitramines identified in the EPA 8330 method; excellent resolution is obtained in a reasonable time. Figure 2 shows the separation using a RRHT 4.6 mm × 100 mm, 1.8 µm, Extend-C18. Resolution of all peaks is baseline or better (Rs > 1.5). High resolution makes it easier to quantify the analytes. For example, the EPA 8330 method warns, *"2,4-DNT and 2,6-DNT elute at similar retention times (Rs < 1.5) and a large concentration of one isomer may mask the other; therefore, if it is not apparent that both isomers are present, an isomeric mixture should be reported"* [1]. When baseline resolution is obtained, retention times differ significantly, avoiding peak masking. If higher resolution is the most important objective, then the Extend-C18 4.6 mm × 100 mm, 1.8-µm column using the conditions in Figure 2 is an excellent choice.



Column:	Rapid Resolution HT Extend-C18, 4.6 mm × 100 mm, 1.8 µm
Mobile phase:	A: 5 mM NH_4COOH (ph 6)
	B: MeOH (75A:25B)
Flow rate:	1.7 mL/min
Temperature:	41 °C
Injection:	2 µL × (0.5 µg ea/mL)
Pressure:	520 bar

Figure 2. EPA 8330 explosive standard high-resolution separation on Extend-C18.

Table 1 names the 14 explosives and their abbreviations used in the figures.

Table 1. EPA 8330 Explosives and Their Abbreviations

Name	Abbreviation
Cyclotetramethylene-tetranitramine	HMX
Cyclotrimethylene-trinitramine	RDX
1,3,5-trinitrobenzene	135TNB
1,3-dinitrobenzene	13DNB
Nitrobenzene	NB
2,4,6-trinitrophenyl-N-methylnitramine	tetryl
2,4,6-trinitrotoluene	TNT
2-amino-4,6-dinitrotoluene	2A DNT
4-amino-2,6-dinitrotoluene	4A DNT
2,4-dinitrotoluene	24 DNT
2,6-dinitrotoluene	26 DNT
2-nitrotoluene	2NT
4-nitrotoluene	4NT
3-nitrotoluene	3NT

If higher throughput is important, isocratic methods can be sped up by increasing flow rate. The 25% methanol mobile phase flowing 1.7 mL/min through the 4.6 mm × 100 mm, 1.8-µm column generates a system pressure of about 500 bar, leaving a small range to increase flow rate. An alternative is to substitute the 1.8-µm column with a 3.5-µm column. Pressure decreases substantially, allowing faster flow rates.

Figure 3 overlays two Extend-C18 chromatograms. The top chromatogram is a 4.6 mm × 100 mm column with 3.5-µm particles at a 2.5 mL/min flow rate. Compared to Figure 2, the 32% increase in flow rate reduces analysis time by roughly 40%. The price for the considerable time savings is less resolution of closely neighboring peaks. Resolution is still sufficient, as a resolution factor (Rs) of 1.25 for equally sized peaks means 99.4% of peak area is not overlapped. If one peak is 1/32 as tall as the other, an Rs of 1.0 still means 99.2% of the peak areas do not overlap [3].

Figure 3's bottom chromatogram is a different column substitution, replacing the 4.6-mm-id column with the Solvent Saver 3.0-mm-id column. Flow rate was reduced from 2.5 to 1.1 mL/min. for equivalent mobile phase linear velocity. The outcome is similar retention and resolution, but only half of the solvent is consumed.

Table 2 summarizes the customization benefits.

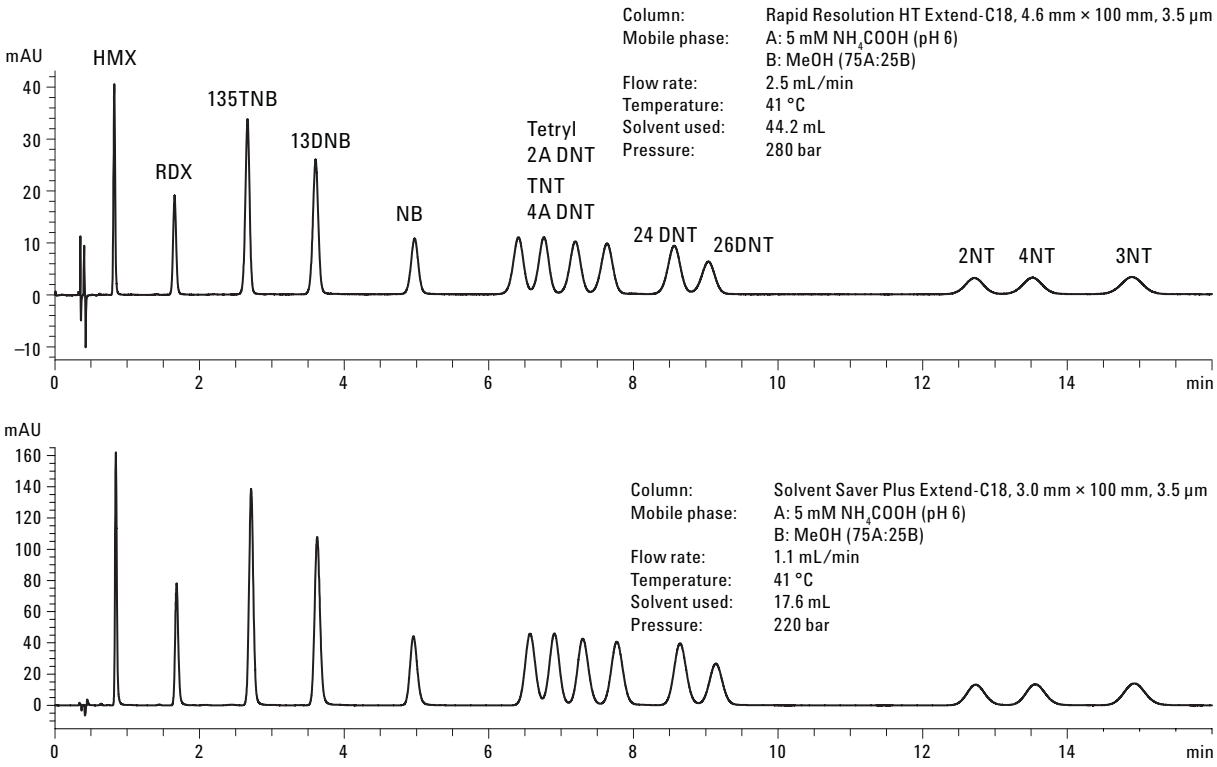


Figure 3. Rapid resolution options for EPA 8330 explosive standard on Extend-C18.

Table 2. Column Dimensions Highlight Resolution, Speed, and Solvent Savings

	RRHT (4.6 mm id, 1.8 µm)	RR (4.6 mm id, 3.5 µm)	Solvent Saver Plus (3.0 mm id, 3.5 µm)
Resolution: Rs 7,6	2.3	1.3	1.3
Resolution: Rs 8,7	1.6	1.6	1.4
Resolution: Rs 9,8	2.3	1.5	1.6
Analysis time	26 min	16 min	16 min
Solvent consumption	44.2 mL/analysis	40 mL/analysis	17.6 mL/analysis

Table 2 suggests that another column configuration could be valuable for this analysis: Solvent Saver HT Extend-C18, 3.0 mm × 100 mm, 1.8-µm column (PN 728975-302). This would produce high resolution like the RRHT column and produce time and solvent savings from the smaller column diameter. The Solvent Saver HT Extend-C18 column was not evaluated in this work.

Conclusions

Highly efficient (1.8 µm) short columns (100 mm) are ideal for method development compared to 5-µm, 150-mm or 250-mm columns because shorter analysis time increases productivity and allows more analyses to be performed in a fixed time frame.

Selectivity is manipulated by changing stationary phase, mobile phase, and temperature.

An isocratic HPLC method for complex mixtures of explosive materials was quickly created from highly efficient 100-mm columns, Extend-C18's unique selectivity, and temperature optimization. The selectivity and column configurations make Extend-C18 a compelling choice for the analysis of explosive substances named in EPA method 8330. Extend-C18's selectivity provides ample resolution with negligible peak coelution; this may eliminate an additional analysis to confirm peak identity.

The ZORBAX column family, including Extend-C18, has consistent stationary-phase chemistry between 3.5- and 1.8-µm particles, enabling simple column substitution for method customization. The high-resolution 4.6 x 100, 1.8-µm configuration, however, requires flexibility to work at operating pressures above 400 bar. The chromatographer can choose benefits such as higher resolution, faster analysis time, or less solvent usage based on column dimensions.

References

1. EPA Method 8330, *Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC),* revision 0, September 1994
2. R. Kinghorn, C. Milner, J. Zweigenbaum, "Analysis of Trace Residues of Explosive Materials by Time-of-Flight LC/MS," Agilent publication 5989-2449EN (2005)
3. L. R. Snyder, J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd ed., pp. 38–42, 1979

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Printed in the USA
November 21, 2007
5989-7632EN

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Parts-per-Trillion Level Calibration of Semivolatiles Using LVI-PTV-GC/MSD

Application

Environmental

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Abstract

The analysis of semivolatiles in the parts-per-trillion range presents challenges due to analyte activity, background contamination, and instrument sensitivity. Method requirements vary worldwide, with the least sensitive specifying 1-µL injections and full scan data acquisition. Lower level calibrations can be achieved using large volume injection (LVI) with a programmable temperature vaporizing (PTV) inlet and the MSD operating in SIM mode. Decreased sample preparation can be used as a trade-off for these lower detection limits.

Introduction

Low-level semivolatiles analysis is used to concurrently measure a mixture of acids, bases, neutrals, and pesticides in drinking water or source water. Most laboratories analyze for > 100 compounds with a chromatographic run time of 25 to 40 minutes. Sample extraction is accomplished using liquid-solid extraction (LSE) with C_{18} disks or catridges. Liquid-liquid extraction with a solvent such as dichloromethane is an alternative technique. Extract injection is typically 1 µL hot splitless with the MSD operating in full scan mode, as specified in some commonly used methods, such as U.S. EPA Method 525.2 [1].

Sensitivity is an area where laboratories are seeking improved performance. Sensitivity can be affected by sample preparation, extract volume injected, instrument tuning, signal acquisition, and overall system activity.

A PTV inlet provides better sensitivity through large-volume injection. Instead of 1 µL, 25 µL of relatively clean sample extracts can be routinely injected. Active analyte degradation is minimized on a PTV, providing lower detection limits than using hot splitless injection.

Methods for semivolatiles usually require identification of analytes with retention time (RT) and ratios of qualifier ions to a target ion. Selected ion monitoring (SIM) acquisition can be used in place of full scan with a sensitivity, or signal-to-noise ratio, increase of 10 to 50x.

A typical calibration range for low-level semivolatiles is 0.1 to 10 ppm as is found in U.S. EPA Method 525. This application note will demonstrate a calibration 1,000x lower and 10x wider that is from 0.1 to 100 ppb. LVI-PTV with SIM data acquisition on a retention time locked (RTL) GC/MSD system was used to achieve this performance. This application is a follow-up note to reference 2, where additional background information can be found.

Experimental

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These conditions may have to be optimized for use in another laboratory.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 7890A or 6890N		
Inlet	EPC PTV		
Mode	Solvent vent		
Temp ramp	°C/min	Next °C	Hold min
Initial		20	0.60
Ramp 1	600	350	1.30
Ramp 2	10	250	0.00
Cryo	On		
Cryo use temp	100 °C		
Cryo timeout	10.00 min (On)		
Cryo fault	On		
Pressure	11.77 psi (On)		
Vent time	0.60 min		
Vent flow	100.0 mL/min		
Vent pressure	0.0 psi		
Purge flow	50.0 mL/min		
Purge time	2.50 min		
Total flow	53.9 mL/min		
Gas saver	Off		
Gas type	Helium		
PTV Liner	Agilent multi-baffle liner, no packing, part number 5183-2037		
Oven	**240V**		
Oven ramp	°C/min	Next °C	Hold min
Initial		40	2.50
Ramp 1	50	110	0.00
Ramp 2	10	320	1.10
Total run time	26 min		
Equilibration time	0.5 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP 5 MSi, part number 19091S-433i		
Length	30.0 m		
Diameter	0.25 mm		
Film thickness	0.25 µm		
Mode	Constant flow		
Pressure	11.77 psi		
Nominal initial flow	1.5 mL/min		
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
RTL	System retention time locked to phenanthrene-d10 at 12.700 min		

Front Injector	
Sample washes	0
Sample pumps	2
Injection volume	25 microliters
Syringe size	50 microliters
Preinj solv A washes	0
Preinj solv B washes	1
Postinj solv A washes	2
Postinj solv B washes	2
Viscosity delay	1 second
Plunger speed	Variable
Injection speed	50 microliters/minute
Draw speed	600 microliters/minute
Dispense speed	6,000 microliters/minute
Preinjection dwell	0 minutes
Postinjection dwell	0 minutes
MSD	Agilent Technologies 5975C, Trace Ion Detection
Drawout lens	6 mm large aperture drawout lens, part number G2589-20045
Solvent delay	4 min
Low mass	45 amu
High mass	450 amu
Threshold	0
Sampling	1
Quad temp	180 °C
Source temp	300 °C
Transfer line temp	280 °C
Tune type	Autotune
EM voltage	Tune voltage, 1,247 V
MSD-SIM	
AutoSIM was used to pick ions, groups, and switching times	
Number of groups	25
Compounds/group	Varied 1 to 22
Ions/group	Varied 2 to 45
Dwell time, msec	Varied 5 or 10
Cycles/peak	Minimum 10

Calibration Standards

Ultra Scientific, North Kingstown, RI. Part number DWK-5252. Four mixtures, codiluted, resulting in 108 compounds at 4 concentration levels, spiked with 3 Internal Standards at 50 ppb and 4 surrogate standards at 50 ppb.

Calibration standards made separately in both dichloromethane and ethyl acetate.

The newer 7890A GC offers significant speed advantage over the older 6890N. Cooldown time from 320 °C to 40 °C is reduced from 7 minutes to 4.3 minutes. The MSD can optionally be mounted in the new rear position on a 7890A GC. With the PTV also installed in the back inlet position, the oven insert or "pillow" can be used to further reduce cooldown time to 3.3 minutes.

The PTV was operated in the Solvent Vent mode. Figure 1 shows the PTV temperature and flow programs together with the oven program. The PTV is held at 20 °C, a temperature below the boiling point of the solvent dichloromethane, 39.8 °C, during the injection period, 0.6 minute. The solvent is slowly evaporated through the vent line, held at 0 psi, with helium flow at 100 mL/minute. At the end of the injection period, the vent line is closed, inlet pressure is raised to 11.77 psi, and the PTV is rapidly heated to 350 °C. The vent line is reopened at the end of the splitless time, 1.3 minutes, and the inlet is purged at 50 mL/min. The PTV is allowed to cool during the run.

While the vent line is closed, the PTV is in the classical splitless mode with respect to flow. Because of the programmed temperature, compounds are vaporized and transferred onto the column at the lowest possible temperature. This significantly reduces loss of active analytes, such as pesticides and bases, which are often specified in semivolatiles methods.

The PTV inlet liner, 5183-2037, is multibaffled and deactivated. It does not contain glass wool, which could contribute to active compound degradation. This liner has sufficient capacity to accommodate the 25-uL injection volume at a correct injection speed.

The oven program relationship to the PTV parameters is shown in Figure 1. The oven starts at 40 °C and is held there during the injection/solvent vent cycle and splitless transfer of analytes onto the column. The oven then programs rapidly to 110 °C followed by a slower ramp for compound separation. The 240V oven was used but a 120V oven can also achieve the ramp rates found in Table 1.

The HP-5MSi column is designed for inertness and is well suited to this method. This is the latest version of the most popular column in environmental laboratories, the HP-5MS. The column was run in constant flow mode at 1.5 mL/min to maintain peak shape and sensitivity.

The system was retention time locked to phenanthrene-d10 at 12.700 minutes. The funda-

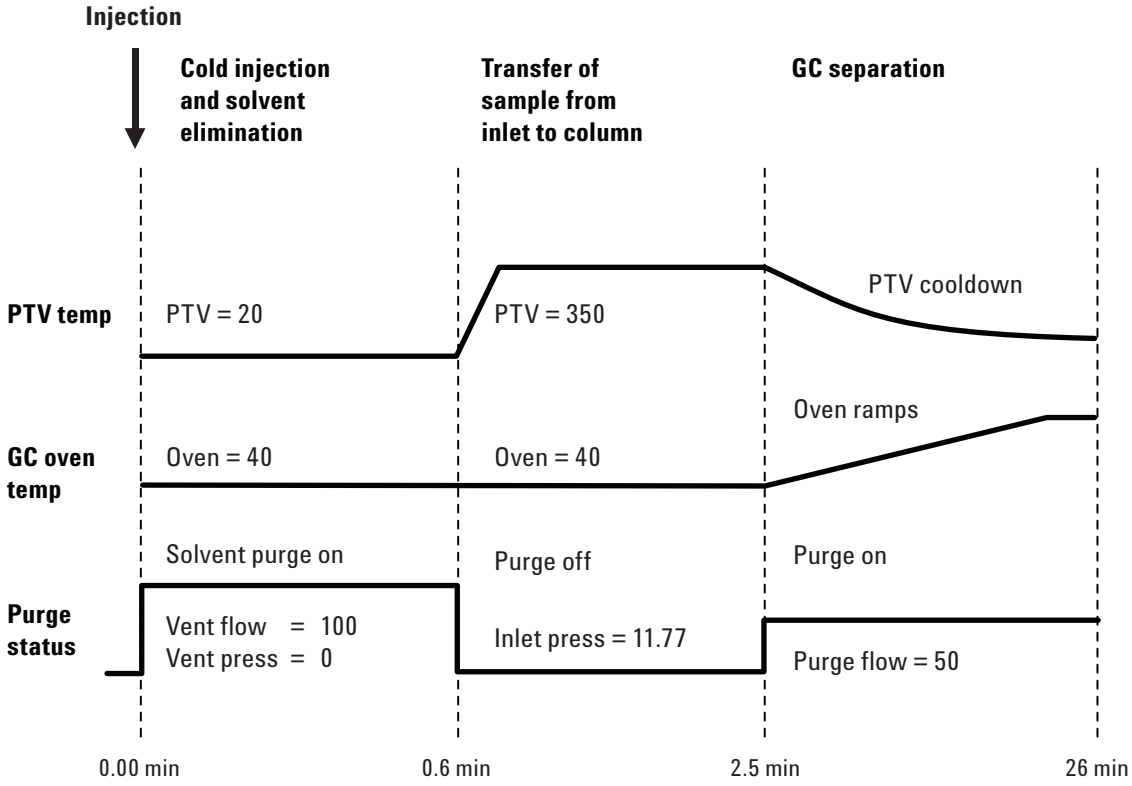

Figure 1. PTV temperature and flow programs.

mentals of RTL for GC/MSD systems can be found in reference 3. The primary benefit of RTL for this analysis is maintaining constant switching times for SIM groups. After clipping the column, a rerun and analysis of the locking standard is all that is needed to restore shifted peak times. Quantitation database and integration events times also do not have to be changed. Additional RTL application notes detailing the numerous benefits of RTL are available at www.agilent.com/chem. It is almost impossible to use a method with this many SIM groups, without RTL, in a productive laboratory.

Previous work has shown improved linearity across a wide calibration range using a 6 mm draw-out lens instead of the standard 3 mm lens [4]. Although this application uses a lower calibration range, the linearity improvement is still valid. The signal/noise loss using the 6 mm lens, even at low levels, was minimal compared to the linearity gain. The 6 mm lens is also included in Agilent Kit part number G2860A.

Scan parameters are listed even though the calibration was done using SIM data. All runs were made in synchronous SIM/scan mode, acquiring both SIM and scan data with a single injection. A sampling rate of 1, combined with the lower noise characteristics of the 5975C, was used to optimize signal/noise. This sampling rate, with a 45 to 450 mass range, resulted in approximately 10 scans across the peaks. The full scan data could be used to identify total unknowns by library searching, if present in sufficient amount. If full scan data is not needed, SIM/scan can be turned off and only SIM data collected. This will provide approximately 2x the number of data points across a peak.

AutoSIM setup was used in combination with the quantitation database to pick ions, groups, and switching times. Details of AutoSIM can be found in reference 5. The SIM acquisition table from AutoSIM was used directly with only two modifications. Tebuthiuron (ion 156) and tricyclazole (ion 189) are known for poor peak shape. Their target and qualifier ions were manually added to the groups across which the peaks eluted. A target ion plus one qualifier ion were used for all internal standards (ISTDs) and surrogate standards (SSs). A target ion plus two qualifier ions were used for all other analytes, if present in sufficient abundance in the spectra. The 10 SIM data points acquired across an average peak were used for calibration.

A source temperature of 300 °C was used instead of the typical 230 °C to 250 °C range. This higher temperature has been used to minimize peak tailing, and therefore increase sensitivity, for PAHs [6] and to improve performance for semivolatiles [2].

Calibration standards were prepared in dichloromethane only for the single-component analytes. Standards were not prepared for toxaphene or the Aroclors. Disulfoton sulfoxide and disulfoton sulfone were not included in the commercially available mixture. A separate set of calibration standards was prepared in ethyl acetate.

Results and Discussion

The system was calibrated at four levels, 0.1, 1.0, 10, and 100 ppb, with the standards in dichloromethane. Tebuthiuron, known to be problematic, was the only analyte that showed insufficient reponse at the lowest level. The SIM total ion chromatogram (TIC) for the 1.0 ppb level run in SIM/scan mode is shown in Figure 2. Each calibration level contained 108 compounds plus three ISTDs and four SSs at 50 ppb. Intermediate calibration levels are specified by some methods but were not needed here to demonstrate system performance.

The best overall performance was accomplished using the PTV parameters in Table 1. Successful PTV injections are a balance of injection speed, temperature, vent flow rate, and vent time.

Injection speeds of 150, 100, and 50 µL/min were tried. Faster injection rates showed decreased abundance for most analytes, regardless of RT. Sample passes through the liner, before solvent evaporation, and is swept out the vent line.

The initial PTV temperature was tested at 10, 20, 30, and 40 °C. Higher temperatures showed loss of the early eluters, those with volatility closer to that of the solvent. Lower temperatures preserve early eluters but hinder solvent venting.

The vent flow was tested at 50, 100, 200, and 300 mL/min. Increasing either the flow rate or vent time can decrease recovery of the early eluters. Decreasing the flow rate or vent time can result in excess solvent on the column and therefore poor chromatography. The minimum vent time must be matched to the injection time. In this case the injection takes 0.5 min (25 µL at 50 µL/min), so a vent time of 0.6 min was used.

Ethyl acetate is used in some methods as a solvent for solid phase extractions. Calibrations with standards in ethyl acetate showed worse performance

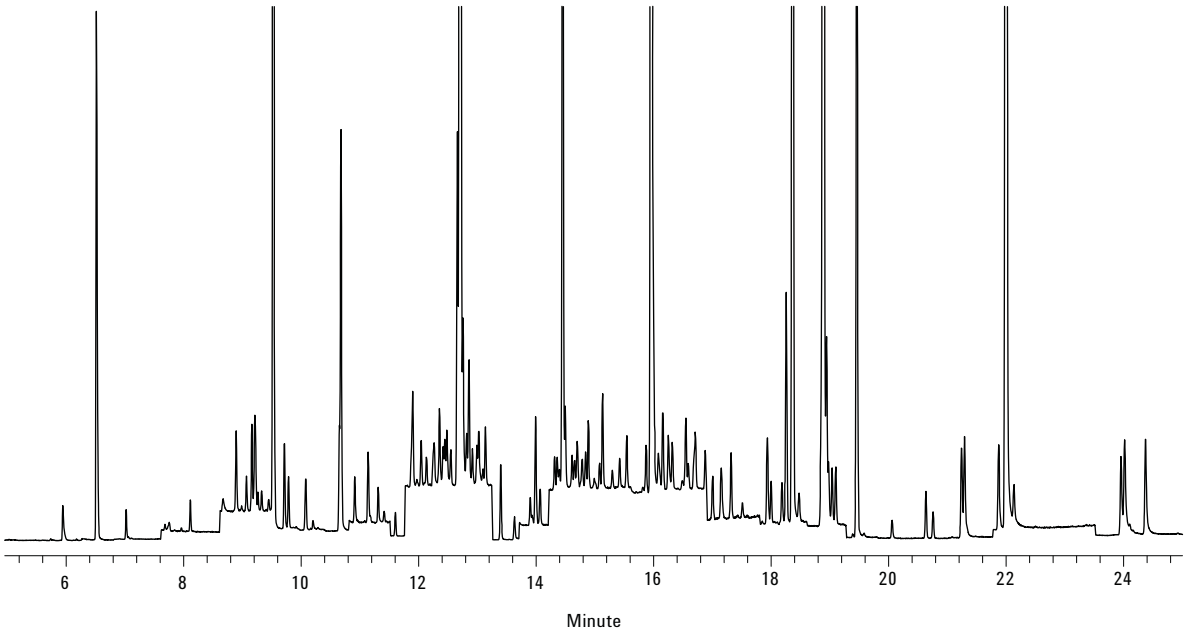

Figure 2. SIM TIC for the 1.0 ppb level run in SIM/scan mode.

than standards in dichloromethane. Ethyl acetate does not wet the stationary phase evenly, resulting in misshapen peaks, as the PTV did not eliminate 100% of the solvent. Adjusting the PTV parameters to account for the higher boiling point of ethyl acetate resulted in measurable losses of the early eluters. As a general rule, the earliest eluter for which quantitative recovery is required should have an elution temperature at least 100 °C greater than the solvent's boiling point. Ethyl acetate can be used successfully, but the lowest calibration point may be higher for some analytes.

Linearity can be determined by the percent relative standard deviation (%RSD) of the relative response factor (RRF) for each compound across the calibration range. The %RSD and the RRFs calculations are done automatically by the GC/MSD ChemStation software and can be reported in Excel. There is no correct %RSD as it is method dependent. As an example, U.S. EPA Method 525 has a criterion of < 30%RSD, but only for a subset of the compound list. The %RSDs of the RRFs for selected compounds are shown in Table 2.

Table 2. Signal-to-Noise and Linearity for Selected Analytes

Compound	RT	Target Ion	S/N 100 ppt	%RSD
Dichlorvos	7.01	109	6.5	4
Mevinphos	8.90	127	7.1	17
Simazine	12.24	201	4.8	6
Atrazine	12.35	200	20	6
Pentachlorophenol	12.48	266	22	24
Chlorpyrifos	14.78	197	2.7	12
2,2',3',4,6'-pentachlorobiphenyl	15.55	326	12	9
Phenamiphos	16.30	303	3.2	25
p,p'-DDT	18.00	235	13	9

At first glance some of the %RSD values appear high, such as pentachlorophenol (PCP) and the organophosphorus pesticides (OPPs). PCP is a known difficult compound and is commonly analyzed at significantly higher levels as in Method 525. The OPPs are very active and system inertness is critical to their successful analysis. Given this and the wide calibration range, the data shown here are excellent. As an additional overall measure of system linearity, the average of all %RSDs was 12% for SIM data in this study. The phthalates, easily detected at low levels, were excluded from this overall number due to common laboratory contamination. The %RSDs of the SSs ranged from 2% to 4%, demonstrating good repeatability.

As a further measure of system inertness, the %RSD for p,p'-DDT is 9%. The breakdown products in an active system are p,p'-DDD and p,p'-DDE. Their %RSDs were 6% and 4%, respectively, indicating minimal breakdown. A separate mixture of p,p'-DDT and endrin was also analyzed for breakdown, using the classical U.S. EPA criteria. The p,p'-DDT % breakdown was 1.2 and Endrin was 1.9, well below the required 15%.

The signal-to-noise values are also shown in Table 2. Peak-to-peak noise was used, as this is what the analyst sees and has to work with. Atrazine and PCP values are sufficiently high that they could be calibrated and measured at a lower concentration. Chlorpyrifos and phenamiphos have S/N values below 5 and are near the limit of reproducible integration and hence quantitation. Extracted ions for PCP and chlorpyrifos are shown in Figure 3. In all cases the analytes exhibited sufficient S/N for successfull calibration at the 100 ppt level.

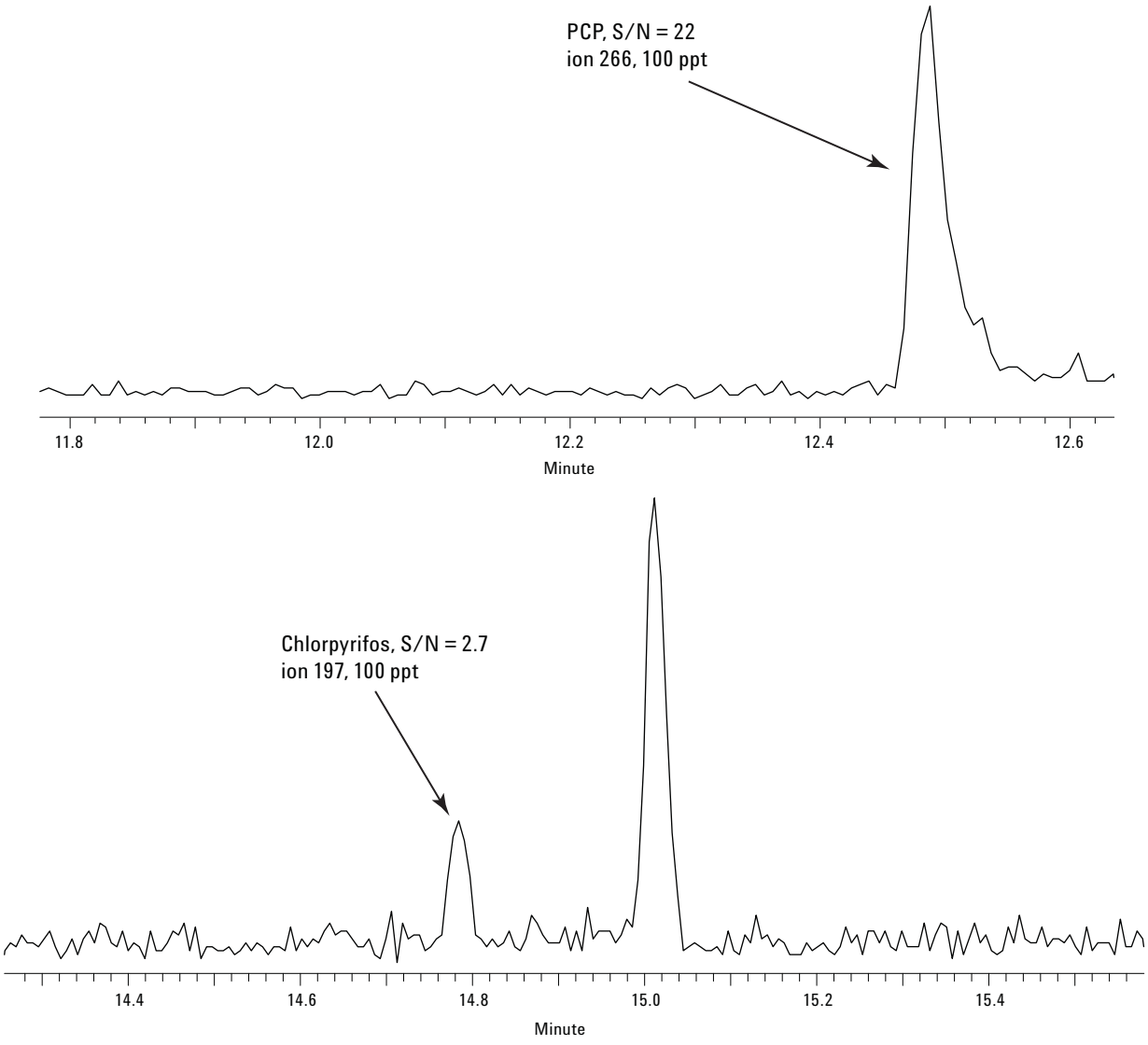

Figure 3. Extracted ions for PCP and chlorpyrifos.

As a trade-off to lower calibration levels and method detection limits, a laboratory could reduce sample preparation, shown in Table 3. The first column, "Traditional," assumes 1 liter of water is extracted, concentrated to 1 mL, and 1 uL is injected. Methods using this approach have a lowest calibration level of 100 ppb (0.1 ppm) in scan mode. As described in this application, the "7890A-5975C" column maintains the same sample preparation but increases the sensitivity by a factor of 1,000, to the ppt level. The "Fast Prep" column shows extracting only 10 mL and still lowering the method limits by 10x compared to Traditional. Extracting 10 mL of sample is significantly easier and faster than 1 liter. Better recoveries may also be realized by needing less concentration of the extract. The Quick Screen extraction is accomplished directly in a 2-mL vial (Agilent p/n 5182-3454) with an integral pointed bottom. The dichloromethane extract is withdrawn from the bottom of the vial by the autosampler syringe. Variations of these examples can be used to maximize sensitivity and minimize sample preparation time.

Conclusions

Traditional semivolatiles methods can be altered to achieve better detection limits. Large volume injection-PTV coupled with SIM allows calibration to the 100-ppt level. Linearity is excellent for the wide calibration range used, even for active analytes. Using RTL saves the analyst time by preserving SIM group switching times. The 7890A reduces cycle times by rapid oven cooling. Laboratories can choose to lower method calibration limits and/or save time through reduced sample preparation.

References

1. U.S. EPA Method 525.2 is available from different sources listed on this Web site: www.epa.gov/OGWDW/methods/where.html
2. M. Szelewski, "Drinking Water Semivolatiles Analysis Using the 6890N/5975B Inert GC/MSD," Agilent Technologies publication 5989-5421EN.
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5. H. Prest and D. Peterson, "New Approaches to the Development of GC/MS Selected Ion Monitoring Acquisition and Quantitation Methods," Agilent Technologies publication 5988-4188.
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Table 3. Sample Preparation and Calibration Limits

	Traditional	7890A-5975C	Fast Prep	Quick Screen
Sample concentration, ppb	0.1	0.0001	0.01	0.02
Lowest cal level, ppb	100	0.1	0.1	0.1
Injection volume, µL	1	25	25	25
Extract volume, mL	1	1	1	0.25
Sample size, mL	1000	1000	10	1.25

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Printed in the USA
April 13, 2007
5989-6589EN

Agilent Technologies

Significant Cycle Time Reduction Using the Agilent 7890A/5975C GC/MSD for EPA Method 8270

Application Brief

Mike Szelewski

U.S. EPA Method 8270 for semivolatiles analysis is used to concurrently measure a mix of 70–100 acids, bases, and neutrals. Laboratories want to reduce the typical 25–60-minute cycle time for productivity increases. The Agilent 7890A/5975C GC/MSD system meets this demand using a smaller id column, faster cooling oven, and backflushing. Criteria for system performance check compounds (SPCCs) and continuing calibration compounds (CCCs) are met using a calibration range of 1–200 ppm.

The system was calibrated at 10 levels using the conditions in Table 1. The SPCCs and CCCs all meet 8270 criteria, the results shown in Table 2. The overall average %RSD for all 77 analytes was 11%.

Cycle time savings are shown in Table 3. Historically, a 30 m × 0.25 mm column is used in 6890 systems. The 20 m × 0.180 mm column used here cuts the run time by 8 min, a 5 ppm standard shown in Figure 1A.

Highlights

- Productivity increases > 55% with the Agilent 7890A/5975C GC/MSD system.
- The oven heats faster, cools down faster, and reduces cycle time.
- Backflushing reduces analysis time and increases column life while reducing maintenance time and frequency.

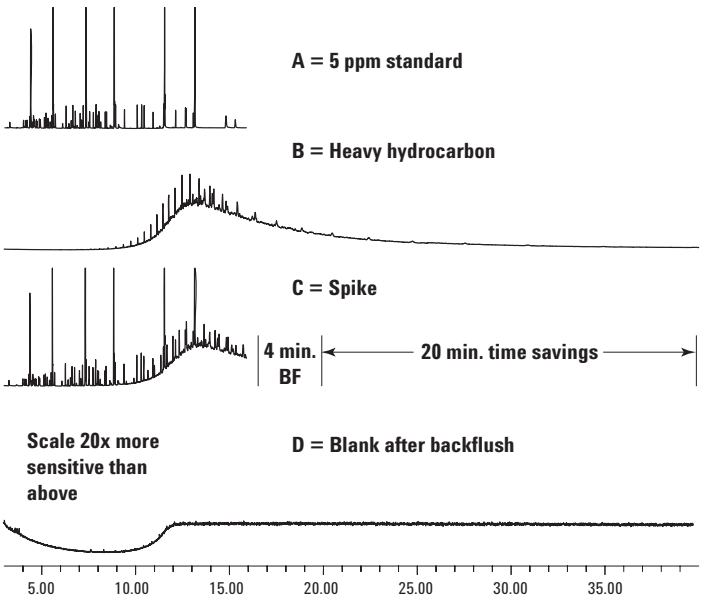

Figure 1. Time savings using backflush.

Bake-out of matrix often takes longer than the run time for analytes of interest. A heavy hydrocarbon, typical of an 8270-type extract, is shown in Figure 1B, eluting in ~40 min. The elution time on a 30-m column is > 50 min. All of this material is usually eluted into the MSD.

A backflush of the heavy material from a sample spiked with standard is shown in Figure 1C. The 4-min backflush is accomplished by raising the pressure in the QuickSwap and lowering the inlet pressure. The matrix elutes into the inlet and is swept out the split vent line.

Figure 1D shows a blank solvent run after the backflush. The heavy hydrocarbon matrix is not on the column nor in the liner.

The Agilent 7890A has faster oven cool-down, from 320–40 °C. Additionally, the MSD can be interfaced in a new rear oven position, along with the inlet in the rear. An oven insert, the "pillow," can occupy the front half of the oven. This allows even faster heating and cooling. The times are shown in Table 3.

Additional time savings are realized by using QuickSwap. Liner and column maintenance or changing can be done without venting the MSD.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 7890A		
Inlet	EPC split/splitless		
Mode	Splitless, 0.5 µL injected		
Inlet temp	300 °C		
Pressure	25.0 psi		
Purge flow	30.0 mL/min		
Purge time	0.75 min		
Gas saver	Off		
Gas type	Helium		
Liner	Agilent Helix single taper liner with a narrow o.d. for both split and splitless, proprietary deactivation, Part # 5188-5397		
Oven	**240V**		
Oven ramp	°C/min	Next °C	Hold min
Initial		40	1.00
Ramp 1	25	320	4.80
Total run time	17.0 min		
Equilibration time	0.5 min		
Oven max temp	325 °C		
Column	Agilent Technologies DB-5.625, Part # 121-5622		
Length	20.0 m		
Diameter	0.18 mm		
Film thickness	0.36 µm		
Mode	Constant flow = 1.1 mL/min		
Inlet	Front		
Outlet	QuickSwap, Agilent Part # G3185B		
QuickSwap pressure	5.0 psi during acquisition, 80.0 psi during backflush with inlet set to 1.0 psi during backflush		
MSD	Agilent Technologies 5975C, Performance Turbo		
Drawout lens	6 mm large aperture drawout lens, Part # G2589-20045		
Solvent delay	2.8 min		
EM voltage	Tune voltage		
Mass range	35–500 amu		
Sampling	1		
Quad temp	180 °C		
Source temp	300 °C		
Transfer line temp	250 °C		
Emission current	25 µamp		

Calibration Standards

Accustandard, New Haven, CT. Part # M-8270-IS-WL-0.25x to 10x

77 compounds at 10 concentration levels with 6 internal standards at 40 ppm

Table 2. SPCC and CCC, Criteria and Results

	8270 criteria	7890A-5975C with QuickSwap (range)
4 SPCCs minimum average RRF	0.050	0.110–0.405
13 CCCs %RSD	< 30%	2% –20%

Table 3. Cycle Time Savings Using the 7890A-5975C

	Typical 6890	7890A	Minutes Saved
Run time without matrix bake-out, includes equib	25	17	8
Run time with matrix bake-out 6890 or QuickSwap 7890A	50	21	29
Cool down time from 320 to 40	7	4.3	2.7
Cool down time from 320 to 40 with pillow	n/a	3.3	3.7
Total time savings using a 7890A-5975C with the 20-m column, QuickSwap for backflush, rear position for MSD and pillow	57	24.3	32.7

Significant cycle time savings can be realized, depending on sample complexity and column and instrument configuration. Analyzing dirty samples on a 30-m column can take 57 minutes or more with a 6890. Using an Agilent 7890A, QuickSwap, the 20-m column, and rear oven position, cycle time is < 25 minutes. This is a direct productivity increase of > 55%.

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Printed in the USA
January 10, 2007
5989-6026EN

Agilent Technologies

Drinking Water Semivolatiles Analysis using the 6890N/5975B inert GC/MSD

Application

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Abstract

The analysis of semivolatiles in drinking water presents challenges due to the required detection limits, desired calibration range, and analyte activity. In the United States (U.S.), method requirements are typically those found in USEPA 525.2 (525), but can vary widely outside the U.S. This application is based on 525 with differences described for laboratories that do not require 525. The 6890N/5975B inert GC/MSD system is designed to meet the criteria for semivolatiles analysis in drinking water through excellent sensitivity, minimal activity, and extended linearity.

Introduction

USEPA Method 525.2 for semivolatiles analysis is used to concurrently measure a mixture of acids, bases, neutrals, and pesticides in drinking water or source water [1]. Most laboratories analyze for >100 compounds with a chromatographic run time of 25 to 40 minutes. Sample extraction is accomplished using liquid-solid extraction (LSE) with C_{18} disks or cartridges. A 1-µL hot splitless injection is specified with the MSD operating in full scan mode.

Sensitivity and linearity are two areas where laboratories are seeking improved performance. Sensitivity can be affected by sample preparation, extract volume injected, instrument tuning, signal processing, and overall system activity. Linearity can be affected by source design, tuning, activity, data acqusition mode, and system reproducibility.

This application will demonstrate the use of the 6890N/5975B inert for USEPA Method 525.2. Performance has been improved through the use of inert columns, high-temperature inert source, and manual tuning. Additional specific areas for improved performance will be discussed for laboratories that are not required to follow 525 mandates.

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized.

Pulsed splitless injection was used to minimize residence times of analytes in the liner, thereby reducing loss of active compounds. The column flow rate alone, without using a pulsed injection, would take too long to sweep the 700-µL liner volume.

The inlet liner, G1544-80700, has shown the best performance for active compounds at low levels. It does not contain glass wool, which would contribute to active compound degradation. This liner

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890N		
Inlet	EPC split/splitless		
Mode	Pulsed splitless, 1.0 µL injected		
Inlet temperature	250 °C		
Pressure	11.56 psi		
Pulse pressure	40.0 psi		
Pulse time	0.10 min		
Purge flow	30.0 mL/min		
Purge time	1.00 min		
Total flow	34.6 mL/min		
Gas saver	Off		
Gas type	Helium		
Inlet liner	Agilent direct connect, dual taper, 4 mm i.d., part number G1544-80700		
Oven	240V		
Oven ramp	°C/min	Next °C	Hold min
Initial		40	1.00
Ramp 1	50	110	0.00
Ramp 2	10	320	0.60
Total run time	24.0 min		
Equilibration time	0.5 min		
Oven max temperature	325 °C		
Column	Agilent Technologies HP 5 MSi, part number 19091S-433i		
Length	30.0 m		
Diameter	0.25 mm		
Film thickness	0.25 µm		
Mode	Constant flow = 1.5 mL/min		
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
RTL	System retention time locked to phenanthrene-d10 at 11.400 min		
MSD	Agilent Technologies 5975B inert		
Drawout lens	6 mm large-aperture drawout lens, part number G2589-20045		
Solvent delay	4.00 min		
EM voltage	Run at Autotune voltage = 1294 V		
Low mass	45 amu		
High mass	450 amu		
Threshold	25		
Sampling	2		
Scans/second	3.54		
Quad temperature	180 °C		
Source temperature	300 °C		
Transfer line temperature	280 °C		
Emission current	Autotune @ 35 µamp		
MSD-SIM			
AutoSIM was used to pick ions, groups, and switching times			
Number of groups	26		
Compounds/group	Varied 1 to 22		
Ions/group	Varied 2 to 55		
Dwell times	Varied 5 to 10 ms		
Cycles/peak	Minimum 10		
Calibration standards			
Ultra Scientific, North Kingstown, RI. Part number DWK-5252.			
Four mixtures codiluted, resulting in 108 compounds at 10 concentration levels, spiked with 3 Internal Standards at 5 ppm and 4 Surrogate Standards at 5 ppm.			

connects directly to the column and has a tapered top, minimizing contact with metal in the inlet. Other liners can be used; a detailed discussion of these can be found in Reference 2.

The 6890N 240V oven was used but a 120V oven can also achieve the ramp rates found in Table 1.

The HP-5MSi column is designed for inertness and is well suited to this method. This is the latest version of the most popular column in environmental laboratories, the HP-5MS. The column was run in constant flow mode at 1.5 mL/min to maintain peak shape and sensitivity.

The system was retention time locked to phenanthrene-d10 at 11.400 min. The fundamentals of retention time locking (RTL) for GC/MSD systems can be found in Reference 3. The primary benefit of RTL for the environmental laboratory is the ability to maintain retention times after clipping or changing the column. Quantitation database and integration events times do not have to be changed. For laboratories performing SIM analyses, switching group times remain constant. Additional RTL application notes detailing the numerous benefits of RTL are available at www.agilent.com/chem.

Previous work has shown improved linearity across a wide calibration range using a 6-mm drawout lens instead of the standard 3-mm lens [1]. Although 525 uses a lower calibration range, the linearity improvement is still valid. The signal/noise loss using the 6-mm lens, even at low levels, was minimal compared to the linearity gain. The 6-mm lens is also included in Agilent Kit part number G2860A.

EPA method 525 requires that the system meet DFTPP tune criteria, but in this case the 5975B inert was tuned using Autotune. A new entrance lens (EL) value was then manually input at half the tune value (16 versus 32). DFTPP was injected and tune criteria were checked. If the injection passed DFTPP criteria, the EL value was raised 4 volts and DFTPP was reinjected. This process continued until the highest EL value that allowed DFTPP to pass criteria was determined.

A sampling rate of 2, combined with the lower noise characteristics of the 5975B inert, was used to optimize signal/noise. This sampling rate resulted in 3.54 scans/sec, with a 45 to 450 scan range, typically yielding 10 data points across the peaks.

A previous publication [4] detailed steps to match sampling rates in tune with those used in data acquisition. This process is no longer necessary. The automatic tuning has been significantly improved in the 5975B inert. Valid tune parameters are stored for all data acquisition sampling rates. These parameters are automatically called and used based on the method sampling rate.

AutoSIM setup was used in combination with the quantitation database to pick ions, groups, and switching times. Details of AutoSIM can be found in Reference 5. The SIM acqusition table from AutoSIM was used directly with only two modifications. Tebuthiuron (ion 156) and tricyclazole (ion 189) are known for poor peak shape. Their ions were manually added to the groups across which the peaks eluted.

A source temperature of 300 °C was used instead of the typical 230 °C to 250 °C range. This higher temperature has been used to minimize peak tailing, and therefore increase sensitivity, for PAHs [6]. Lower source temperatures have historically been used to maintain performance of the active pesticides. At 300 °C, the inert source shows equivalent or better performance for all but 14 compounds, compared to 230 °C. Of the 14 compounds, 10 still had single-digit percent relative standard deviations (%RSDs). More importantly, the average %RSD for all 115 analytes was reduced almost 2× using a source temperature of 300 °C versus 230 °C.

Calibration standards in dichloromethane were prepared only for the single component analytes. Standards were not prepared for toxaphene or the Aroclors. Disulfoton sulfoxide and disulfoton sulfone were not included in the commercially availble mixture.

Alternatives to EPA Method 525

There are many laboratories both within the U.S. and in other geographies that are not required to follow 525 mandates. Many of these laboratories use 525 as a framework or starting point for drinking water analyses. This section will discuss areas in 525 that can be modified for improved performance. None of these have been approved as alternatives to Method 525 by the USEPA.

LSE is required by 525 for sample preparation, with ethyl acetate and dichloromethane (DCM) as

the final extract solvents. An alternative is a liquid-liquid extraction (LLE) using only DCM. Specific analtye recoveries should be determined just as they are with LSE and 525. The initial sample and final extract volumes are sized to meet the laboratory's detection limits. Traditionally, a liter or more of water is extracted with 3×100 mL aliquots of DCM. The aliqouts are combined and concentrated. Some laboratories use 40-mL screw-cap vials, extracting 30 mL of water with 3 mL of DCM, with extract concentration as needed. Other laboratories, for screening purposes, use a tapered bottom 2-mL sample vial. A 1.25-mL water sample is extracted with 0.25 mL of DCM. The DCM layer is injected directly from the bottom taper of the vial. LLE of small volumes of water increases laboratory productivity compared to LSE.

A 1-µL hot splitless injection is specified by 525. A cool-on-column inlet is allowed, with the same 1-µL injection volume. Lower detection limits can be achieved using a programmable temperature vaporizing (PTV) inlet and large-volume injection (LVI) inlet [7]. A single 25-µL injection of a DCM extract can easily be made using the PTV in solvent evaporation mode. Additionally, thermally labile compounds are less likely to degrade. The PTV is held near the solvent's boiling point during injection. As the inlet is programmed, analytes are transferred onto the column at the lowest possible temperature. This can result in better performance for active compounds.

Full scan data acquisition is mandated by 525. As an alternative, selected ion monitoring (SIM) could be used. With a defined compound list, quantitation (target) ions and qualifier ions/ratios are known or can be easily determined. Using SIM typically results in a sensitivity increase of 10 to 100× compared to scan [7]. Using either PTV or SIM increases laboratory throughput by allowing a wider calibration range on the GC/MSD system. Sample and extract volumes need less manipulation and fewer reruns are required to have results fall within the calibration range. Using SIM and PTV together can lower the detection limit up to 2,500× compared to a 1-µL injection in scan mode [7].

The 6890N/5975B inert GC/MSD can acquire both SIM and scan data in the same run [6]. This is an attractive alternative for laboratories that want a wider calibration range (SIM) but also want full scan spectra for confimation. A SIM acquisition table is easily constructed using AutoSIM setup. A check box is selected in data acquisition and both signals are collected. If SIM/scan is used, typically the scan sampling rate should be halved – in this case from 2 to 1.

Tuning to meet DFTPP criteria is required by 525. This enhances the response at lower *m/z* values but decreases the response at higher *m/z* values. With enhanced responses for higher *m/z* values as a result of Autotune, more unique ions can be used for identification. Most laboratories have found that DFTPP tuning also decreases the overall instrument sensitivity 3 to 6× compared to Autotune. To maintain maximum instrument sensitivity, Autotune should be used. Qualifier ion ratios are established for each system as a routine laboratory practice and will stay consistent using Autotune. Library matches against NIST are excellent using Autotune, as most NIST spectra were not obtained under DFTPP tune conditions.

Results

The 5975B inert passed DFTPP tune criteria for 525 with a 1-µL splitless injection of 5 ppm. This was accomplished using the procedure discussed in the Instrument Operating Parameters section instead of using the DFTPP menu item. The sensitivity loss of 3 to 6× usually seen with DFTPP tuning was less than 2× with this manual procedure. Sensitivity loss is usually a result of DFTPP tuning with a low repeller in combination with the entrance lens offset (ELO) values. In the manual procedure, the repeller stays at its Autotune value, which is best above 20. The EL value is lowered only enough to allow passing.

The system was calibrated at seven levels, 0.1, 0.2, 0.5, 1, 2, 5, and 10 ppm in scan mode, with the 0.2 ppm level not required by 525. The total ion chromatogram (TIC) for the 0.5 ppm level in scan mode is shown in Figure 1. Each calibration level contained 108 compounds plus 3 internal standards (ISTDs) and 4 surrogate standards (SSs) at 5 ppm. Not all compounds showed a response at all levels as is stated in 525 and therefore expected. A listing of problem compounds and reasons can be found in Method 525.2, Section 13.2.

Full method-detection limits must be established in each laboratory using the sample preparation procedure as described in 525.2, Section 9.

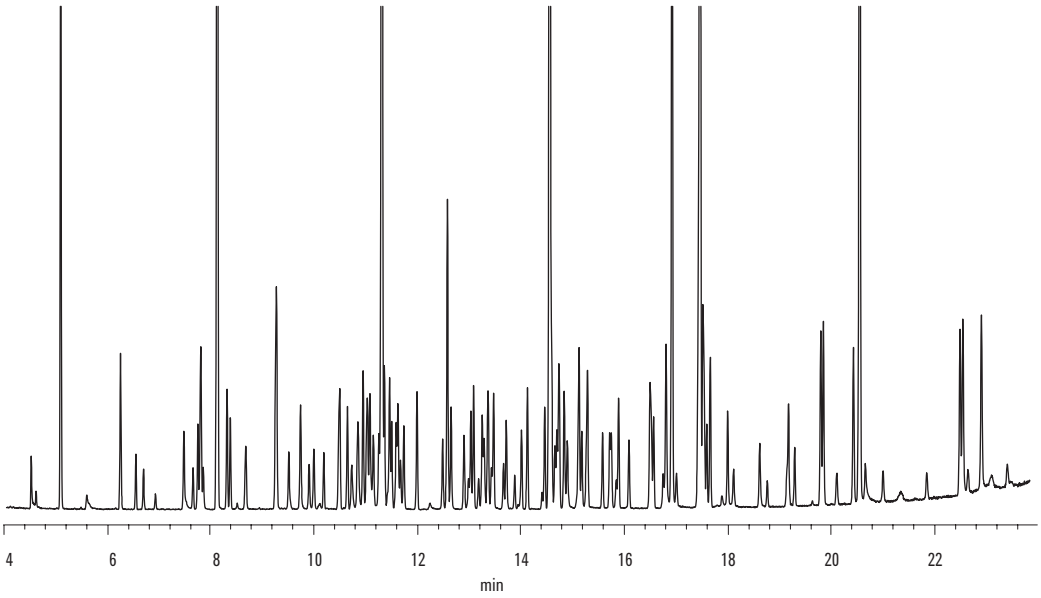

Figure 1. TIC for 0.5 ppm 525 semivolatiles – scan 45 to 450 *m/z* for 115 compounds

For SIM acquisition, three additional calibration levels were run: 0.01, 0.02, and 0.05 ppm. The TIC for the 0.05 ppm level in SIM mode is shown in Figure 2.

USEPA Method 525 does not specify minimum RRFs for system performance check compounds (SPCCs). USEPA Method 8270 for semivolatiles in wastewater does have criteria for SPCCs and should not be confused with 525.

Linearity can be determined by the %RSD of the relative response factor (RRF) for each compound across the calibration range. The %RSD and the RRF calculations are done automatically by the GC/MSD Chemstation software in conjunction with Microsoft Excel. Not all compounds meet the 525 criteria of <30%, which is allowed, with the most active compounds showing the highest %RSD. However, as an overall measure of system linearity, the average of all %RSDs was 8% for scan data. The %RSDs are summarized in Table 2.

Linear regression is allowed by 525 as an alternative to mean RRF and %RSD. When linear regression is used, a continuing calibration check (CCC) must report values of ± 30% of the true value for each compound. A CCC was run and all but one of the 108 compounds were within the criteria. USEPA method 525 allows up to 10% of the compounds to miss the CCC criteria on a single day.

The SIM data were reduced two ways, using a seven- level calibration or a 10-level calibration, including three lower concentrations. For the seven-level SIM, the average %RSD of all 108 compounds was 6%. The ISTDs were 4% and SS ranged from 1 to 3%. For the 10-level SIM, the average %RSD for all compounds was 11%; ISTDs at 4% and SS ranged from 1to 5%.

Table 2. Summary of Linearity for Scan and SIM Calibrations

	Avg %RSD 108 Analytes	%RSD Range 3 ISTDS	%RSD Range 4 SS
Scan 7-level cal	8	6 to 8	1 to 3
SIM 7-level cal	6	4	1 to 3
SIM 10-level cal	11	4	1 to 5

Conclusions

The 6890N/5975B inert meets USEPA Method 525.2 criteria. Analysis of 108 analytes and 7 ISTDs/SSs is accomplished in 24 minutes. The 525 DFTPP tune criteria are routinely achieved, and sensitivity is increased through manual tuning. Linearity is met over the method calibration range in scan mode; CCC requirements of ± 30% are also attained. Laboratories that are not required to follow 525 mandates can achieve productivity gains. Autotune can be used for better sensitivity compared to DFTPP tuning. PTV can reduce sample preparation and reduce detection limits. SIM futher lowers detection limits, extends the calibration range, and improves

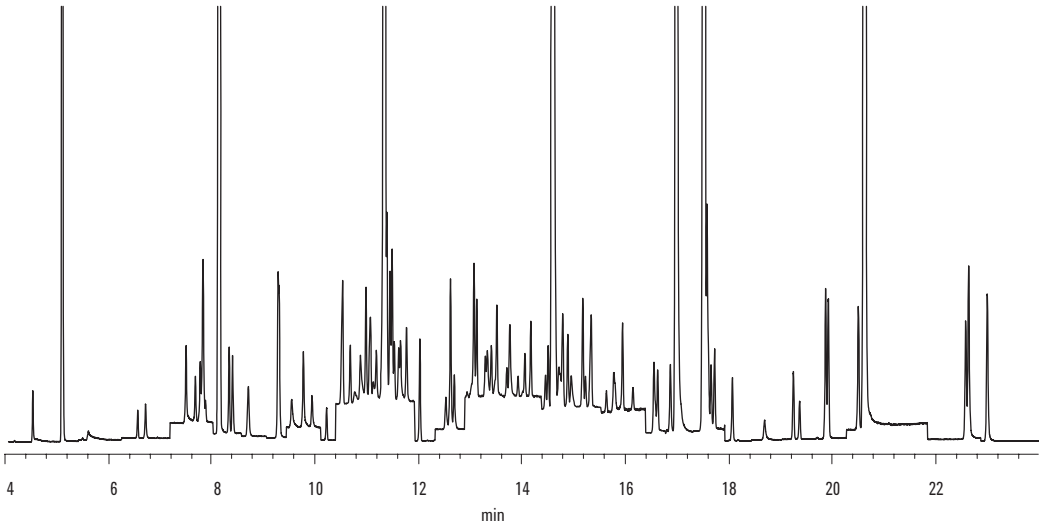

Figure 2. TIC for 0.05 ppm 525 semivolatiles — SIM 115 compounds in 26 groups

linearity. SIM/scan mode has all of the benefits of SIM with full scan spectra available for analyte confirmation.

References

1. USEPA Method 525.2 is available from different sources listed on this Web site: www.epa.gov/OGWDW/methods/where.html.
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Printed in the USA
July 31, 2006
5989-5421EN

Synchronous SIM/Scan Low-Level PAH Analysis Using the Agilent Technologies 6890/5975 inert GC/MSD

Application

Environmental

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Abstract

The analysis of Polynuclear Aromatic Hydrocarbons (PAHs) presents challenges due to the tendency of the PAHs to adsorb on surfaces in the chromatographic system. Selected Ion Monitoring (SIM) analysis is needed for low-level analysis, while scan data are desired for confirmation. The 6890/5975 inert GC/MSD system is designed for improved PAH analysis using synchronous SIM/scan while maintaining linearity across a wide calibration range.

Introduction

PAHs are produced during combustion of organic material and are suspected carcinogens. The high amounts and widespread occurrence of these compounds in our environment requires reliable, sensitive, and very robust analytical methods.

PAHs tend to be adsorbed on any active or cold site in a GC/MSD system, such as inlets and ion sources. The 6890/5975 inert includes the inert source with high temperature filaments described previously [1]. Using the proper inlet liner also improves chromatographic peak shape and sensitivity.

Many laboratories calibrate for PAHs from 0.1 ppm to 10 ppm using SIM for low-level work. Historically, SIM has been necessary because of instrument sensitivity and loss of PAHs at the lower concentration levels. Full scan data is preferred for further confirmation of the compounds. The 5975 inert can acquire both SIM and scan data in a single run.

This application note will show the performance of the 6890/5975 inert for PAHs using a calibration range of 0.01 ppm–10.0 ppm in synchronous SIM/scan mode with linearity equal to that of many SIM only methods.

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890			
Inlet	EPC Split/Splitless			
Mode	Pulsed Splitless, 1 µL injected			
Inlet temp	300 °C			
Pressure	13.00 psi			
Pulse pressure	40.0 psi			
Pulse time	0.20 min			
Purge flow	30.0 mL/min			
Purge time	0.75 min			
Total flow	34.6 mL/min			
Gas saver	Off			
Gas type	Helium			
Inlet Liner	**Description**			**Agilent part number**
	Direct connect, dual-taper, 4-mm id			G1544-80700
	or Splitless liner, single-taper, 4-mm id			5181-3316
Oven 240 V				
Oven ramp	**°C/min**	**Next °C**	**Hold min**	
Initial		55	1.00	
Ramp 1	25	320	3.00	
Total run time	14.60 min			
Equilibration time	0.5 min			
Oven max temp	325 °C			
Column	Agilent Technologies HP-5MS			19091S-433
Length	30.0 m			
Diameter	250 µm			
Film thickness	0.25 µm			
Mode	Constant Flow = 1.5 mL/min			
Inlet	Front			
Outlet	MSD			
Outlet pressure	Vacuum			
RTL	System Retention Time Locked to Triphenyl phosphate at 10.530 min			
MSD	Agilent Technologies 5975			
Drawout lens	6-mm ultra-large aperture			G2589-20045
Solvent delay	4.00 min			
EM voltage	Run at Autotune voltage = 1294 V			
Low mass scan	45 amu			
High mass scan	450 amu			
SIM	12 groups, 3–6 ions/group, 10 ms dwell/ion			
Threshold	0			
Sampling	1			
Cycles/s	5.55 each, SIM and scan			
Quad temp	180 °C			
Source temp	300 °C			
Transfer line temp	280 °C			
Emission current	Autotune value = 34.6 µamp			

Calibration Standards

Calibration standards were diluted in dichloromethane from a stock mix of 16 PAHs. The 10 levels made were 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02 and 0.01 ppm. The perylene-d12 internal standard and the three surrogate standards, 1,3-dimethyl-2-nitrobenzene, pyrene-d10 and triphenylphosphate, were added to each calibration level at 1.0 ppm.

The 6890 inlet temperature was set to 300 °C, instead of the typical 250 °C, to minimize compounds adsorbing on the liner surface. Pulsed injection was used to facilitate quantitative transfer of the heavier PAHs onto the column, minimizing inlet discrimination. Pulsed injection parameters are easily set in the ChemStation software and are automatically controlled by the EPC (Electronic Pneumatic Control) module.

The Direct Connect inlet liner allows for complete transfer of analytes onto the column. The column inlet end attaches to the liner and minimizes analyte exposure to the stainless steel annular volume in the inlet. The splitless liner, 5181-3316, yields better peak shapes for early eluters at the expense of lower amounts of analytes transferred to the column. Neither of these liners is well suited for split injections. Higher concentration samples requiring split injection would need a cyclosplitter-type liner, also suitable for splitless.

The 6890N 240V oven was necessary for the 25 °C/min ramp used up to the final temperature of 320 °C. A 120 V oven will achieve 20 °C/min at these higher temperatures and could be used, resulting in slightly longer run times.

The HP-5MS column is the most widely used column for environmental analysis. It has excellent lifetime and stability at elevated temperatures.

The system was Retention Time Locked to Triphenyl phosphate at 10.530 min. See the fundamentals of Retention Time Locking (RTL) for GC/MSD systems [2]. The primary benefit of RTL for the environmental laboratory is the ability to maintain retention times after clipping or changing the column. Quant database and integration events times do not have to be changed. For laboratories performing PAH SIM analyses, reproducible retention times are a must so SIM group times remain constant. Additional RTL application notes are available at www.agilent.com/chem, detailing the numerous benefits of RTL.

The 5975 inert was tuned using Autotune. The automatic DFTPP target tune, as required by some government methods, can also be used. The ultra-large aperture drawout lens was used to maintain linearity across the wide calibration range of 0.01–10.0 ppm. Source temperature was set to 300 °C, which is now possible with the high temperature filaments. This higher source temperature in combination with the new source material produces better peak shapes for the PAHs.

Data were collected using the synchronous SIM/scan mode available with the 5975 inert. A quant database is first setup using full scan data. SIM ions and groups are then determined automatically using Generate AutoSIM Method. A checkbox in data acqusition is used to acquire SIM and scan data in the same run. For details of synchronous SIM/scan, see reference 3.

Results

The system was calibrated at 10 levels: 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 ppm using the SIM data from SIM/scan acquisition. The calibration table allows the user to choose either the SIM or scan data. The TIC (Total Ion Chromatogram) for the 0.2 ppm level is shown in Figure 1, both SIM and scan traces. Each calibration level contained 16 PAHs, perylene-d12 (ISTD) and the three surrogate standards, 1,3-dimethyl-2-nitrobenzene, pyrene-d10, and triphenyl phosphate.

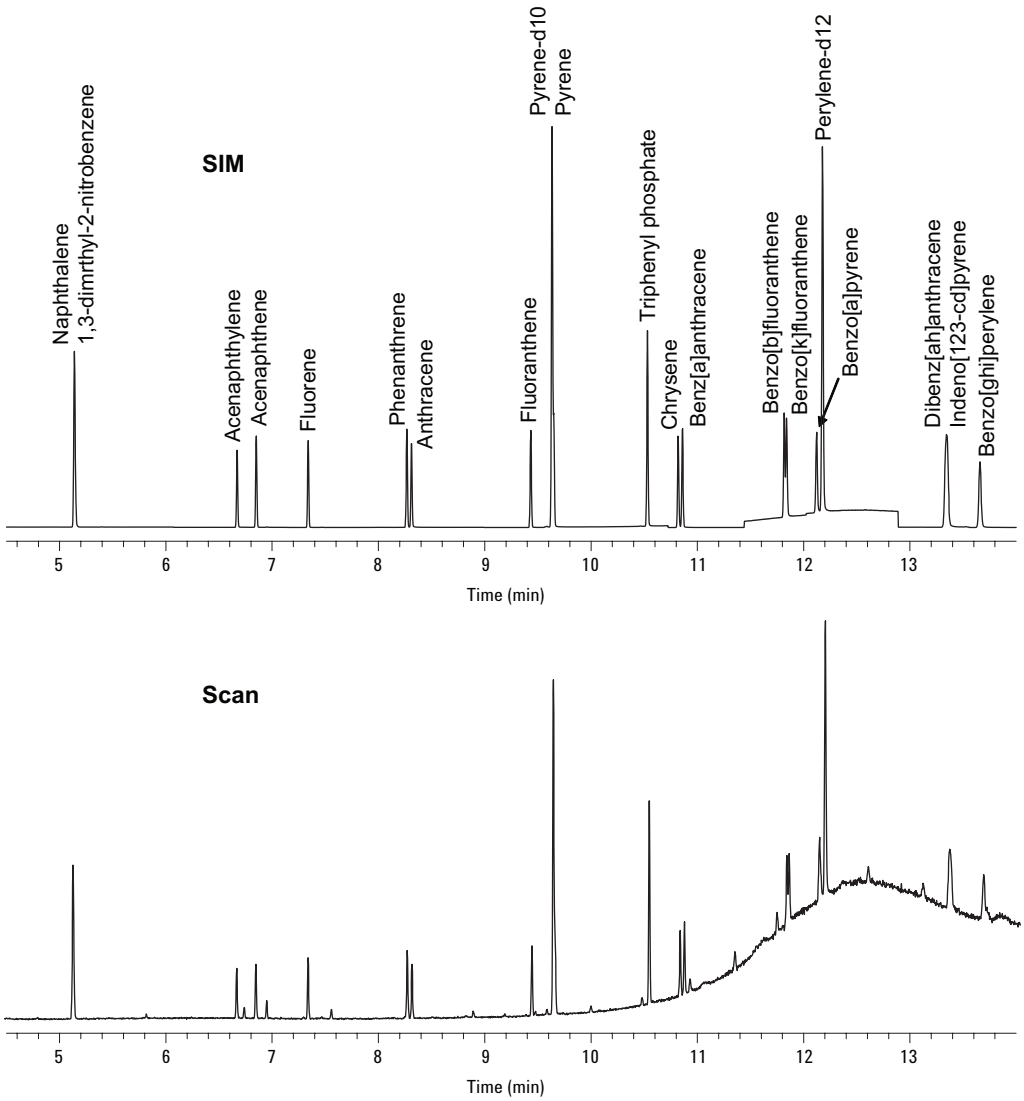

Figure 1. Sixteen PAHs at 0.2 ppm each with surrogates and ISTD at 1.0 ppm each, using synchronous SIM/scan mode.

The RRF (relative response factor) was calculated automatically for each compound at each level by the GC/MSD ChemStation software. Linearity was determined by calculating the %RSD (percent relative standard deviation) of the RRFs across the calibration range for each compound. This is also done automatically by the software in conjunction with Excel.

Linearity is excellent with the average of all %RSDs = 6 %. This compares favorably with other methods that are SIM only or those that only calibrate down to 0.1 ppm.

There were 5.55 SIM cycles/s and 5.55 scans/s acquired throughout the run. This yields 11 SIM data points and 11 scan data points across a typical peak.

Full scan data are also available for further PAH confirmation using library searching. Figure 2 shows a full scan spectrum from benzo[ghi]perylene, together with its library match. Unknown peaks for which SIM data were not acquired can also be library searched. A more reliable, faster method for identifying all the peaks is the use of Deconvolution Reporting Software [4].

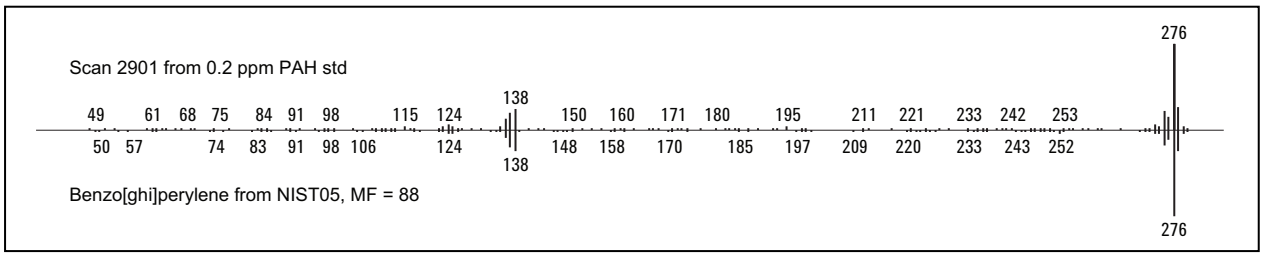

Figure 2. Spectrum from scan at 13.662 min with NIST05 Library match.

Conclusions

The 6890/5975 inert shows much improved response and peak shape for PAHs due to the inert source material and higher allowable source temperature. This improved response gives better linearity across the calibration range. Analysis of PAHs can be accomplished using synchronous SIM/scan data acquisition over a calibration range of 0.01 ppm to 10 ppm, while maintaining performance similar to SIM methods. Sensitivity of SIM is achieved while providing full scan data for confirmation of PAHs and identification of unknowns in a single run.

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2. K. Weiner, N. Mata and P. Wylie, “Retention Time Locking with the G1701BA MSD Productivity ChemStation”, Agilent Technologies, publication 5968-3433E, www.agilent.com/chem.
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Printed in the USA
November 9, 2005
5989-4184EN

Agilent Technologies

Applying the 5975 inert MSD to the Higher Molecular Weight Polybrominated Diphenyl Ethers (PBDEs)

Application

Environmental, Component Testing

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Abstract

A previous application note presented results for analysis of the polybrominated diphenyl ethers (PBDEs) in polymers using the 5973N inert MSD [1]. Mass spectra were presented and interpreted for all of the important PBDEs. The new 5975 inert MSD provides many new features and improvements with expanded mass range to 1050 u being but one. This note presents the full spectra of the octa-, nona and decabrominated biphenyls ethers including ions that appear beyond the mass range of the previous 5973 MSD platform.

Introduction

PBDEs have become the "new PCBs" due to their widespread detection throughout the ecosystem. They have some structural and consequently mass spectral features in common with the polychlorinated biphenyls (PCBs) as well. The series of fragments formed by loss of chlorines (M-nCl_2) generates a number of intense ions useful in their determination. The PCBs also show relatively intense molecular ion clusters that assist in distinguishing the congeners. Similar attributes are expected and hoped for the PBDEs which show much more analytical difficulty than the PCBs.

This note presents the full scan spectra obtained for the PBDEs over the extended mass range of the 5975 inert MSD. The polymeric sample preparation and extraction protocols are cited elsewhere and supply two approaches to PBDE determinations [1].

Experimental

PBDE standards were acquired from Cambridge Isotope Laboratories (Andover, MA) and AccuStandard (New Haven, CT).

Instrumental Configuration and Conditions

The 6890 GC configuration and conditions are given in the previous application note [1]. The 5975 inert MSD system was operated in scan mode for acquisition of the PBDE spectra. The MSD scan operating parameters are cited in Table 1.

Table 1. 5975 inert MSD Configuration and Parameters

Mass spectrometer parameters	
Ionization mode	Electron impact
Ionization energy	70 eV
Tune parameters	Autotune
Electron multiplier voltage	Autotune + 400V
Scan mode	200–1000 u
Quadrupole temperature	150 °C
Inert source temperature	300 °C

Full conditions and parameters, as appropriate to the polymer analysis cited in reference 1, are available in the eMethod for this analysis (www.agilent.com/chem/emethods).

Results

EI Spectra of the Higher Molecular Weight PBDEs

Figures 1, 2, and 3 present the full-scan spectra of an octa-, nona- and the decabromodiphenyl ether. Note that most intense ions in all cases are the $[M–Br_2]^+$ and the corresponding to $[M–Br_2]^{+2}$ ions. The relative abundance of the molecular ion clusters $[M]^+$ are under 30%. Figure 4 compares the theoretical isotopic pattern to that experimentally obtained by the 5975 inert MSD. Agreement is good in both the abundance of the isotopes and the mass accuracy using the standard system Autotune. Mass accuracy agrees to within 0.2 *m/z* of the theoretical and experimental values. Table 2 presents the important ions for the PBDEs greater than the dibromoDE. These ions are those most important to characterizing the technical mixtures used as additives to polymers.

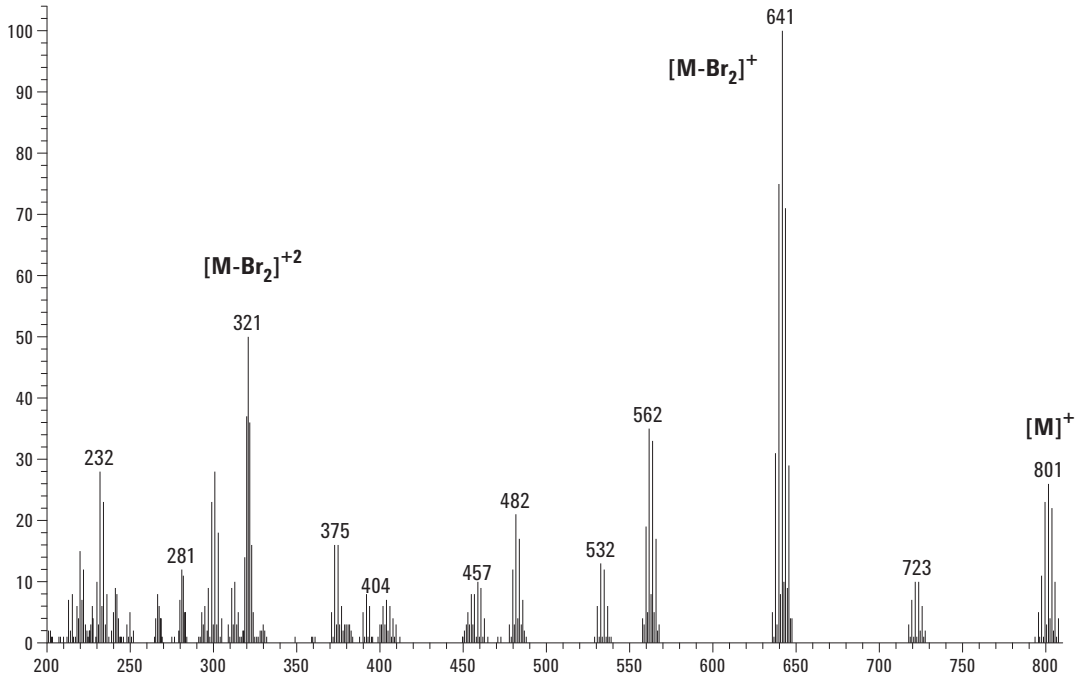

Figure 1. Electron impact ionization spectrum of an octabromodiphenyl ether (PBDE-203) from 200 to 810 *m/z*.

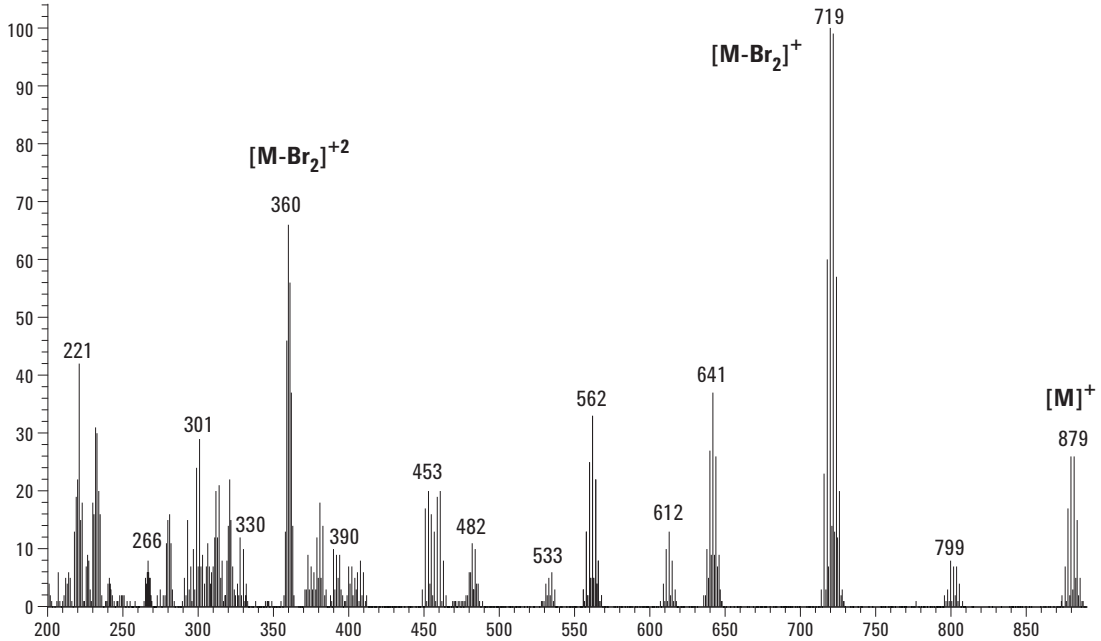

Figure 2. Electron impact ionization spectrum of a nonabromodiphenyl ether (PBDE-208) from 200 to 890 *m/z*.

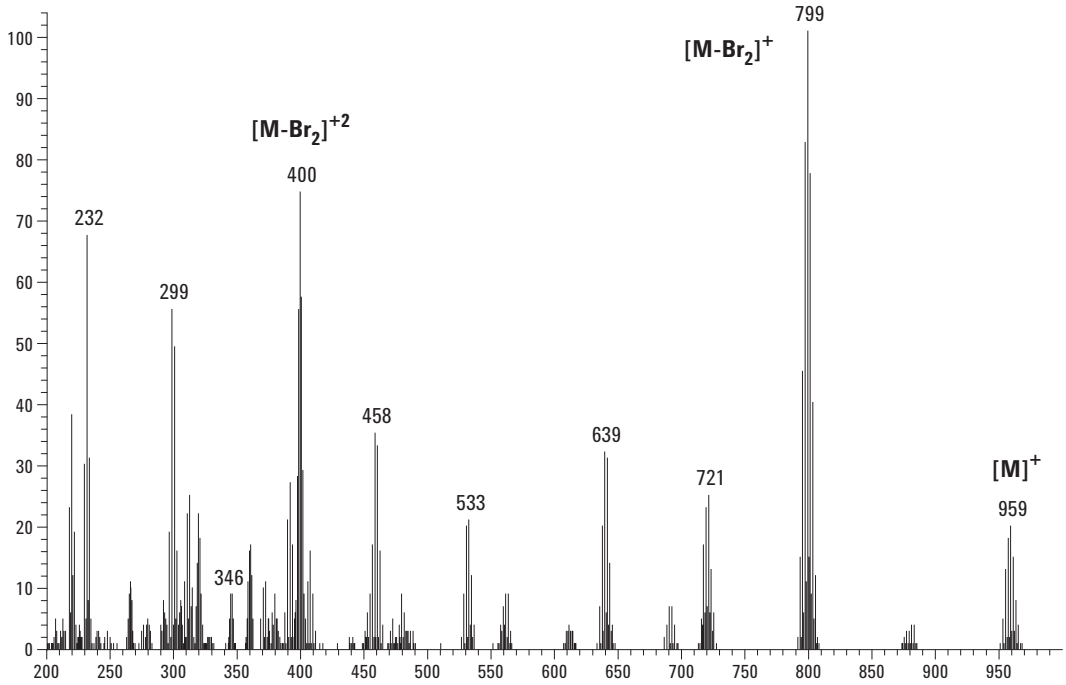

Figure 3. Electron impact ionization spectrum of the decabromodiphenyl ether (PBDE-209) from 200 to 1000 *m/z*.

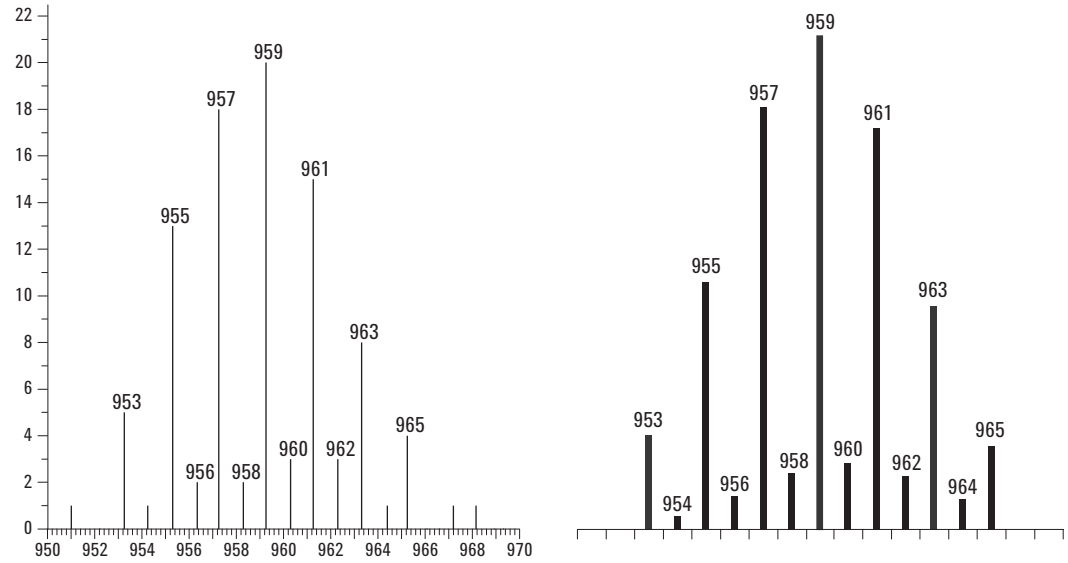

Figure 4. Experimental spectrum of the decabromodiphenyl ether (PBDE-209) molecular ion cluster $[M]^+$ versus theory.

Table 2. Important Ions for the PB_nDEs (n>2)

PBDE bromination	$[M]^+$	$[M\text{-}Br_2]^+$	$[M\text{-}Br_2]^{+2}$
3	405.8	246.0	123.0
4	485.7	325.9	162.9
5	563.6	403.8	201.9
6	643.5	483.7	241.9
7	721.5	561.6	(280.8 **)
8	801.4	641.5	320.8
9	879.3	719.4	359.7
10	959.2	799.3	399.7

**The 280.8 and 281.8 *m/z* ions can be compromised by column bleed interferences so these have not been used in acquisition although they provide a useful diagnostic for column degradation.

The user should note the ion source and quadrupole temperature settings in Table 1. Figure 5 presents SIM acquisitions of several higher molecular weight PBDEs at source temperatures of 300 °C and 230 °C. Notice the signal height roughly doubles on average for the PBDEs at the higher ion source temperature. The insert in the figure shows the improvement in the peak shape for the hexabrominated diphenyl ether. This peak sharpening accounts for the increase in signal height. Since these compounds elute at higher temperatures

among other high boiling components that belong to the matrix, heating the quadrupole is important for robust and low maintenance operation in samples.

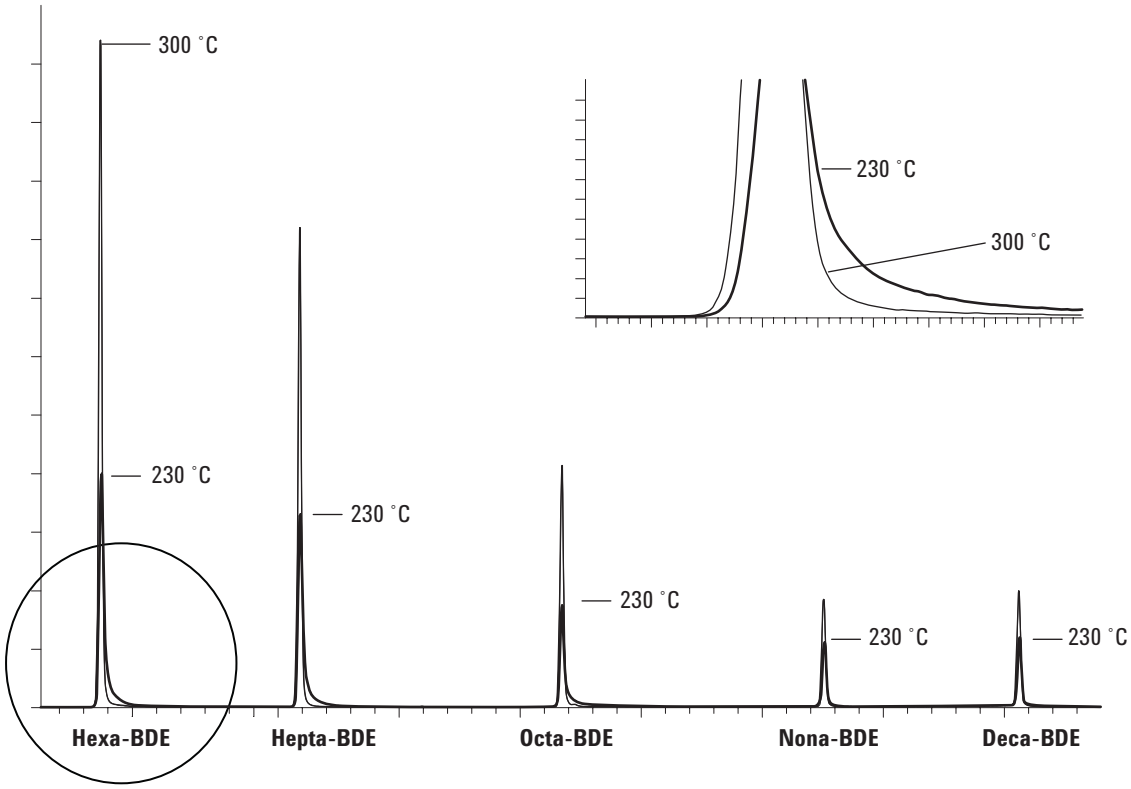

Figure 5. Overlaid RIC SIM acquisitions of five PBDEs at ion source temperatures of 230 °C and 300 °C. Insert is expanded view of hexa-BDE overlays near baseline.

Conclusions

The new 5975 inert MSD has an expanded set of features including mass range. High mass accuracy under standard autotuning is obtained even at the high masses typical of the brominated diphenyl ethers. As users survey higher mass compounds, the heated quadrupole and high temperature capabilities of the 5975 inert MSD will become even more important to rugged and robust analyses in complicated samples.

More details on the other relevant instrumental parameters are available in the eMethod (www.agilent.com/chem/emethods).

Reference

1. C. Tu, and H. Prest, Determination of polybrominated diphenyl ethers in polymeric materials using the 6890 GC/5973N Inert MSD with electron impact ionization. Agilent Technologies, publication 5989-2850EN, www.agilent.com/chem

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Printed in the USA
June 13, 2005
5989-3142EN

Fast USEPA 8270 Semivolatiles Analysis Using the 6890N/5975 inert GC/MSD

Application

Environmental

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Abstract

The analysis of semivolatiles using EPA Method 8270 presents challenges due to the simultaneous measurement of acids, bases, and neutrals over a wide concentration range. Due to productivity demands, laboratories want to run faster while maintaining linearity and sensitivity for even the most active compounds. The 6890N/5975 inert GC/MSD system is designed to meet the criteria for fast analysis, while minimizing activity and maintaining linearity.

Introduction

USEPA Method 8270 for semivolatiles analysis is used to concurrently measure a mix of acids, bases and neutrals. Most laboratories analyze for 70–100 compounds with a chromatographic run time of 25–40 minutes. Laboratories want to reduce this run time for productivity increases. The calibration range required for the analysis varies dependent on a particular laboratory's statement of work. Historically, a range of 20–160 ppm (parts-per-million) has been used. With the increased sensitivity of newer GC/MS systems, laboratories are moving toward lower minimum detection limits (MDLs) and pushing the calibration range down to 1 ppm.

The 6890N/5975 inert GC/MSD (gas chromatograph/mass selective detector) system was designed to meet the demand for faster runs and lower MDLs. Faster scan rates without loss of signal are now possible. This allows the use of smaller id columns, such as 0.18 mm, resulting in shorter runs, while maintaining sufficient data points across narrower chromatographic peaks.

The inert source allows for less material injected onto the column while maintaining mass spectrometer performance. Injection volume can therefore be matched to the 0.18-mm column. Performance comparisons using the inert source were previously published [1, 2, and 3].

This application note will demonstrate the use of the 6890N/5975 inert for USEPA Method 8270. Smaller id columns with faster scan rates yield run times of 15 minutes while meeting 8270 method criteria.

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized.

Pulsed splitless injection was used to minimize residence times of analytes in the liner, thereby reducing loss of active compounds. The column flow rate alone, without using a pulsed injection, would take too long to sweep the 900-µL liner volume.

The inlet liner, Agilent p/n G1544-80700, has shown the best performance for active compounds at low levels. It does not contain glass wool, which would contribute to active compound degradation. Other liners can be used and a detailed discussion of these can be found in Reference 1.

The 6890N 240 V oven was necessary for the 25 °C/min ramp used up to the final temperature of 320 °C/min. A 120 V oven will achieve 20 °C/min at these higher temperatures and could be used resulting in slightly longer run times.

The DB-5.625 column was recently introduced in the dimensions listed. A 0.5-µL injection volume is well suited to this column. The excellent resolution from this column allows a higher than normal initial temperature (55 °C versus 40 °C). This higher temperature shortens cool-down time by more than 5 minutes, resulting in productivity increases for the laboratory. Benzo[b]fluoranthene and benzo[k]fluoranthene met 8270 resolution requirements at the 80-ppm calibration level and lower, using the operating parameters in Table 1.

The system was Retention Time Locked to Phenanthrene-d10 at 8.700 minutes. The fundamentals of Retention Time Locking (RTL) for GC/MSD systems can be found in Reference 4. The primary benefit of RTL for the environmental laboratory is the ability to maintain retention times after clipping or changing the column. Quantitative database and integration events times do not have to be changed. For laboratories performing SIM analyses, switching group times remain constant. Additional RTL application notes detailing the numerous benefits of RTL are available at www.agilent.com/chem.

Previous work [1] showed improved linearity across a wide calibration range using a 6-mm drawout lens instead of the standard 3-mm lens. Although not shown here, that comparison was repeated on this 5975 inert system and is still valid. The 6-mm lens is also included in Agilent Kit p/n G2860A.

The 5975 inert was tuned using the automatic DFTPP target tune. A previous publication [3] detailed steps to match sampling rates to those used in data acquisition. This process is no longer necessary. The automatic tuning was significantly improved in the 5975 inert. Valid tune parameters are stored for all data acquisition sampling rates. These parameters are automatically called and used based on the method sampling rate.

Previous work showed improved linearity across a wide calibration range using a 25-µamp emission current, instead of the default 35 µamp. The emission current can be set by the user in the Tune Wizard. A mass 50 target = 0.7% was also set in the Tune Wizard.

The sampling rate was changed from the default of 2 to 1, while preserving sufficient sensitivity. The resultant 5.92 scans/s typically yield 10 data points across the peaks.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890N		
Inlet	EPC Split/Splitless		
Mode	Pulsed splitless, 0.5-µL injected		
Inlet temp	250 °C		
Pressure	21.29 psi		
Pulse pressure	40.0 psi		
Pulse time	0.20 min		
Purge flow	30.0 mL/min		
Purge time	0.75 min		
Total flow	34.0 mL/min		
Gas saver	Off		
Gas type	Helium		
Inlet liner	Agilent direct connect, dual taper, 4-mm id, p/n G1544-80700		
Oven	240 V		
Oven ramp	°C/min	Next °C	Hold min
Initial		55	1.00
Ramp 1	25	320	3.80
Total run time	15.4 min		
Equilibration time	0.5 min		
Oven max temp	325 °C		
Column	Agilent Technologies DB-5.625, p/n 121-5622		
Length	20.0 m		
Diameter	0.18 mm		
Film thickness	0.36 µm		
Mode	Constant Flow = 1.0 mL/min		
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
RTL	System Retention Time Locked to Phenanthrene-d10 at 8.700 min		
MSD	Agilent Technologies 5975 inert		
Drawout lens	6-mm Large Aperture Drawout lens p/n G2589-20045		
Solvent delay	1.90 min		
EM voltage	Run at DFTPP tune voltage - 153 V = 1282 V		
Low mass	35 amu		
High mass	500 amu		
Threshold	0		
Sampling	1		
Scans/s	5.92		
Quad temp	180 °C		
Source temp	230 °C		
Transfer line temp	280 °C		
Emission current	DFTPP tune @ 25 µamp		
Calibration standards	Accustandard, New Haven, CT. p/n M-8270-IS-WL 0.25X to 10X, 77 compounds at 10 concentration levels with 6 Internal Standards at 40 ppm		

Results

The 5975 inert passed DFTPP tune criteria for Method 8270 at both 50 ppm and 5 ppm.

The system was calibrated at 10 levels: 1, 2, 5, 10, 20, 50, 80, 120, 160, and 200 ppm. The TIC (total ion chromatogram) for the 10-ppm level is shown in Figure 1. Each calibration level contained 77 compounds together with six ISTDs (internal standards) at 40 ppm.

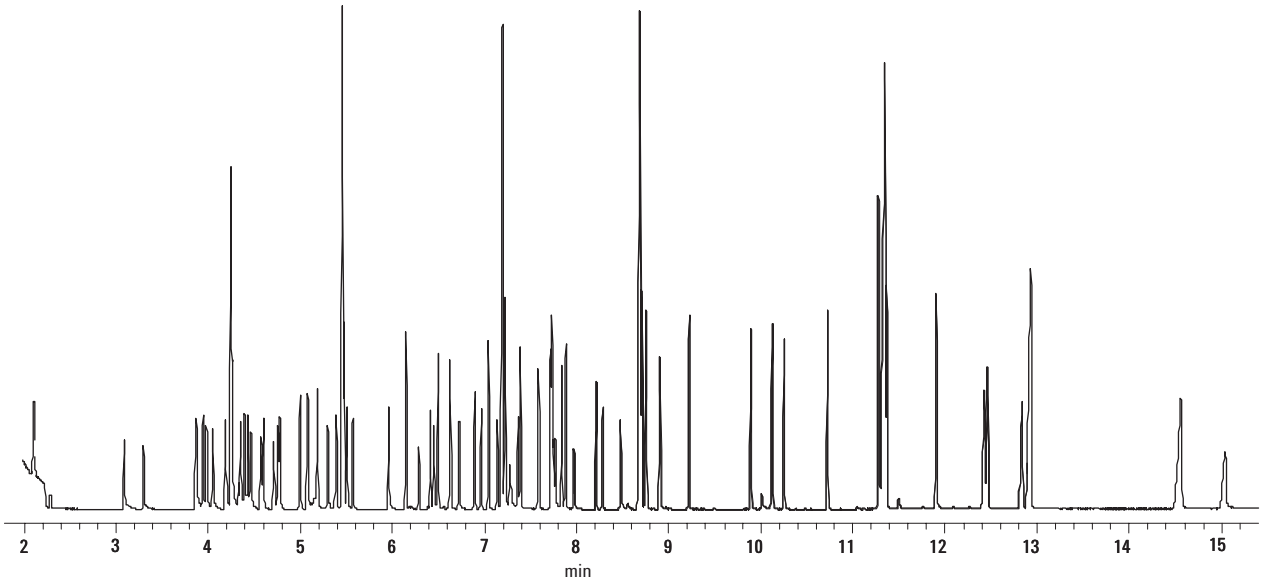

Figure 1. TIC for 8270 semivolatiles at 10 ppm.

The RRF (relative response factor) was calculated automatically for each compound by the GC/MSD ChemStation software. Linearity was determined by calculating the %RSD (percent relative standard deviation) of the RRFs across the calibration range for each compound. This is also done automatically by the software in conjunction with Excel.

USEPA Method 8270D specifies criteria for suitable RRFs and %RSD. Minimum system performance is determined by four active compounds, the SPCCs (system performance check compounds), and is measured by the average RRF.

Table 2 lists the Method 8270D SPCC criteria, and performance of the 5975 inert. The 5975 inert data easily exceeds 8270D criteria and are very good considering the low end of the calibration range. The average RRF of 0.156 for 2,4-Dinitrophenol is difficult to achieve in other systems. This performance margin allows more samples to run before system maintenance is necessary.

Table 2. SPCCs, Comparison of Average RRF

	8270 D Criteria	1-200 ppm 5975 inert
N-Nitroso-di-n-propylamine	0.050	1.270
Hexachlorocyclopentadiene	0.050	0.243
2,4-Dinitrophenol	0.050	0.156
4-Nitrophenol	0.050	0.172

Linearity is shown in Table 3. Method 8270D specifies that this group of Calibration Check Compounds (CCCs) meet a 30% RSD criteria. The %RSD is calculated across the RRFs determined at each calibration level. All CCCs pass criteria using the full calibration range of 1–200 ppm. Pentachlorophenol is a very difficult compound on which to pass criteria. The average of all 77 compound %RSDs is 7%, significantly better than the method criteria of 15%.

Table 3. CCC %RSD of RRFs from 1–200 ppm

	%RSD
Phenol	6
1,4-Dichlorobenzene	3
2-Nitrophenol	5
2,4-Dichlorophenol	8
Hexachlorobutadiene	3
4-Chloro-3-methylphenol	9
2,4,6-Trichlorophenol	16
Acenaphthylene	6
Diphenylamine	5
Pentachlorophenol	25
Fluoranthene	5
Benzo[a]pyrene	3

The excellent system linearity shown here is due to many factors including tuning, the large aperture drawout, and the new electronics. The new electronics allow using a scan rate of 2^1, while maximizing sensitivty. This improved signal/noise together with more data points across a peak yields easier and more reproducible peak integration.

Conclusions

The 6890N/5975 inert meets USEPA Method 8270D criteria. Faster scan rates allow using 0.18-mm id columns for faster runs and shorter cool-down times. Analysis of 77 analytes and six ISTDs can be accomplished in 15 minutes. USEPA Method 8270D tune criteria are routinely achieved. SPCC performance and CCC linearity can be met over a wider calibration range than that historically used. Productivity increases are possible through faster runs, faster cool-down, easier peak integration, and use of a wide calibration range.

References

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2. M. Szelewski, "Fast Semivolatiles Analysis using the Agilent Technologies 6890/5973 inert GC/MSD", Agilent Tecnologies, publication 5989-0207EN, www.agilent.com/chem.
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4. K. Weiner, N. Mata and P. Wylie, "Retention Time Locking with the G1701BA MSD Productivity ChemStation" Agilent Technologies, publication 5968-3433E, www.agilent.com/chem.

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Printed in the USA
April 27, 2005
5989-2981EN

Agilent Technologies

Determination of Polybrominated Diphenyl Ethers in Polymeric Materials Using the 6890 GC/5973N inert MSD with Electron Impact Ionization

Application

Environmental, Component Testing

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Abstract

Due to their ubiquitous appearance in the ecosphere, various polybrominated diphenyl ether formulations have been banned. A major application of PBDEs is to impart fire retardancy to plastics used in electronics and electrical applications. This application note details an approach to determining the PBDEs present in the technical formulations in polymers. The instrumental analysis uses GC/MS with selected-ion monitoring (SIM) to determine tri-BDEs through the decaBDE in 15 minutes. Full scan spectra are presented for the PBDEs with interpretation and to provide an explanation of the choices in SIM ions. To insure correct identification of the PBDE isomers and allow rapid and convenient implementation in the laboratory, Retention Time Locking is applied to an internal standard. A sample preparation scheme referenced in this document provides two flexible and simple approaches to processing polymeric materials for this instrumental technique. PentaBDE, OctaBDE and DecaBDE technical formulations are characterized under the method and results for a typical high-impact polystyrene sample are also presented.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a major issue in discussions of persistent organic contaminants. The detection of PBDEs in essentially all compartments of the ecosystem, including human serum and breast milk, has resulted in a ban of the manufacture and use of certain PBDE formulations by the European Union (EU). Some companies have made it a policy not to allow these compounds in their components and have insisted their suppliers comply. Because the PBDEs are added at percent concentrations (as w/w), the usage of these formulations has been prodigious. Global consumption in 2001 was estimated at 7500, 3790, 56100 metric tons, for the PentaBDE, OctaBDE and DecaBDE technical formulations, respectively.

PBDE analysis even at these relatively high concentrations is challenging in several respects. The PBDEs are a complicated class of compounds and their utility in suppressing combustion also makes them relatively fragile and subject to degradation in GC analysis. This was demonstrated by using shorter GC columns to improve PBDE responses, the most significant improvement being for the deca-BDE (BDE 209) [1]. The loss in congener resolution is less important in this application because the technical mixtures most frequently applied in polymers predominantly consist of isomers extending from the tri-BDEs to the deca-BDE and far less than the 209 possible congeners. Distinguishing congeners on the basis of their electron impact (EI) mass spectrum may be possible since there appears to be some differences in their spectra, however the most reliable index remains retention time (RT). For this reason, compound

Retention Time Locking (RTL) is used to simplify identification and reproduction of the method in the user's laboratory.

Another complication is in sample preparation. There are several methods for extracting PBDEs from polymers each with advantages and disadvantages [2]. Of the many methods, the two approaches applied in processing samples for this application note are relatively inexpensive, simple, universal in application and in their acceptance, and allow for high sample throughput with minimal polymeric interferences. They are polymer dissolution and soxhlet extraction.

Experimental

Polymer samples were obtained from Agilent customers in the electrical and electronic component industries. Specific details of the polymer dissolution and soxhlet extraction methods are presented elsewhere [3]. In summary, the methods extract PBDEs from the sample via solvent, a dilution is made into toluene and PCB 209 is added to follow the dilution factor. Prior to injection, PCB 207 is added as an internal (injection) standard. Standards were made taking into account the potential percent concentration range of the PBDEs in polymeric samples and dilution factors used in the method.

PBDE standards were acquired from Cambridge Isotope Laboratories (Andover, MA) and AccuStandard (New Haven, CT). PCBs 209 and 207 were acquired from AccuStandard (New Haven, CT). Solutions were made in toluene of Burdick & Jackson solvent (VWR Scientific, San Francisco, CA).

Instrumental Configuration and Conditions

The 6890 GC and 5793N-inert MSD (mass selective detector) system configuration and conditions are given in Table 1. The GC is operated under constant flow conditions developed by applying RTL to lock the PCB 209 internal standard RT at 9.350 minutes. The 5973N inert MSD was equipped with the new Performance Electronics upgrade and allowed a single SIM group containing 24 ions to be used. The SIM ions are listed in Table 1 and were acquired with a dwell of 10-ms. This single SIM group method can be used to develop a preliminary method that can be further refined into multiple SIM groups by applying the AUTOSIM utility if the user wishes [4]. This is recommended for 5973-MSDs using standard electronics and targeting only congeners known to predominate in the particular technical mixture.

Table 1. GC and MSD Configuration and Parameters

Injection parameters

Injection mode	Pulsed splitless	
Injection volume	1 µL	
Injection port temperature	320 °C	
Pulse pressure and time	15.8 psi	1.80 min
Purge flow and time	50.0 mL/min	2.00 min
Gas saver flow and time	20.0 mL/min	3.00 min

DB-5ms Column and oven parameters

GC column	DB-5ms (15 m × 0.25 mm id, 0.1 µm film) (p/n: 122-5511)		
Flow and mode	1.8 mL/min	Constant flow	
RTL parameters	9.350 min	RTL compound PCB 209	
Detector and outlet pressure	MSD	Vacuum	
Oven temperature program	90 °C 20 °C/min	1.00 min 340 °C	 2.00 min
Oven equilibrium time	1.0 min		
Total program time	15.5 min		
MSD transfer line temp	320 °C		

Mass spectrometer parameters

Tune parameters	Autotune
Electron multiplier voltage	Autotune + 400V
Solvent delay	6.5 min
Quadrupole temperature	150 °C
Inert source temperature	300 °C

Mass spectrometer SIM ions for single group

405.8	246.0	123.0
485.7	325.9	162.9
563.6	403.8	201.9
643.5	483.7	241.9
721.5	561.6	320.8
799.4	641.5	360.7
719.4	461.7	399.7
463.7	497.7	499.7

*Optional addition of *m/z* 280.8

Miscellaneous parts

Septa	5182-0739	BTO septa (400 °C)
Liner	5181-3315	Deactivated 4-mm id double taper
GC column ferrule	5181-3323	250 µm Vespel/Graphite
MSD interface ferrule	5062-3508	0.4-mm id preconditioned vespel/graphite

Results

Chromatography

After evaluating a series of columns the DB-5ms phase seems the best choice overall, which is consistent with the literature [1]. The literature shows that the shorter columns and thinner films are of benefit to improving the PBDE responses, especially deca-BDE (PBDE-209) [1] and this approach is applied here. The benefit appears in both response and also in shorter analysis times; elution of deca-BDE occurs in less than 15 minutes. The separation on the DB-5ms phase seems sufficient for characterizing PBDE additives in polymers since the desire is not so much the complete separation as it is the overall composition and contribution of the various isomers [5]. Nonetheless, the short analysis time makes RT reproducibility and accuracy more critical for correct assignments of the various PBDE isomers and this is greatly enhanced by applying RTL. A list of the Retention Time Locked elutions of the most prominent PBDEs is presented in Table 2. For reference, Figures 1, 2 and 3 present chromatograms of PentaBDE, OctaBDE, and DecaBDE technical mixtures with approximate elution windows of the various isomers.

Table 2. Prominent PBDE Congeners and their Locked RTs

Compound name	RTL RT (min)
PCB 207	8.69
PCB 209 (locking compound)	9.350
PBDE 17 (tri Br)	6.89
PBDE 28 (tri Br)	7.08
PBDE 71 (tetra Br)	7.97
PBDE 47 (tetra Br)	8.09
PBDE 66 (tetra Br)	8.25
PBDE 100 (penta Br)	8.82
PBDE 99 (penta Br)	9.06
PBDE 85 (penta Br)	9.43
PBDE 154 (hexa Br)	9.62
PBDE 153 (hexa Br)	9.93
PBDE 138 (hexa Br)	10.31
PBDE 183 (hepta Br)	10.73
? hepta PBDE	11.07
PBDE 190 (hepta Br)	11.23
PBDE 204 (octa)	11.62
PBDE 203 (octa)	11.78
? PBDE 196 (octa)	11.84
PBDE 205 (octa)	12.00
PBDE 208 (nona)	12.56
PBDE 207 (nona)	12.64
PBDE 209 (deca Br)	13.60

Note - tentative identification of PBDE 196 was based on reference [1]

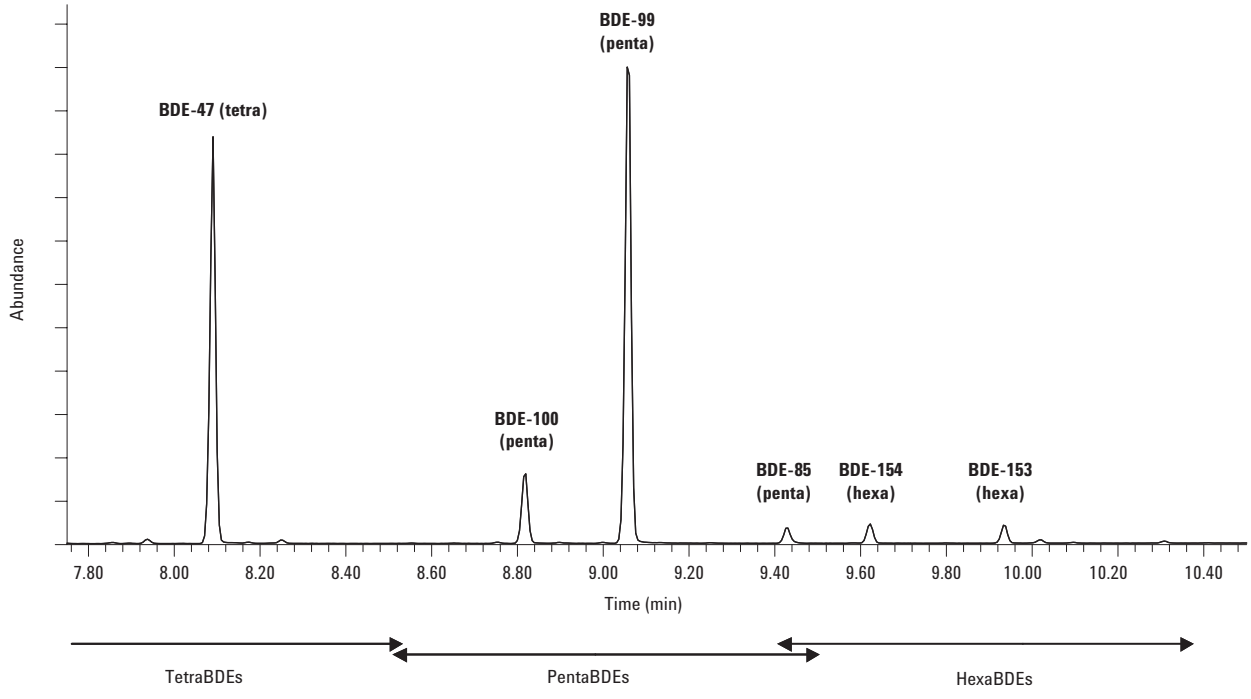

Figure 1 Reconstructed ion chromatogram (RIC) for the GC/MS EI-SIM acquisition of a PentaBDE technical mixture (Cambridge Isotope Laboratories).

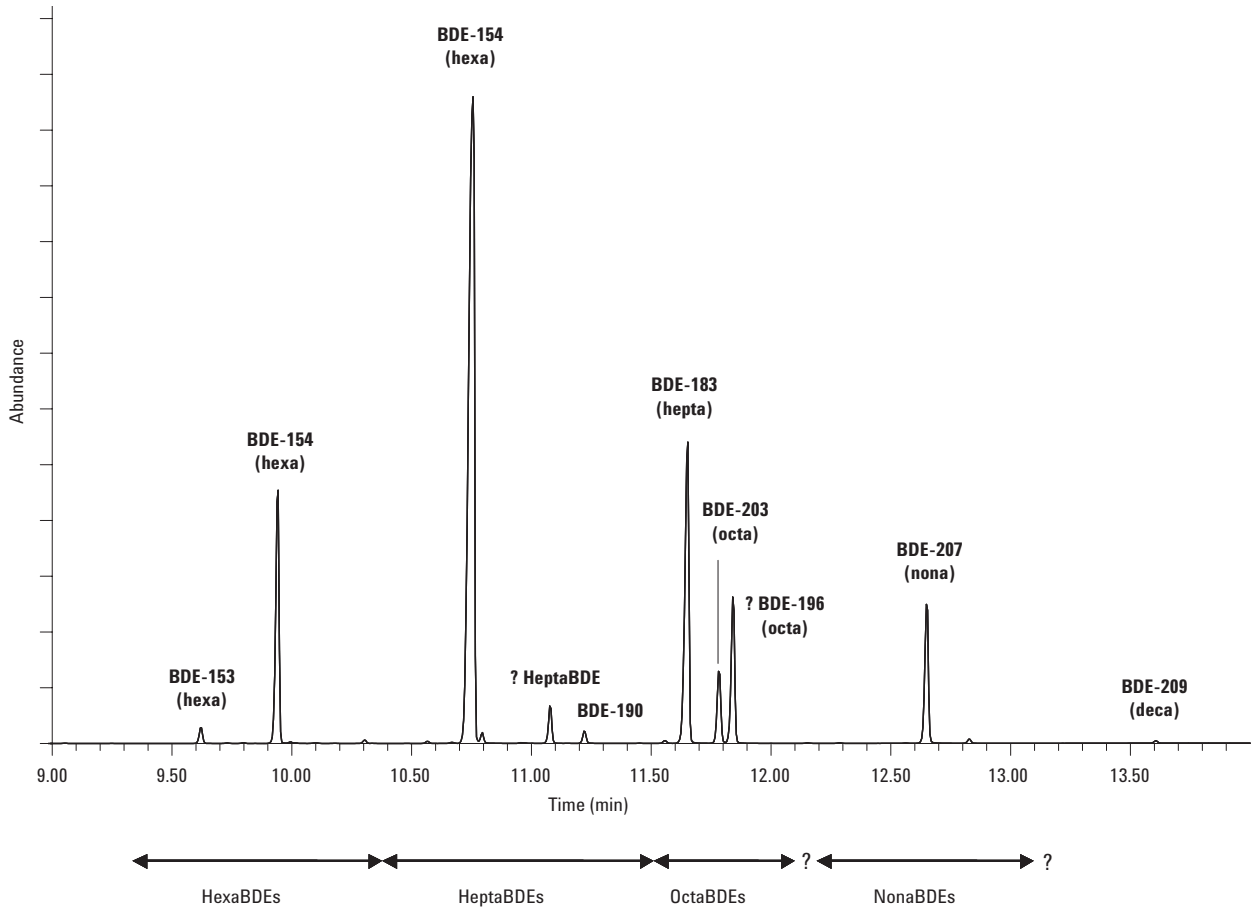

Figure 2 RIC for the GC/MS EI-SIM acquisition of a OctaBDE technical mixture (Cambridge Isotope Laboratories)

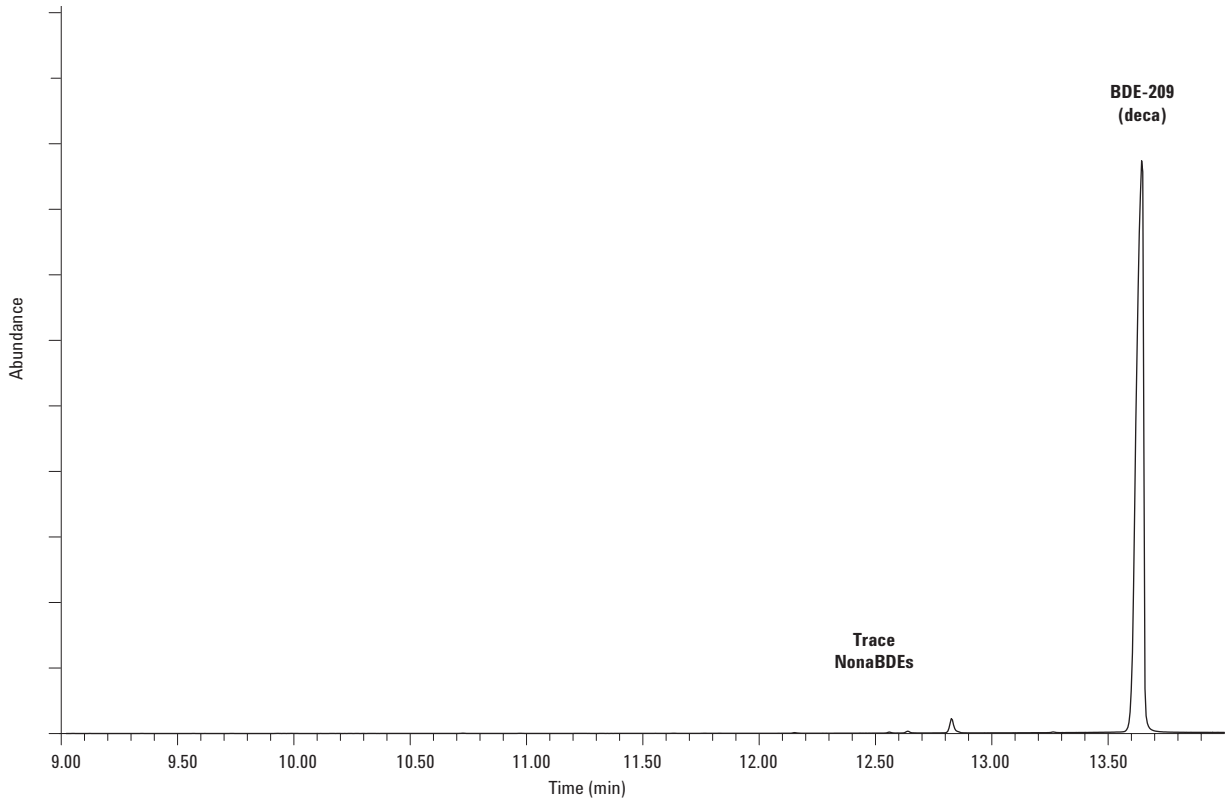

Figure 3 RIC for the GC/MS EI-SIM acquisition of a DecaBDE technical mixture (Cambridge Isotope Laboratories).

PBDE Spectral Interpretation

The EI ionization mass spectra of the PBDE congeners are rich in details and partially described in the literature [7]. Among the isomers the spectra are expected to be approximately identical in pattern and fragmentation pathway. Figure 4 presents a full scan spectrum of a hexabrominated-DE, PBDE-138, obtained at a source temperature of 300 °C. The spectrum shows the isotope cluster due to the molecular ion (643 *m/z*) and an intense cluster (484 *m/z*) consistent with the loss of Br_2. The mass assignment of the *m/z* 484 cluster is consistent with the result of $[M\text{-}Br_2]^+$, that is, $[C_{12}H_4OBr_4]^+$, and shows the tetrabrominated pattern (18 : 69 : 100 : 65 : 16). The next highest abundance isotope cluster appears around 242 *m/z*. Figure 4 shows this cluster and the cluster at *m/z* 484, $[M\text{-}Br_2]^+$. The isotope cluster patterns are similar, which suggests the same degree of bromination, but the fragment mass assignments are half those of the 484 cluster and mass spacing is not 2 but 1 *m/z* unit. While it is possible this is due to overlapping fragments, the close correspondence in patterns lead the authors to propose that this isotope cluster is due to double-charged fragments; that is, $[M\text{-}Br_2]^{+2}$. Recently, this assignment was confirmed by high-resolution MS and the results will be published elsewhere [8]. This $[M\text{-}Br_2]^{+2}$ fragment is common among the PBDEs congeners and grows in relative abundance as the degree of bromination increases: approximately in 10% tetraBDEs; 15% in pentaBDEs; 20%–25% in hexaBDEs and heptaBDEs; 45% in octaBDEs; 60% in nonaBDEs; and > 80% in decaBDE. Figures 5, 6, 7, 8 and 9 show spectra for several PBDEs. We have also observed the same phenomena for the polybrominated biphenyls (PBBs). We also find the ratios vary within an isomeric series more than in PCBs.

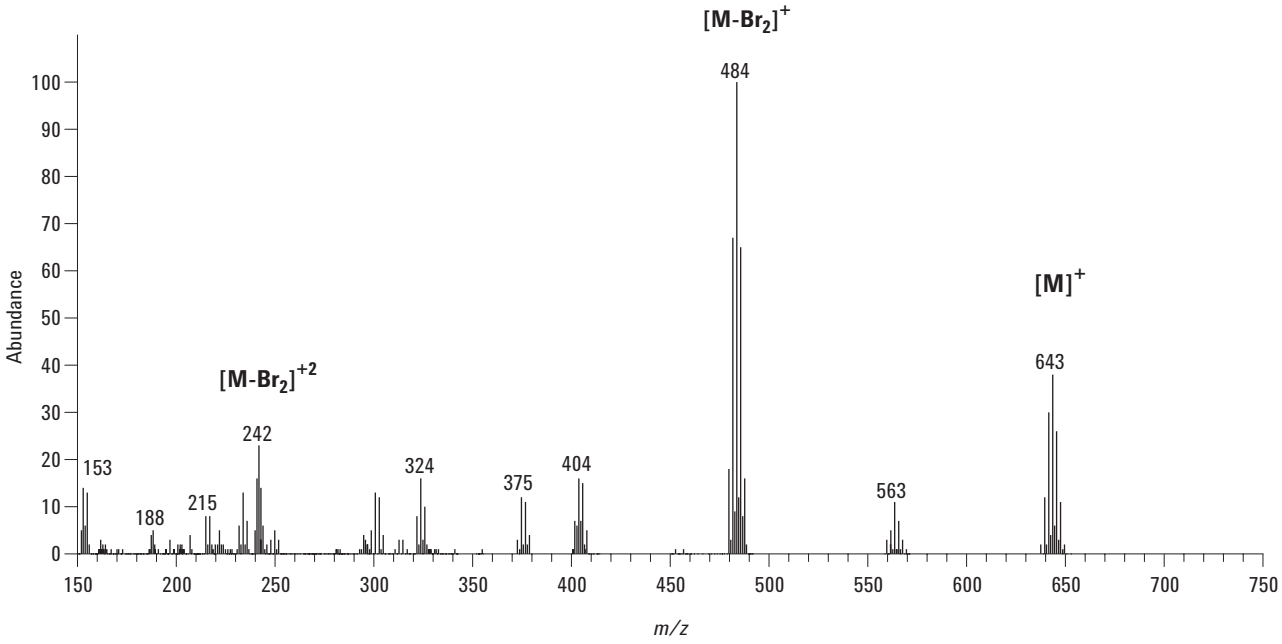

Figure 4 Normalized EI mass spectrum of a hexabrominated-DE, PBDE-138, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

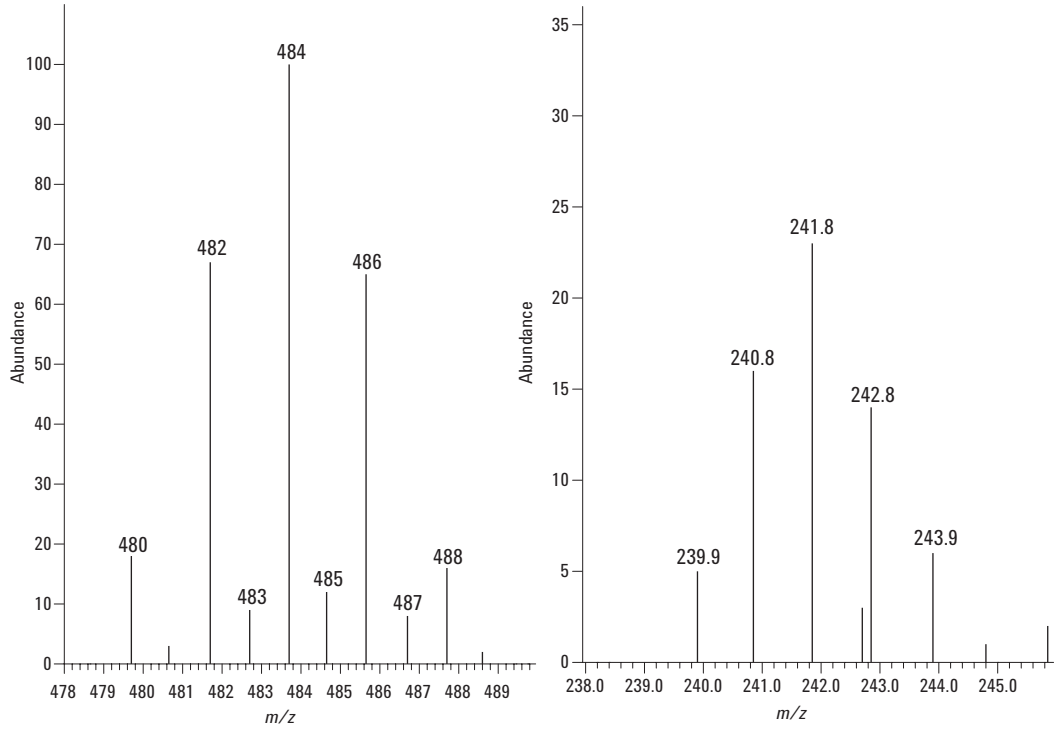

Figure 5 **Sections of the normalized EI mass spectrum of the hexabrominated-DE, PBDE-138, for the $[M\text{-}Br_2]^+$ and proposed $[M\text{-}Br_2]^{+2}$ clusters.**

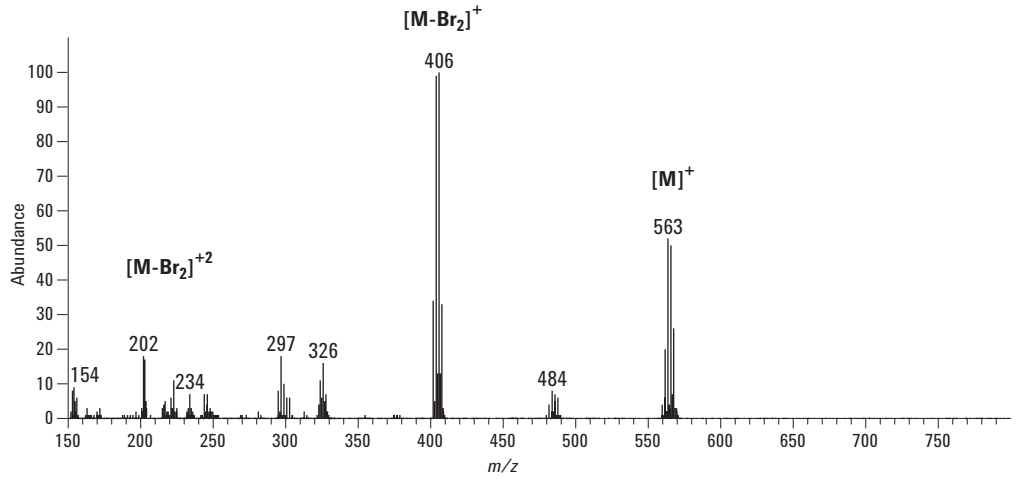

Figure 6 **Normalized EI mass spectrum of a pentabrominated-DE obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.**

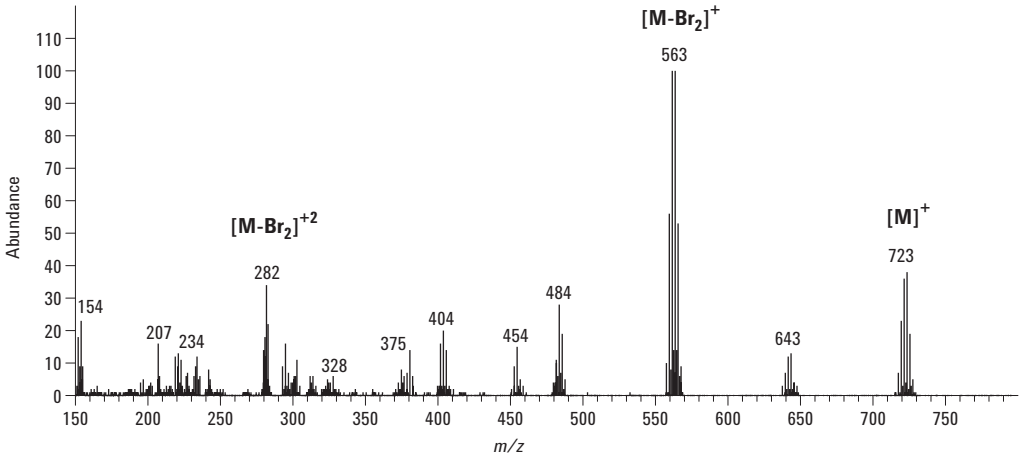

Figure 7 Normalized EI mass spectrum of a heptabrominated-DE obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

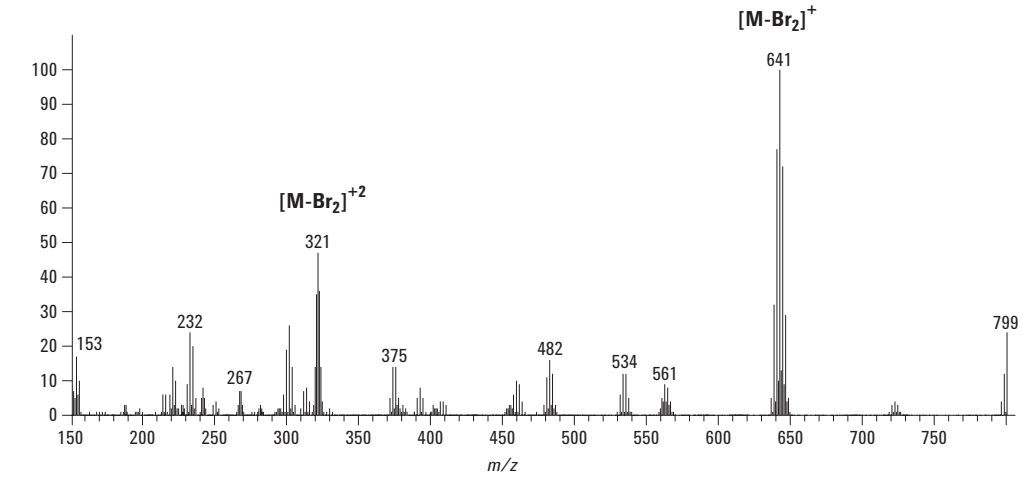

Figure 8 Normalized EI mass spectrum of a octabrominated-DE, PDBE-203, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

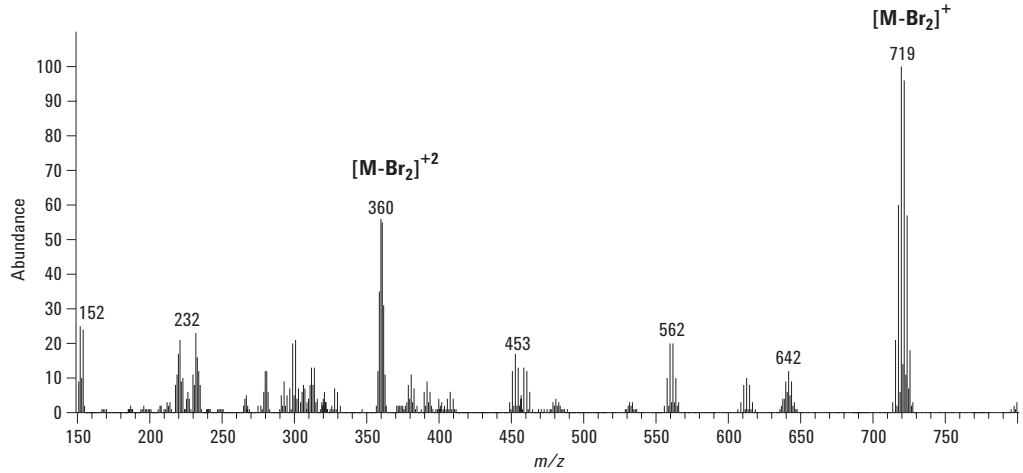

Figure 9 Normalized EI mass spectrum of a nonabrominated-DE, PDBE-208, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

In considering the EI spectrum of the decabromodiphenyl ether, PBDE-209, the same observations apply, Figure 10. Although the cluster of the molecular ion at 959 u, eludes the mass range limitation of the 5973N-MSD, the loss of Br_2 forms an intense isotope cluster at *m/z* 799, $[M\text{-}Br_2]^+$ and the doubly charged fragment(s) for the $[M\text{-}Br_2]^{+2}$ at *m/z* 400 (399.6) as shown in Figure 11. Other data has shown that the intensity of the molecular ion cluster (959 u) is far less than that of the fragments at *m/z* 799 as is the trend for the PBDEs.

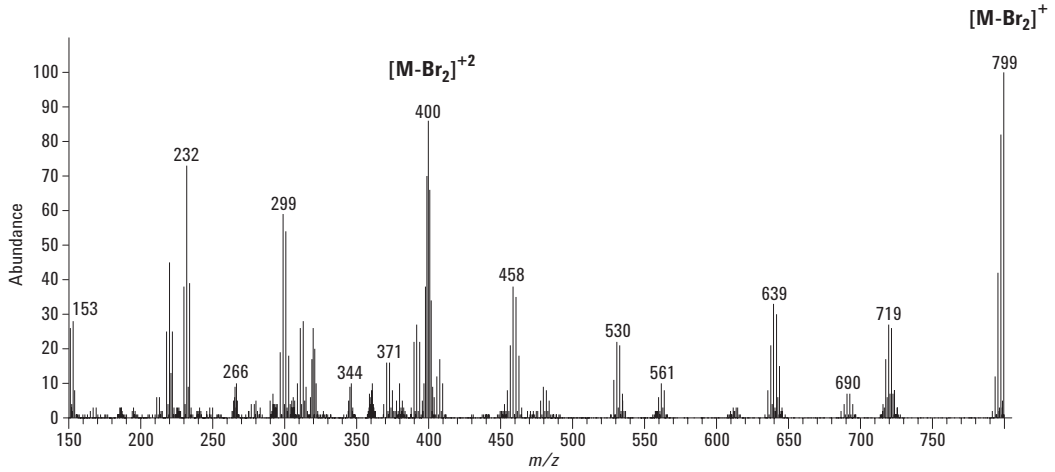

Figure 10 Normalized EI mass spectrum of the decabrominated-DE, PDBE-209, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

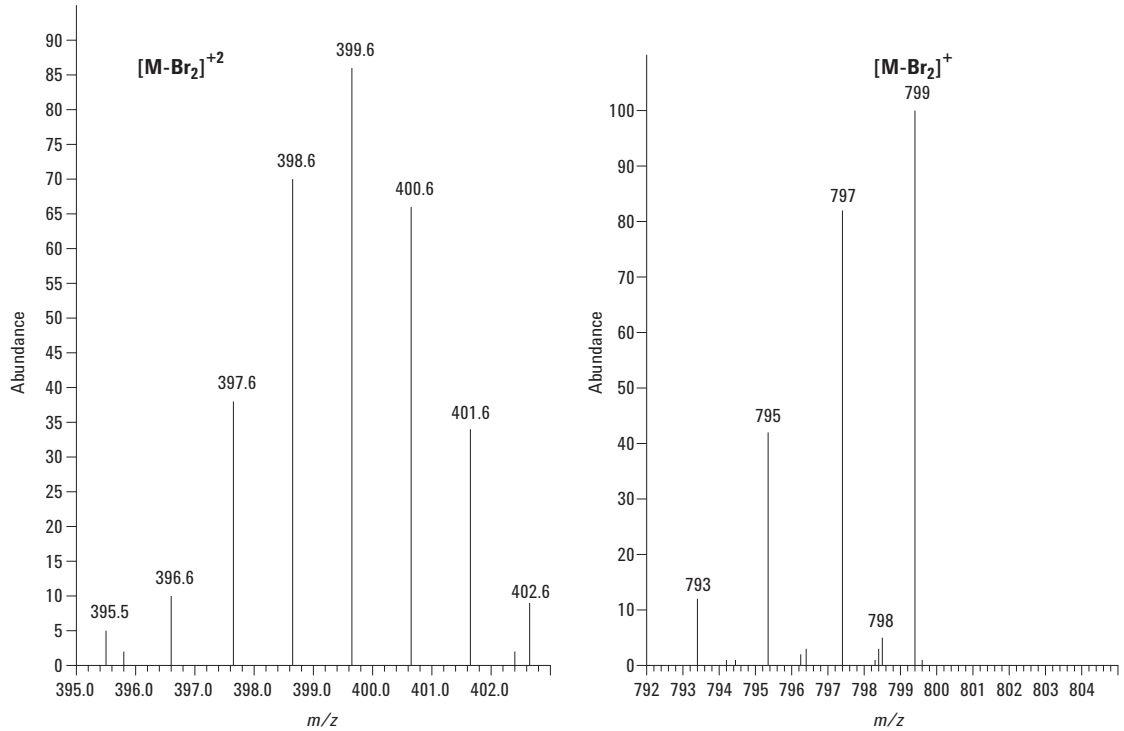

Figure 11 Normalized EI mass spectrum of the decabrominated-DE, PDBE-209, obtained in scan from 150–800 *m/z* at a source temperature of 300 °C.

Therefore these ions (that is, $[M\text{-}Br_2]^+$, $[M\text{-}Br_2]^{+2}$ and $[M]^+$ where available), and compound RTs, identify and allow determination of the deca-BDE and other PBDEs to the ability of the 15-m column to separate the isomers, which appears quite effective and sufficient for characterizing additives. The monitored ions are given in Table 3 with the ions for the internal standards used in this analysis. Obviously, the bromines provide other ions displaced in mass by two units (except for the doubly-charged ions) that offer other additional ions for quantitation or confirmation.

Using the ions listed in Table 3 to identify the PBDE isomers, the regions in the chromatograms presented in Figures 1, 2 and 3 were labeled with the isomer elution windows. These ions and their ratios were also used to characterize PBDEs not available in the standards but found to occur within the samples and technical mixtures (for example, PBDE 196).

Results for Polymeric Samples

Extracting PBDEs from polymers requires that the entrained PBDEs permeate the polymer into the extracting medium. Apparently "melting" the polymer closes the transport corridors in the polymer and impedes extraction. However, "swelling" the polymer with a proper solvent, greatly improves the kinetics of extraction. Beyond deciding the proper solvent, the optimal time of the extraction must be experimentally determined for each plastic based on its consistency and response to the solvent. For the polymer dissolution and soxhlet extraction methods used here, solvent contact times or the number of soxhlet cycles for near complete extraction was determined by serial extraction. Other concerns are described in the sample preparation protocols [3].

Figure 12 shows the chromatogram for an extracted HIPS (high-impact polystyrene) polymer sample supplied by an Agilent customer and Table 4 shows the results for replicate extractions and analysis. Note the chromatogram and its major components closely resembles the chromatogram for the OctaBDE technical mixture (Figure 2) and indicates the specificity of the selected ions and most importantly, the lack of polymeric interferences. The reproducibility of the component compositions is a testament to the reproducibility of the total method. A good portion of the variance is introduced by the high dilution factors used in the method to bring the polymer extract concentrations with the scale of the PBDE standards and therefore discriminates against the lower abundance components producing a higher degree of variation and absolute detection. A series of 25 replicate injections of an extracted sample showed negligible degradation in response or chromatography. The robust performance is largely due to the high MSD ion source and quadrupole operating temperatures of 300 °C and 150 °C, respectively. These high temperatures mitigate the effect of co-extracted polymeric residues on the ion source optics to render robust performance. The high operating temperature of the quadrupole provides a very long lifetime without cleaning or maintenance even when analyzing very dirty matrices such as these.

Table 3. Quantitation and Confirmation Ions for the PB_nDEs (n>2)

PBDE bromination	$[M]^+$	$[M\text{-}Br_2]^+$	$[M\text{-}Br_2]^{+2}$	Confirmation ion
3	405.8	246.0	123.0	403.8
4	485.7	325.9	162.9	483.7
5	563.6	403.8	201.9	561.6
6	643.5	483.7	241.9	641.5
7	721.5	561.6	(280.8 **)	563.6/719.4
8	799.4	641.5	320.8	643.5
9	–	719.4	360.7	721.5
10	–	799.4	399.7	–
PCB 207	463.7	461.7	–	–
PCB 209	497.7	499.7	–	–

**The 280.8 and 281.8 *m/z* ions can be compromised by column bleed interferences so these were not used in acquisition although they provide a useful diagnostic for column degradation.

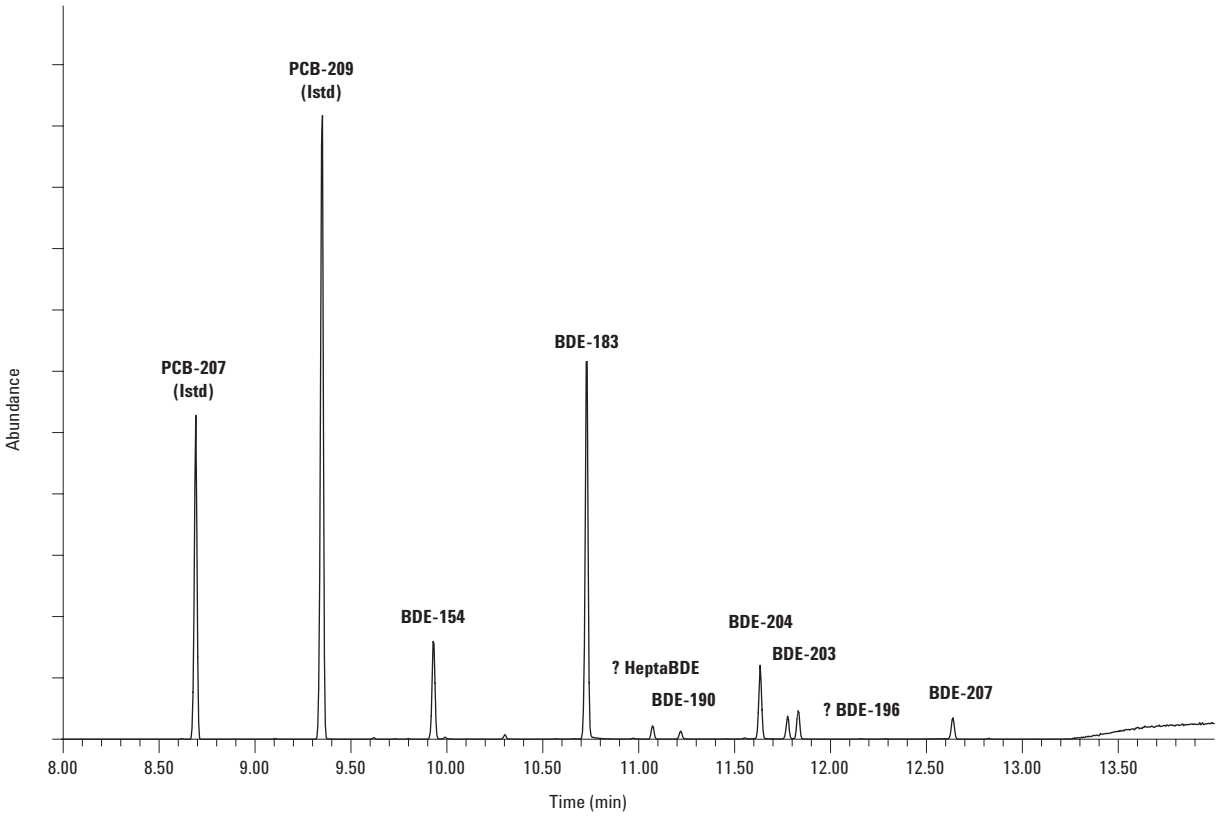

Figure 12 RIC of the GC/MS SIM acquisition of an extracted HIPS polymer sample.

Table 4. Extraction Results for Replicate Analysis of a Polymer Sample for PBDE Composition Using the Two Extraction and Sample Preparation Protocols [3]

Soxhlet polymer extraction protocol results

Sums	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Replicate 4 (%)	Replicate 5 (%)	SD
HexaBDEs	9.1	9.5	8.9	8.7	9.1	0.3
HeptaBDEs	53.3	52.5	51.7	53.1	53.1	0.7
OctaBDEs	29.5	29.5	30.7	29.5	29.8	0.5
NonaBDEs	8.0	8.4	8.6	8.7	8.1	0.3

Polymer Dissolution Extraction Protocol Results*

Sums	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	SD
HexaBDEs	9.9	10.0	9.7	0.2
HeptaBDEs	55.3	56.2	55.9	0.5
OctaBDEs	34.8	33.8	34.4	0.5

SD standard deviation

No tri-DEs, tetraBDEs, pentaBDEs, or decaBDE were detected.

*A difference in analyte lists used to quantitate the soxhlet extracts slightly skews the results, specifically the addition of the nona-BDE analytes. Removing this group, the results agree within 3%.

Remarks

Figure 13 presents two overlaid reconstructed ion chromatograms of the SIM acquisitions of two splits of a single PBDE standard. One of the splits was contained in a clear vial and was exposed to laboratory light for about a week and the other split was stored in amber vial and in a freezer as a reference. The most impressive feature is the dramatic loss of the decaBDE and the possible appearance of another intense nonaBDE (around 11.8 minutes). Note the nonaBDEs in the standard showed no degradation while the octaBDEs and heptaBDEs showed varying degrees of loss in concentration. A number of small peaks appear in the baseline that suggest, on the basis of their fragments, ion ratios, and proximity to existing PBDEs in the standard, the presence of other BDE isomers. Assigning any identification in SIM without a standard reference compound to confirm RT and fragment ratios, or a full scan acquisition, must be considered highly speculative. However, the data does indicate a degradation of the decaBDE and some other PBDEs, and suggests possible isomerization of the some PBDEs under the influence of typical laboratory fluorescent lights. Time and resources do not allow us to pursue this matter, but we provide these observations since there are implications in sample handling and standard preparation and storage.

Conclusions

The 5973N inert MSD equipped with performance electronics allows a single SIM group to survey for PBDE isomers important to characterizing the technical formulations of the PBDEs. Using a single group has the advantages of allowing many formulations to be studied without regard to the particular elution of the congeners (which would require careful maintenance of SIM windows), simplified setup and very rapid analysis. Implementing RTL allows specific congeners to be characterized and quantitated with high confidence. The intense fragmentation of the PBDEs and their universal propensity to form $[M\text{-}Br_2]^+$ and $[M\text{-}Br_2]^{+2}$ ions provides a unique fingerprint for each degree of bromination. The 15-m column used here provides rapid analysis and sufficient class separation. The method is universally applicable regardless of the sample preparation scheme as demonstrated here by replicate polymer analysis by two techniques, soxhlet extraction and polymer dissolution.

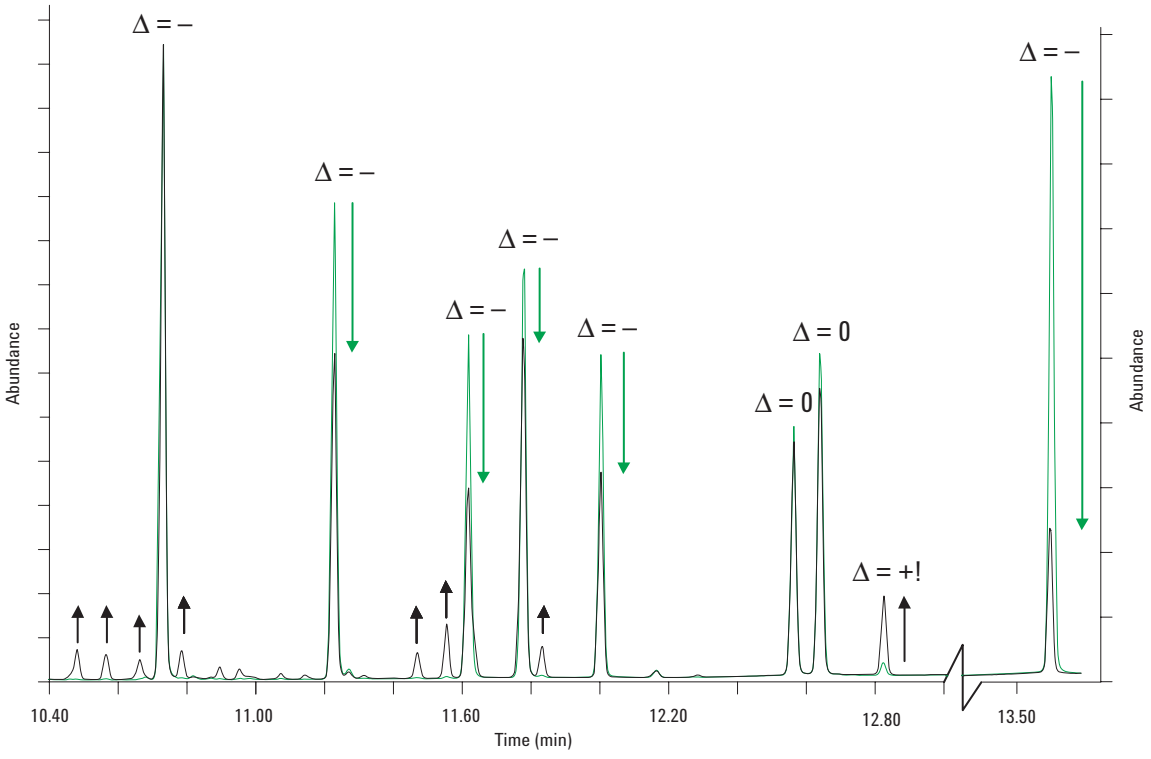

Figure 13. PBDE standard unexposed (green) and exposed to laboratory light. Delta (Δ) indicates change in response as Exposed-Unexposed (with negative signs indicating loss in response and positive an increased response).

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Printed in the USA
April 5, 2005
5989-2850EN

Agilent Technologies

Effect of Sample Matrix on Suppression of Ionization in Water Samples Using LC-ESI-MS

Application

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Abstract

Liquid chromatography electrospray ionization mass spectrometry is a very powerful technique that combines the liquid chromatography separation with highly selective and sensitive mass detection, but the ionization process is susceptible to matrix signal suppression. Signal suppression, therefore, presents a challenge in doing quantitative liquid chromatography/mass spectrometry applications. Using the Agilent Technologies 1100 series quadrupole mass spectrometry system coupled to an Agilent Technologies 1100 series liquid chromatography system revealed that the greatest part of the ion suppression phenomenon is largely due to compounds in the extracted sample that were carried through the extraction.

Introduction

Liquid chromatography (LC) electrospray ionization mass spectrometry (ESI-MS) is a very powerful technique in analyzing organic compounds. The technique combines the LC separation with highly selective and sensitive mass detection. However, one drawback of ESI-MS is that the ionization process is susceptible to matrix signal suppression. The liquid chromatography/mass spectrometry (LC/MS) response obtained from a standard can differ significantly from matrix samples. Therefore, signal suppression presents a challenge in doing quantitative LC/MS applications.

Previous studies have shown that the ion suppression effects are due mainly to the competition between matrix components and the analytes to get charges or to gain access to the droplet surface for gas-phase emission [1]. Other factors include surface tension [2, 3], sample pH [4], and compound polarity [5]. Published approaches to minimize the ion suppression effects include: using a more selective extraction procedure for matrix cleanup [6], providing more chromatographic retention of analytes [6, 7], changing buffer and its concentration [3, 8, 9], flow-splitting [10] or nanospray [11], post-column addition [12], and 2-dimensional chromatography [13]. All the approaches to minimize suppression seem to develop a uniform spray with fine droplets.

In this application note, a target compound in different water matrices was used to study the effects of sample matrix on signal suppression. In the case of potable waters, in particular water samples derived from surface water sources, the suppression was likely to be due to humic and fulvic acids, which elute very early in high aqueous mobile phase conditions [4]. These acids are organic acids that arise naturally in decomposing organic material called humus. The ionization suppression can lead to an apparent reduction in recovery values for certain compounds. The suppression effect in different water matrices was reported recently [4, 14].

Imazapyr (see Figure 1) is a compound that has shown differences in overall recovery with varying water matrices [15]; it has shown, a 73% recovery from groundwater and 48% recovery from potable water derived from a surface water source.

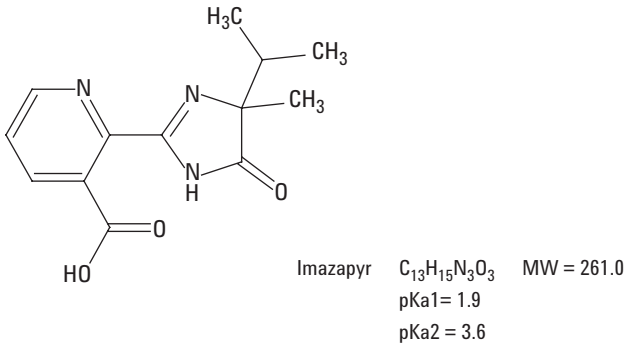

Figure 1. The structure of Imazapyr, the chemical standard used in this study.

A series of experiments were conducted to study the effects of varying sample matrix as measured by Total Organic Carbon (TOC) content to show if indeed sample matrix was the cause of apparent variation in overall compound recovery and also to observe if any other parameters affect the suppression.

Experimental

All analyses were performed using an Agilent 1100 series quadrupole MS system coupled to an Agilent 1100 series LC system consisting of a vacuum degasser, binary pump, autosampler, and diode array detector (DAD). The quadrupole mass spectrometer was operated with the Agilent atmospheric pressure electrospray ionization (API-ESI) source. Figure 2 shows the mass spectrum of 10 mg/L Imazapyr in negative ion mode at fragmentor voltage of 75 V. This spectrum was obtained using the flow injection analysis (FIA) mode while varying the fragmentor voltage.

The TOC of all sample matrices was determined using an automated Labtoc TOC analyzer. The samples are first acidified using concentrated orthophosphoric acid and purged with helium to remove inorganic carbon. The samples are then digested with sodium persulphate and ultraviolet (UV) light to convert the organic carbon to carbon dioxide. The carbon dioxide concentration is measured using infrared detection.

Four sample matrices were chosen for the initial work:

- Deionized water, with a TOC measurement of less than 0.1 mg/L
- Borehole water with TOC measurement equal to 0.5 mg/L
- Potable tap water with a TOC measure equal to 5 mg/L
- River water with a TOC measurement equal to 15.5 mg/L

The first three matrices were also used for the matrix stripping experiments.

All samples were spiked at 5 µg/L with Imazapyr. At this concentration, the samples were within the working range of the DAD and could be analyzed by the MS in the full scan mode.

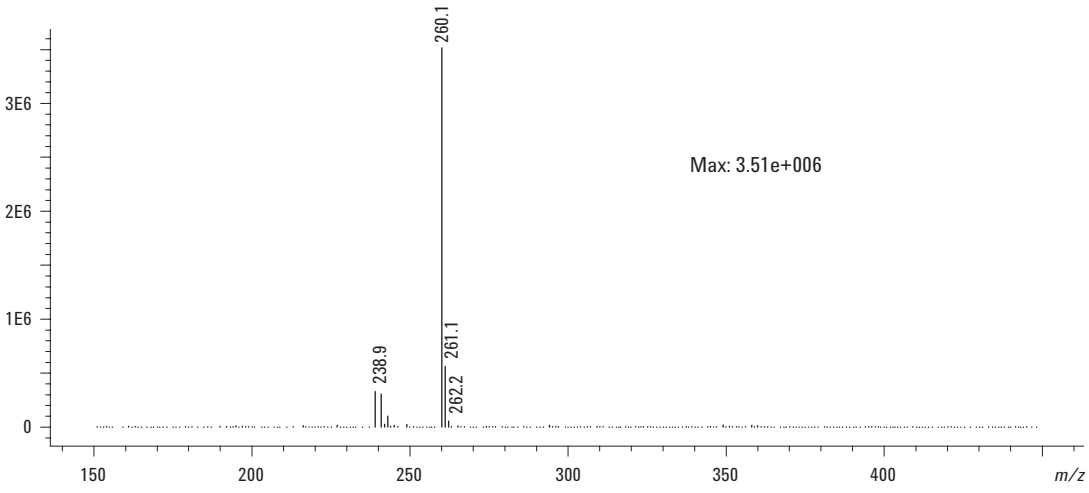

Figure 2. The mass spectrum of Imazapyr at 10 mg/L in negative ion mode at fragmentor voltage of 75 V.

The chemicals and consumables used for this study include:

TBME, 99.8%	HPLC Grade	Sigma- Aldrich part number 29,321-0
Methanol	HPLC Grade	Rathburn Chemicals Ltd part number RH1019
Acetonitrile	HPLC Grade	Rathburn Chemicals Ltd part number RH1015
Water	HPLC Grade	Rathburn Chemicals Ltd part number 1020
Formic acid, 98%	AR Grade	Fisher Scientific part number F/1900/PB08
Hydrochloric acid, 37%		J. T. Baker part number 6011/2.5
Imazapyr standard, 99%		Qmx Laboratories part number C142830
Oasis HLB (60 mg, 3 mL) cartridges		Waters Corporation part number WAT094226
Oasis MAX (60 mg, 3 mL) LP cartridges		Waters Corporation part number 186000368

Sample Preparation

Fully automated solid phase extraction (SPE) using Tekmar Autotrace SPE workstations and Oasis HLB, 60 mg, 3-mL cartridges and a set of solutions:

a. 90:10 *tert*-butyl methyl ether (TBME):methanol (0.01% formic acid)

b. Methanol (0.01% formic acid)

c. HPLC grade water

d. HPLC grade water (0.25% hydrochloric acid)

1. Initially condition each cartridge with sequential additions.

Solvent	mL of solution
a	5
b	5
c	3
d	3

2. Dilute an aqueous sample of 50 mL to 200 mL with deionized water and acidify with 0.75 mL of concentrated hydrochloric acid. This step is done to reduce matrix effects and to give enough sample to pump through the cartridges.
3. Pump a diluted sample of 100 mL through the conditioned cartridge at 10 mL/min.
4. Dry the cartridge for 10 minutes, using forced air.
5. Elute the cartridge with solution (a) twice using 1.5 mL and once using 1 mL, total 4 mL.
6. Evaporate the extract to dryness on a 45 °C heated block, using a gentle air stream.
7. Dissolve the residue in 0.25 mL of 90:10 water (0.01% formic acid):methanol solution.

Matrix Stripping Procedures

Ion suppression phenomena often appear to be extraction recovery effects. To decouple the effects of ion suppression from extraction recovery a series of experiments was conducted on matrix that had been stripped by an SPE procedure.

Two different stripping chemistries were investigated for their impact on ion suppression.

MAX (anion) cartridges
MAX cartridges were used to remove suspected humic acids from the matrix. The MAX cartridges (60 mg, 3 mL) were conditioned with 3-mL methanol and 3-mL HPLC grade water. The sample was pumped through the cartridge to collect the water. The collected water was spiked with Imazapyr at 5 µg/L and re-extracted as per standard method.

HLB cartridges
The different water matrices were pumped through HLB cartridges that were conditioned using the standard method [15] and the water samples were collected. The collected water was spiked with Imazapyr at 5.0 µg/L and re-extracted as per standard method.

Liquid Chromatograph Conditions

Column	Zorbax Eclipse XDB-C18, 2.1 mm × 150 mm × 3.5 µm at 60 °C	
Column flow	Binary pump, isocratic mode	
Mobile phase A	0.01% Formic acid in water	85%
Mobile phase B	Acetonitrile	15%
Flow rate	0.3 mL/min	
Sample size injected	50 µL	
DAD	230 nm (Spectra acquired from 200–400 nm)	

Mass Spectrometer Conditions

V_{cap}	2500 V (Negative), 3000 V (Positive)
Fragmentor voltage	75 V
Full scan range	100–400 amu
Nebulizer pressure	50 psi
Drying gas flow rate	13 L/min
Drying gas temperature	350 °C

Results and Discussion

The ratio of the mass selective detector (MSD) recovery result to the LC DAD result was used to measure the ion suppression effect. The recovery of the target compound based on UV measurement was not significantly affected by any of the matrices tested. The recovery value for the UV reference measurement was 84.1% ±1.7% over all samples and matrix types (Table 1). This method precision suggests an extraction and measurement system that will yield repeatable results in distilled, tap, and borehole waters, as well as stripped water samples. The deionized water extracts were spiked at relatively high value of 5.0 µg/L for three reasons. First, to avoid complicating the investigation of the suppression effect with low level extraction non-linearities. Second, by working at this concentration it was possible to work in scan mode thereby saving the data from the spectral domain for all the experiments. It would then be possible to see whether the causes of ion suppression could be explained by spectral data. Third, at this level it was possible to use the measurement by DAD to provide a predictable, repeatable, and matrix-independent reference-recovery value. Consequently, the expected value obtained for both MSD and DAD should be *circa* 5.0 µg/L.

The MS parameters listed in the application note [15] were the starting point for this study. Under the conditions of these experiments the presence of a cluster ion at *m/z* 195 in the background spectra indicated that the desolvation characteristics of the source required some optimization. The presence of this cluster ion in the background was predictive of low response in the negative ion for the target analyte. Under source conditions that exhibited inadequate desolvation and cluster formation, the system exhibited a low-recovery result for the deionized water sample (MSD/DAD = 0.77); the MSD result in negative ion was 77% of the reference DAD recovery result. Therefore, the nebulizer pressure, drying gas temperature, and flow rate were adjusted until the cluster ion was no longer present. Under source conditions that produced no cluster ion at *m/z* 195, the MSD recovery result for the deionized water sample, as compared to the reference DAD recovery result, improved significantly to 91%.

Table 1. DAD and MSD Recovery Data from Different Matrices

Water samples		Samples spiked before SPE						Matrix stripped with MAX			Matrix stripped with HLB		
		DAD	Neg. ESI	Ratio	DAD	Pos. ESI	Ratio	DAD	Neg. ESI	Ratio	DAD	Neg. ESI	Ratio
Deionized	MEAN	**4.17**	**4.16**	**1.00**	**4.10**	**4.03**	**0.98**	**4.21**	**4.1**	**0.97**	**4.22**	**4.15**	**0.98**
	SD	0.12	0.13		0.08	0.14		0.16	0.09		0.11	0.24	
	%RSD	3	3.2		2.06	3.38		3.8	2.2		2.6	5.8	
Borehole	MEAN	**4.35**	**2.96**	**0.68**	**3.98**	**4.03**	**1.01**	**4.1**	**3.91**	**0.95**	**4.3**	**4.04**	**0.94**
TOC=0.5 mg/L	SD	0.15	0.05		0.11	0.19		0.12	0.13		0.18	0.29	
	%RSD	3.4	1.6		2.76	4.66		2.9	3.2		4.1	7.1	
Tap	MEAN	**4.29**	**1.55**	**0.36**	**3.94**	**4.14**	**1.05**	**4.13**	**2.45**	**0.59**	**4.17**	**3.57**	**0.86**
TOC=5 mg/L	SD	0.15	0.14		0.13	0.11		0.09	0.09		0.07	0.17	
	%RSD	3.4	8.7		3.30	2.71		2.2	3.6		1.8	4.8	
River	MEAN	**3.04**	**0.9**	**0.30**	**3.04**	**2.88**	**0.95**						
TOC=15.5 mg/L	SD	0.1	0.05		0.1	0.11							
	%RSD	3.4	6		3.4	3.9							

A thorough source cleaning of the metal surfaces and a rinse with 50:50 isopropanol:water followed by a mass axis calibration and ion transmission tune, increased the MSD recovery result for the deionized water sample, as compared to the reference DAD recovery result to 97%. The %RSD for these ratios was approximately 3%. Clearly, the desolvation and declustering characteristics of the source had a large impact on the instrumental contribution to the suppression effect for clean samples.

As can be seen from Table 1, the positive ion results for this compound, as extracted from spiked samples, show no evidence of ion suppression regardless of matrix. In addition to the reference characteristics of the DAD data, the positive ion results provide an ESI-based reference for the study of the origin of these suppression effects in ESI. Comparison of negative ion ESI results to both the DAD and positive ion ESI results permits a closer correlation between the magnitude of the suppression and the chemical nature of the suppressant in the matrix.

The nature of the negative ion suppression can be investigated from the data presented in Table 1. No suppression is observed for samples extracted from distilled water. The TOC for this water is at least an order of magnitude lower than the next highest content matrix. The negative ion ESI spectrum (Figure 3) was not significantly greater than background. Moreover, there were few significant ions other than those in the background.

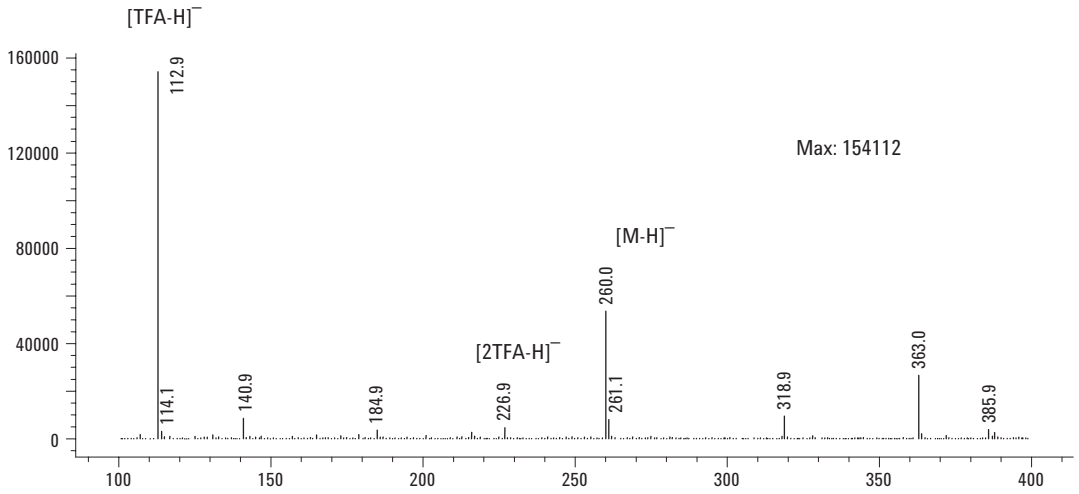

Figure 3. The mass spectrum of Imazapyr in deionized water spiked at 5.0 µg/L.

Borehole water has a TOC measurement of 0.5 mg/L. Recovery results obtained from the MSD were 68% of those obtained from the reference DAD signal. This represents significant ion suppression. In addition, the spectral data (Figure 4) indicated that there were significant numbers of background ions from mass 300 and higher due to the sample matrix. The spectrum above *m/z* 300 shows the beginnings of what appears to be a spectral envelope analogous to the ESI spectra observed for complex polymers.

Tap water (TOC at 5 mg/L) recovery results obtained from the MSD were 36% of those obtained from the reference DAD signal. The spectral data (Figure 5) indicated that there were significant numbers of background ions from mass 200 and higher due to the sample matrix. The impression of the low-mass end of a polymer's spectral envelope is further strengthened. Both the intensity and the number of ion have increased relative to the previous sample.

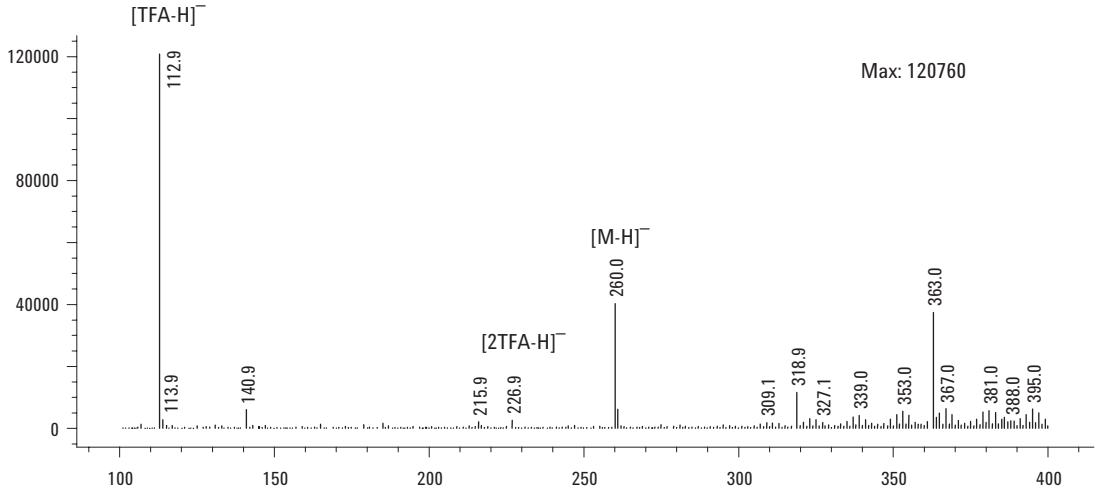

Figure 4. The mass spectrum of Imazapyr in borehole water spiked at 5.0 µg/L. Notice the significant numbers of background ions from *m/z* 300 and up due to the sample matrix.

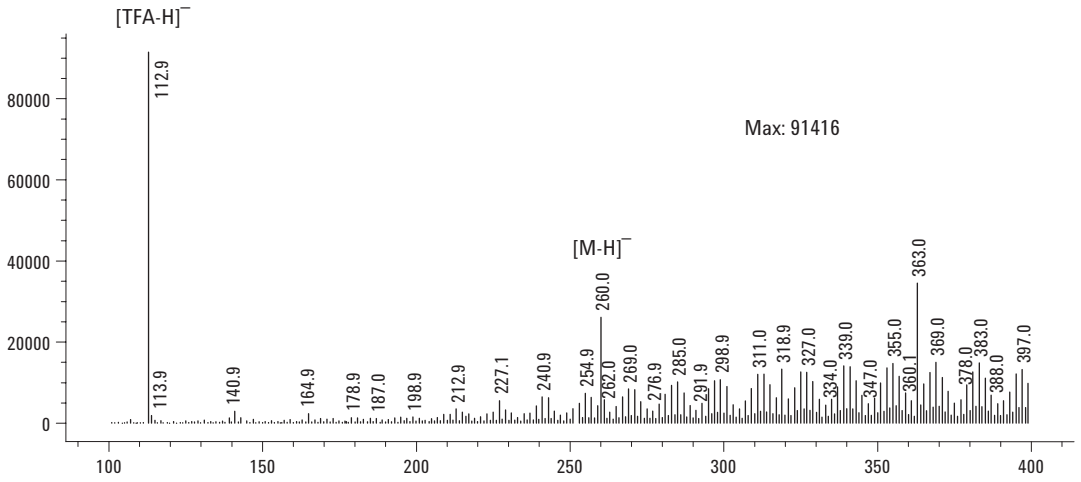

Figure 5. The mass spectrum of Imazapyr in tap water spiked at 5.0 µg/L. From *m/z* 200 upward, there was a vast increase in the number of ions due to the increase in matrix content.

To further explore the effect of matrix, a high TOC (15.5 mg/L) river water sample was collected and analyzed without any matrix stripping. The sample was spiked with Imazapyr at 5.0 µg/L and extracted, as described in the sample preparation section. The extracted sample was analyzed in both positive and negative ionization modes as well as with the DAD. The extraction method was not developed with raw river water in mind, so some differences were expected as compared to the simpler matrices. The only major difference between the results from the river water samples and the simpler matrices was extraction efficiency. Consequently, the DAD recovery results were significantly lower due to the extraction efficiency from the high organic load matrix. Despite the lower DAD recovery results, the chromatographic and ESI spectral data were excellent at the concentration of 5 µg/L. The recovery relationships are expected to hold even though the method was not specifically engineered for river water. Comparison of the positive ion MSD recovery to the DAD recovery results showed that, while extraction efficiency was lower in these river water samples, the high organic content sample exhibited no noticeable suppression in the positive ion mode. The positive ion mode spectrum of this extracted sample was very similar to that of tap water as shown in Figure 6.

However, the negative ion mode showed a large suppression effect. The ratio of negative ion MSD recovery to the reference DAD recovery results was only at 30% obtained by the DAD.

At the same level of extraction efficiency, DAD recovery results showed very similar values for all matrices, as did the positive ion results. Therefore, the ion suppression observed was directly related to the negative ion ESI process and the chemical nature of the species producing the spectral envelope observed in Figures 4 and 5. The suppression, negative ion spectral complexity and negative ion spectral envelope intensity increased with increasing organic content in the matrix as measured by the TOC. The relationship between TOC and recovery ratio as well as the deviation from back-calculated data is graphed in Figure 7 and 8. As can be seen from the graph, even a low TOC level can result in significant suppression.

To further test these observations, a matrix-stripping experiment was carried out for each of distilled, borehole, and tap waters using two different stripping chemistries.

The HLB cartridge was used to test the stripping effect under the same extraction conditions, while the Oasis MAX chemistry was used to investigate the effect of an anion exchanger on the suppression.

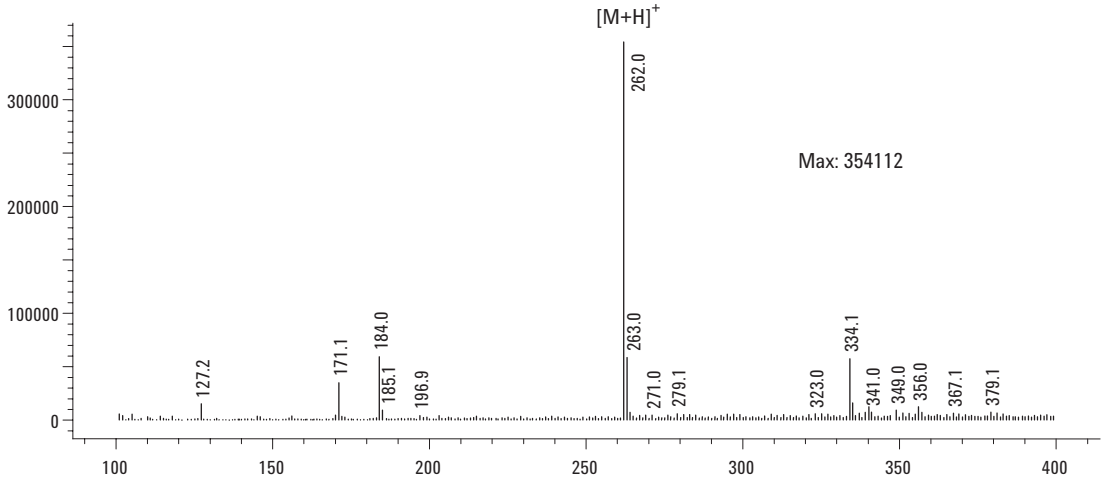

Figure 6. The mass spectrum of Imazapyr in HLB cartridge extracted tap water, positive ion mode, showing low background ion abundances for *m/z* 200 and higher.

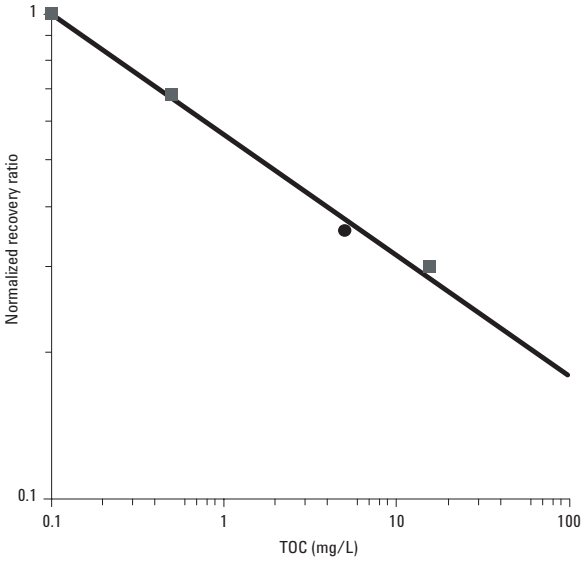

Figure 7. Normalized recovery ratio versus TOC.

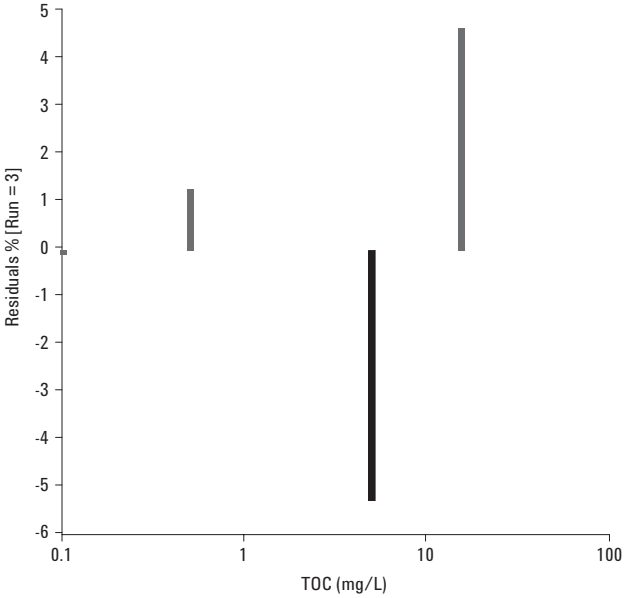

Figure 8. Residuals from back calculated data for Figure 7.

In all negative ion ESI cases the matrix stripping prior to analyte spiking dramatically increased the ratio of the ESI recovery result to the reference DAD recovery result regardless of stripping chemistry. In all cases the stripping chemistry reduced the intensity of the ion continuum above *m/z* 200 from 50% to 90%. The greater the intensity reduction of this spectral envelope, the greater the ratio of MSD-to-DAD recovery result and the smaller the extent of ion suppression. This is strong evidence that the portion of the suppression effect not due to inadequate desolvation, is largely a matter of matrix compounds bleeding through the extraction procedure into the extracted sample. The chemical nature of these suppressant compounds would have to be remarkable given their persistence under a wide range of extraction chemistries.

It was expected that the agents causing the suppression effects were humic acids and were expected to be preferentially removed by the anion exchange chemistry of the MAX cartridge. This proved not to be the case. The HLB cartridge stripping chemistry proved more effective at reducing the suppression than was the MAX chemistry. Either the humic acids were not effectively removed due to extraction conditions or the compounds causing suppression were not entirely humic acids.

Ion suppression in the ESI-MS analysis of water samples was reported under a wide variety of extraction and measurement conditions. A compound class, complex enough to produce the higher mass ion continuum seen in this work, would likely be extremely diverse. Such a diverse mixture could carry through virtually any simple extraction procedure. An ideal technique would have to be extremely discriminating to be free from members of such a compound class.

Conclusions

Two main points of conclusion about the ion suppression effect in negative ion ESI analysis of water samples can be suggested. The first is that ion suppression is aggravated by instrumental conditions that favor incomplete desolvation and that such conditions can be indicated by the presence of extensive clustering.

The second conclusion is that the greatest part of the ion suppression phenomenon is largely due to compounds in the sample vial that were carried through the extraction. The presence of these compounds is evidenced in the spectrometry. The suppressants are seen as a higher mass continuum of ions starting at about *m/z* 200 and above in negative ion mode. The majority of the continuum exists at higher *m/z* than is used in measurement and so its effects are felt in the recovery results as ion suppression but not seen in the chromatographic signal. The effect would be especially frustrating in highly specific tandem techniques. The higher the intensity of this ion continuum, the greater the suppression. In these samples the positive ion response of the ions in the continuum were much lower as compared to the negative ion mode. Interestingly enough there is no ion suppression in positive ion mode.

This work underscores the often-repeated message that a clean sample extract is essential in producing reliable methodologies. A clean extract reduces the need for tandem techniques and often rare and expensive isotopically labelled internal standards.

Future work would focus on a more precise identification of this compound class and on isolation techniques that embody a more powerful discrimination than simple SPE.

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Printed in the USA
February 18, 2004
5989-0371EN

Agilent Technologies

Full-Scan Low-Level Polynuclear Aromatic Hydrocarbon Analysis Using the Agilent Technologies 6890/5973 inert Gas Chromatograph/Mass Selective Detector

Application

Environmental

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Abstract

The analysis of polynuclear aromatic hydrocarbons presents challenges due to the tendency of the polynuclear aromatic hydrocarbons to adsorb on surfaces in the chromatographic system. This results in calibrations that are not linear and the need to run selected ion monitoring for low-level analysis. The Agilent Technologies 6890/5973 inert gas chromatograph/mass selective detector system is designed for improved polynuclear aromatic hydrocarbons analysis using full scan while maintaining linearity across a wide calibration range.

Introduction

Polynuclear aromatic hydrocarbons (PAHs) are produced during combustion of organic material and are suspected carcinogens. The high amounts and widespread occurrence of these compounds in our environment requires reliable, sensitive, and very robust analytical methods.

PAHs, especially the high molecular weight ones, tend to be adsorbed on any active or cold site in a gas chromatographic system. Additionally occurring inlet discrimination often further reduces the number of compounds with higher boiling points that are transferred onto the column. Therefore, typical PAH analyses on a gas chromatography (GC) or gas chromatography/mass selective detector (GC/MSD) system show decreasing response and sensitivity with increasing molecular weight.

The Agilent 6890/5973 inert GC/MSD system has features to overcome this negative trend, including a new uncoated solid-source material and higher temperature filaments. Using a direct-connect inlet liner also improves chromatographic peak shape and sensitivity.

Many laboratories calibrate for PAHs from 0.1 to 10 ppm using Selected Ion Monitoring (SIM) for low level work. Historically, SIM has been necessary because of instrument sensitivity considerations and loss of PAHs at the lower concentration levels, although full scan data is preferred for further confirmation of the compounds.

This application note will show the performance of the Agilent 6890/5973 inert for PAHs using a calibration range of 0.1 to 10.0 ppm in full scan mode with linearity equal to that of many SIM methods.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890		
Inlet	EPC split/splitless		
Mode	Pulsed splitless, 1 µL injected		
Inlet temperature	300 °C		
Pressure	12.64 psi		
Pulse presssure	30.0 psi		
Pulse time	0.30 min		
Purge flow	30.0 mL/min		
Purge time	1.0 min		
Total flow	34.6 mL/min		
Gas saver	Off		
Gas type	Helium		
Inlet liner	Direct Connect, deactivated, 4-mm id, Agilent part number G1544-80700		
Oven			
Oven ramp	°C/min	Next °C	Hold min
Initial		50	1.00
Ramp 1	25	200	0.00
Ramp 2	8	316	0.00
Total run time	21.50 min		
Equilibration time	0.5 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP-5MS part number 19091S-433		
Length	30.0 m		
Diameter	250 µm		
Film thickness	0.25 µm		
Mode	Constant flow		
Flow	1.5 mL/min		
Initial pressure	12.64 psi		
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
MSD	Agilent Technologies 5973 inert		
Drawout lens	6-mm ultralarge aperture, Agilent part number G2589-20045		
Solvent delay	3.00 min		
EM voltage	Run at DFTPP tune voltage = 1000 V		
Low mass	45 amu		
High mass	450 amu		
Threshold	0		
Sampling	2		
Scans/s	3.58		
Quad temp	180 °C		
Source temp	300 °C		
Transfer line temperature	280 °C		
Repeller voltage	DFTPP tune value		
Emission current	DFTPP tune value = 34.6 µamp		

Calibration standards

Calibration standards were diluted in dichloromethane from a stock mix of the 13 PAHs. The seven levels made were 10, 5, 2, 1, 0.5, 0.2 and 0.1 ppm. The perylene-d12 internal standard (ISTD) and the two surrogate standards, 1,3-dimethyl-2-nitrobenzene and triphenylphosphate, were added to each calibration level at 2.0 ppm.

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions that may have to be optimized.

The Agilent 6890 inlet temperature was set to 300 °C, instead of the typical 250 °C, to minimize compounds adsorbing on the liner surface. Pulsed injection was used to facilitate quantitative transfer of the heavier PAHs onto the column, minimizing inlet discrimination. Pulsed injection parameters are easily set in the ChemStation software and are automatically controlled by the electronic pneumatic control (EPC) module.

The Direct Connect inlet liner allows for complete transfer of analytes onto the column. The column inlet end attaches to the liner and minimizes analyte exposure to the stainless steel annular volume in the inlet.

The Agilent 5973 inert was tuned using the automatic DFTPP target tune, as required by some Government methods. The ultralarge aperture drawout lens was used to maintain linearity across the wide calibration range. Source temperature was set to 300 °C, which is now possible with the high temperature filaments. This higher source temperature in combination with the new source material produces better peak shapes for the PAHs.

Results

The system was calibrated at seven levels, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 ppm using full scan data acquisition. The total ion chromatogram (TIC) for the 0.2-ppm level is shown in Figure 1. Each calibration level contained 13 PAHs, perylene-d12 internal standard (ISTD) and the 2 surrogate standards, 1,3-dimethyl- 2-nitrobenzene and triphenylphosphate.

The relative response factor (RRF) was calculated automatically for each compound by the GC/MSD ChemStation software. Linearity was determined by calculating the percent relative standard deviation (%RSD) of the RRFs across the calibration range for each compound. This is also done automatically by the software in conjunction with Microsoft® Excel.

Linearity is shown in Table 2. The %RSD of the RRFs are shown for each of the PAHs. All RSDs are less than 5%. This level of performance is equal to that of most SIM methods for PAHs.

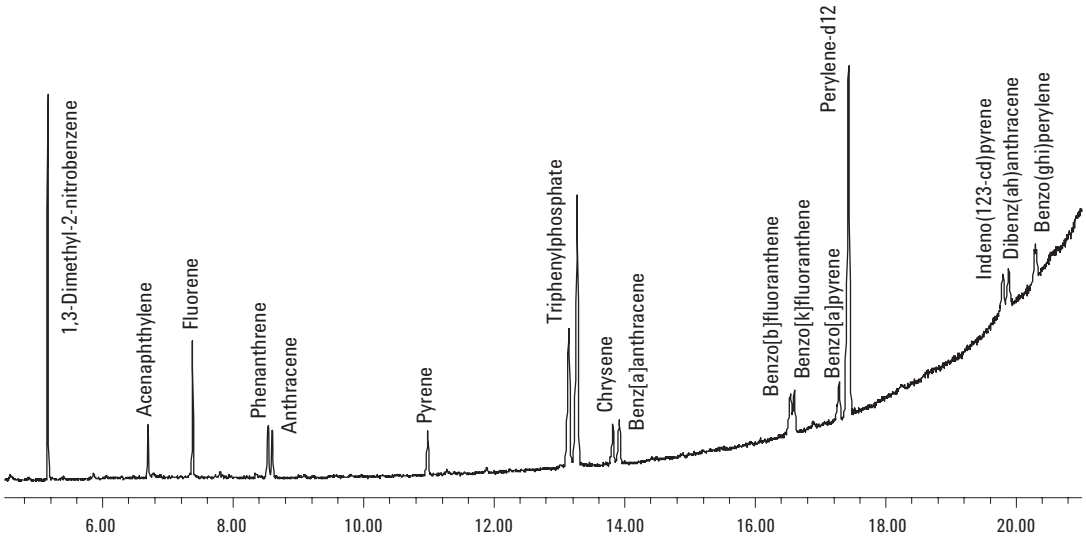

Figure 1. Thirteen PAHs at 0.2 ppm each with surrogates and ISTD at 2.0 ppm each.

As further proof of system inertness and sensitivity, a 0.01-ppm level spike was analyzed. This sample was quantitated against the seven level calibration curve using average response factor. The results are shown in Table 2. These results are excellent considering this is full scan data and the spike level was 10× lower than the lowest calibration point.

Table 2. %RSD of RRF from Seven Level Calibration and 0.01-ppm Spike Results

	%RSD	0.01 ppm Spike
Perylene-d12	3	–
1,3-dimethyl-2-nitrobenzene	1	2.100
Acenaphthylene	3	0.011
Fluorene	3	0.010
Phenanthrene	3	0.010
Anthracene	3	0.011
Pyrene	3	0.010
Triphenylphosphate	1	1.940
Chrysene	2	0.009
Benz[a]anthracene	3	0.010
Benzo[b]fluoranthene	2	0.009
Benzo[k]fluoranthene	4	0.010
Benzo[a]pyrene	2	0.010
Indeno(123-cd)pyrene	4	0.010
Dibenz(ah)anthracene	2	0.007
Benzo(ghi)perylene	3	0.011

Conclusions

The Agilent 6890/5973 inert shows much improved response and peak shape for PAHs due to the inert source material and higher allowable source temperature. This improved response gives better linearity across the calibration range. Analysis of PAHs can be accomplished using full scan data acquisition over a calibration range of 0.1 to 10 ppm, while maintaining performance similar to SIM methods.

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Printed in the USA
November 11, 2003
5989-0264EN

Agilent Technologies

Fast Semivolatiles Analysis using the Agilent Technologies 6890/5973 inert GC/MSD

Application

Environmental

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Abstract

The analysis of semivolatiles presents challenges due to the simultaneous measurement of acids, bases, and neutrals over a wide concentration range. Due to productivity demands, laboratories want to run faster while maintaining linearity and sensitivity for even the most active compounds. The Agilent Technologies 6890/5973 inert gas chromatography/mass selective detector system is designed to meet the criteria for fast analysis, while minimizing activity and maintaining linearity.

Introduction

Semivolatiles analysis concurrently measures a mix of acids, bases, and neutrals. This mix presents a challenge for instrument design due to the interaction of the analytes with the instrument and consumables. Most laboratories analyze for 70–100 compounds with a chromatographic run time of 25–40 minutes. The calibration range required for the analysis varies dependent on a particular laboratory's statement of work. Historically a range of 20–160 ng was used. With the increased sensitivity of newer gas chromatography/mass spectrometry (GC/MS) systems, laboratories are moving toward lower minimum detection limits (MDLs) and pushing the calibration range down to 5 ng.

The Agilent 6890/5973 inert gas chromatograph/mass selective detector (GC/MSD) system was designed to meet the demand for these lower MDLs. A new uncoated solid source material has shown improved performance for the most active compounds, such as 2,4-dinitrophenol.

This inert source allows for less material injected onto the column while maintaining mass spectrometer performance. Split injections are possible where only splitless would suffice before. The ability to do split injections matches very well with smaller diameter columns such as 100 µm. These smaller columns provide for run times of 10 minutes or less.

This application note will show the performance of the Agilent 6890/5973 inert for semivolatiles using a 100-µm id column with a run time of 7.5 minutes and a calibration range of 5–200 ng.

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890		
Inlet	150 psi EPC split/splitless		
Mode	Split, 1 µL injected		
Split ratio	10:1		
Inlet temp	250 °C		
Pressure	118 psi		
Split flow	22.8 mL/min		
Total flow	26.9 mL/min		
Gas saver	Off		
Inlet liner	Siltek™ Cyclosplitter, 4-mm id, Restek part number 20706-214.1		
Oven	240 V		
Oven ramp	°C/min	Next °C	Hold min
Initial		40	0.20
Ramp 1	45	320	1.58
Total run time	8.0 min		
Equilibration time	0.5 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP-5MS Custom		
Length	12.5 m		
Diameter	100 µm		
Film thickness	0.1 µm		
Mode	Ramped flow		
Flow	mL/min[2]	mL/min	Hold min
Initial		2.3	0.10
Ramp 1	10	0.8	0.00
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
MSD	Agilent Technologies 5973 inert		
Solvent delay	0.95 min		
EM voltage	Run at DFTPP tune voltage = 1200 V		
Low mass	35 amu		
High mass	500 amu		
Threshold	0		
Sampling	1		
Scans/s	5.92		
Quad temp	150 °C		
Source temp	230 °C		
Transfer line temp	280 °C		
Repeller voltage	DFTPP tune value		
Emission current	DFTPP tune at 35 µamp, run at 25 µamp		

Calibration standards

Accustandard, New Haven, CT. Part number M-8270-IS-WL-0.25x to 10x, 77 compounds at eight concentration levels with six ISTDs at 40 ppm.

The Agilent 6890 with a 150 psi inlet (option) is necessary for both the initial high flow during injection and to maintain constant flow during the run. A 10:1 split is used to match the column capacity to the calibration concentration range. Higher splits can be used but splitting less or using splitless will cause peak overload and too much distortion for good integration.

The inlet liner was found to be of low activity, as it does not contain glass wool. Proper mixing for split injections is done by the internal liner geometry. This liner was also found to perform adequately for higher split ratios and for splitless.

The Agilent 6890 240 V oven was necessary for the 45 °C/min oven program ramp used.

The custom order HP-5MS column was obtained in a 20 m length and cut down to 12.5 m. The ramp flow allows for faster transfer of analytes onto the column to minimize exposure to the inlet liner. Ramp flows are easily set by the software and are accomplished with electronic pneumatic control (EPC).

The Agilent 5973 inert was tuned using the automatic DFTPP target tune, as required by some Government methods. After tuning, the emission current was manually set to 25 µamp. This was done to maximize linearity for easily ionized compounds. The sampling rate was changed from the default of 2 to 1, while preserving sufficient sensitivity. The resultant 5.92 scans/s yields a minimum of eight data points across the narrowest peaks.

Results

The system was calibrated at eight levels, 5, 10, 20, 50, 80, 120, 160, and 200 ppm. The total ion chromatogram (TIC) for the 5-ppm level is shown in Figure 1. Each calibration level contained 77 compounds together with six internal standards (ISTDs) at 40 ppm.

The relative response factor (RRF) was calculated automatically for each compound by the GC/MSD ChemStation software. Linearity was determined by calculating the percent relative standard deviation (%RSD) of the RRFs across the calibration range for each compound and was performed automatically by the software in conjunction with Microsoft® Excel.

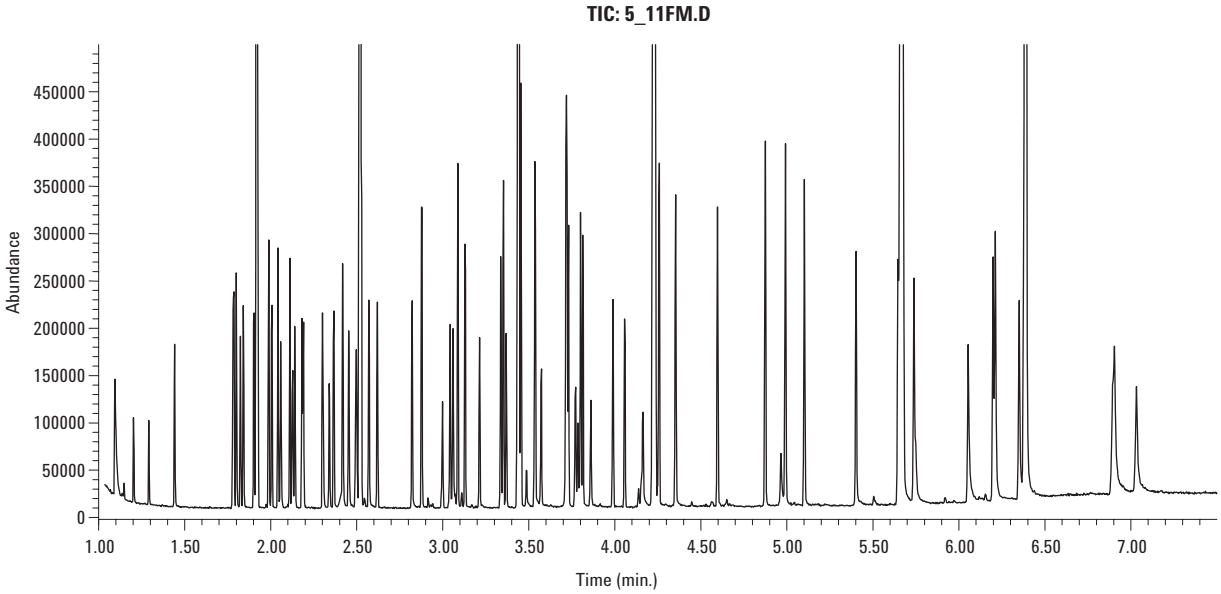

Figure 1. Five ppm each 77 semivolatiles and six ISTDs at 40 ppm each.

There are published Government Methods, such as USEPA Method 8270D for Semivolatiles, that specify criteria for suitable RRFs and %RSD. In Method 8270D, minimum system performance of four active compounds, the system performance check compounds (SPCCs) is measured by the average RRF.

Table 2 lists the Method 8270D SPCC criteria, and performance of the Agilent 5973 inert together with an Agilent 5973 system. The Agilent 5973 inert data exceeds the 8270D criteria. The Agilent 5973 inert also shows exceptional results compared to the Agilent 5973. These results are superior because they were run 10:1 split, putting 10× less compound on column than those run on the Agilent 5973.

Table 2. SPCCs, Comparison of Average RRF

	8270D Criteria	Agilent 5973 inert	Agilent 5973
Calibration range, ppm		0.5–20	5–160
N-Nitroso-di-n-propyl amine	0.050	1.146	0.970
Hexachlorocyclopentadiene	0.050	0.284	0.253
2,4-Dinitrophenol	0.050	0.188	0.075
4-Nitrophenol	0.050	0.236	0.162

Linearity is shown in Table 3. The 77 compounds were grouped as indicated. The RSDs of the RRFs were averaged to show performance for entire compound classes, not just a few selected analytes. The linearity of the Agilent 5973 inert is significantly better than the Agilent 5973 across the same concentration range and across an extended range.

Table 3. Average RSDs of RRFs by Compound Class

	Agilent 5973 inert	Agilent 5973 inert	Agilent 5973
Calibration range, ppm	0.5–20	2–16	20–160
Miscellaneous base neutrals (19)	8	5	11
Acids (17 phenols, dinitrophenols)	8	5	11
Bases (12)	8	6	12
Phthalates, ethers (13)	9	6	12
PAHs (16)	7	5	8

Conclusions

The Agilent 6890/5973 inert shows improved response for active compounds such as nitrophenols at low levels. This improved response gives better linearity across the calibration range. Split injections are now possible while maintaining sufficient response and fast analysis can be done using 100-µm columns. Analysis of 77 analytes and six ISTDs can be accomplished in less than 8 minutes over an extended calibration range of 0.5 ppm to 20 ppm.

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Printed in the USA
October 29, 2003
5989-0207EN

Solid-phase Extraction and Retention-Time Locked GC/MS Analysis of Selected Polycyclic Aromatic Hydrocarbons (PAHs)

Application

Environment

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Abstract

Many of the compounds referred to as polycyclic aromatic hydrocarbons have been characterized as carcinogens, mutagens and teratogens. Therefore the presence of polycyclic aromatic hydrocarbons in water is regulated and monitored around the world by environmental agencies. Determinations of aqueous levels of polycyclic aromatic hydrocarbons typically employ solid phase extraction which offers a simplified approach to the concentration of compounds present at trace levels in water. This work describes the application of Retention-Time Locking (RTL) to the determination of polycyclic aromatic hydrocarbons by GC/MS. Thirty-five common, native polycyclic aromatic hydrocarbons, and 27 surrogate polycyclic aromatic hydrocarbons are characterized under RTL conditions on the DB-5ms column in a runtime of less than 18 minutes. Using this RTL-GC/MS method, spike and recovery experiments were conducted at 50 ppt using 16 common (U.S. EPA listed) polycyclic aromatic hydrocarbons and solid-phase extraction with the AccuBond™ ODS-C18 cartridge. The method allows a 1 L water sample to be processed in less than 50 minutes using the ODS-C18 cartridge with analyte reproducibilities and accuracy better than 5% at 50 ppt. Description is given of a tube heater concentration technique which was used to condense and solvent exchange the ODS-C18 dichloromethane eluant into isooctane. This method has advantages over nitrogen blow-down in reducing time and labor as well as the potential for recovering the dichloromethane to prevent its release.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment. Both naturally occurring and man-made PAHs appear to have similar origins; pyrolytic transformation of organic materials. During the European industrial revolution, coal replaced wood as a fuel source and Dr. Percival Pott (1775) connected the testicular cancers appearing in English chimney sweeps to their exposure to soot (the combustion product). More recent work has demonstrated the carcinogenic, mutagenic and teratogenic behavior of many of the PAHs. In view of this, PAHs are considered compounds of concern by every environmental organization and, to protect human health and the ecosystem, their concentrations in waters are strictly regulated.

The colloquial terms “polycyclic aromatic hydrocarbons”, “polynuclear aromatic hydrocarbons”, etc., have been used to refer to this group of fused, unsaturated rings that may or may not contain other elements beyond carbon and hydrogen, such as sulfur or nitrogen. Strictly speaking, only structures following Hückel’s 4n+2 rule should be considered aromatic so a more appropriate term for this class of compounds would be benzenoid hydrocarbons. In view of the widespread use of the term “PAHs”, that abbreviation will be used herein as the generic term for this class of compounds.

The PAHs selected for extraction studies are those of the minimal list of 16 PAHs designated as "priority pollutants" and regulated by the U.S. Environmental Protection Agency (EPA). Table 1 lists the compounds and some of their properties. This wide range in PAH properties, especially volatility and solubility, makes consistent extraction and concentration difficult. The higher molecular weight PAHs tend to be insoluble and readily adsorbed on surfaces. The lower molecular weight compounds tend to be volatile and readily lost during concentration steps. It is worth noting the extremely low solubilities, (i.e., sub-ppb) of the larger multi-ring PAHs when contemplating spike and recovery experiments.

A more expanded list of PAH compounds is characterized by Retention Time Locking (RTL) in this work [1-3]. The more expanded list contains PAHs likely to be encountered by extraction of typical environmental samples and will assist analysts in the identification of additional isomers that may be misidentified without the use of RTL. This method should also serve as a framework to build a larger and more complete analytical list by adding those components that are routinely appearing in samples. For example, the retention time and ions for 2,3,5-trimethylnapthalene are included and intended to represent the C_3-naphthalenes in general. Extension of the extraction methodology to these additional compounds is straightforward.

In view of the comments on the range of PAH properties, extensive use is made, and should be made of deuterated surrogates or carbon-13 labeled internal standards for the PAHs. PAH properties vary greatly between isomers and even within isomers. For example, personal experience has shown calculated amounts of methyl-naphthalenes will be over-estimated if a correction by d_8-naphthalene surrogate recoveries is applied. Similarly, anthracene and phenanthrene, and other higher-ring isomers have different behaviors, especially with respect to their photochemical stability, and require individual deuterated surrogates. These improvements in the analytical method allow full advantage of the additional dimension of information that mass spectrometry provides and results in higher quality data

Table 1. Physicochemical Properties of the 16 US EPA "Priority Pollutant" PAHs [4]

Compound	CAS Number	Molecular formula	Nominal molecular mass	$\log_{10} K_{ow}$	Henry's Law constant (Pa m^3/mol)	Aqueous solubility mg/L (25 °C)
Naphthalene	91-20-3	$C_{10}H_8$	128	3.4	49	30
Acenaphthene	83-32-9	$C_{12}H_{10}$	154	3.9	6.4	3.9
Acenaphthylene	208-96-8	$C_{12}H_8$	152	4	11.4	3.9
Fluorene	86-73-7	$C_{13}H_{10}$	166	4.2	10	2
Phenanthrene	85-01-8	$C_{14}H_{10}$	178	4.5	4	1.2
Anthracene	120-12-7	$C_{14}H_{10}$	178	4.6	16	0.07
Fluoranthene	206-44-0	$C_{16}H_{10}$	202	5.2		0.26
Pyrene	129-00-0	$C_{16}H_{10}$	202	5.2	6	0.13
Benzo[a]anthracene	56-55-3	$C_{18}H_{12}$	228	5.9		0.01
Chrysene	218-01-9	$C_{18}H_{12}$	228	5.7		0.002
Benzo[b]fluoranthene	205-99-2	$C_{20}H_{12}$	252	6.2	3.55	0.014
Benzo[k]fluoranthene	207-08-9	$C_{20}H_{12}$	252	6.2		0.008
Benzo[a]pyrene	50-32-8	$C_{20}H_{12}$	252	6		0.004
Indeno[1,2,3-cd]pyrene	193-39-5	$C_{22}H_{12}$	276			
Dibenzo[a,h]anthracene	53-70-3	$C_{22}H_{14}$	278	6.2		.0005
Benzo[g,h,i]perylene	191-24-2	$C_{22}H_{12}$	276	7		.00026

Experimental

PAH standards were obtained as mixtures from a number of sources. The 16 PAHs for the spike and extraction studies were obtained from Ultra Scientific (North Kingston, RI). Deuterated PAHs were purchased from Cambridge Isotope Laboratories (Andover, MA). Dilutions were made in acetone or isooctane (pesticide grade, VWR Scientific, San Francisco, CA) appropriate to their purpose as spikes and surrogates or standards and injection internal standards, respectively. Dichloromethane, hexane and methanol for SPE elution and conditioning also were of pesticide grade. Sodium sulfate (analytical grade, VWR Scientific, San Francisco, CA) was kilned at 500 °C and stored in a vacuum bottle and desiccator.

Empty 6-mL cartridges and frits were obtained from Agilent Technologies Inc. (Wilmington, DE) for use as drying cartridges. AccuBond^{II} ODS-C18 Cartridges containing 500 milligrams of octadecyl sorbent in a 6-mL cartridge were obtained from Agilent Technologies Inc. (Wilmington, DE). A summary of the equipment and consumables is given in Table 2.

Table 2. Equpiment and Consumables Summary

Component	Agilent part number
Silanized amber vials	5183-4496
Vial crimp caps	5181-1210
AccuBond^{II} ODS-C18 (500 mg) 6-mL cartridge, box of 30	188-1356
Empty SPE cartridges reservoirs, 6 mL, box of 50	700-4006
Frits for 6-mL cartridges reservoirs, 100/pk	700-4031
Stopcock valves, 10/pk	5185-5758
SPE Manifold, 10-port	5185-5754
SPE Manifold, 20-port	5185-7565

Solid-phase Extraction Experiments

For an initial demonstration of the accuracy and precision of the ODS-C18 extraction approach, a 1.0 L deionized, reverse osmosis (RO) water sample was spiked with the 16 PAHs at 50 ng/L or 0.05 ppb (as 25 µL of 2 ng PAHs/µL acetone). Deuterated PAHs corresponding to each of the 16 PAHs were added at 250 ng/L as recovery surrogates (as 50 µL of 5 ng d_x-PAHs/µL acetone). “Calibrators” were also made at that time by adding the spike and surrogates to an amber GC vial.

The ODS-SPE cartridge was conditioned by sequentially rinsing with three cartridge volumes of dichloromethane (DCM), hexane, methanol, and RO water. At no time after the initial addition of DCM was the SPE column allowed to run dry.

The 1 L water sample was then pulled through the SPE cartridge at a flow between 20 and 25 mL/min such that the sample was processed in less than 50 minutes. The SPE cartridge was dried briefly by drawing clean laboratory air through the cartridge for about 5 minutes while occasionally tapping the cartridge body to dislodge bound water. DCM rinses were made of the amber sample bottle and applied to elute the cartridge. The SPE cartridge was eluted with approximately 8 to 9 mL of DCM. The DCM eluant was dried using a cartridge filled with anhydrous sodium sulfate.

At this stage the usual approach is to condense the DCM eluant to 1-mL final volume by nitrogen blowdown. However in this work the dried DCM eluant was condensed and solvent exchanged into isooctane by use of a tube heater. This process is described in Appendix 1. Samples and calibrators were adjusted to 1 mL with isooctane and spiked to 20 ng dibromooctafluorobiphenyl/mL (as 10 µL of 2 ng/µL isooctane) which served as the injection internal standard.

A method blank, the calibrators and samples were then analyzed using an Agilent 6890 Plus GC and 5973N MSD according to operating parameters listed in Table 3. A press-fit connector joined the DB-5ms capillary column to a 5-meter uncoated, deactivated guard column.

Table 3. GC Injection, Oven and MSD Parameters

Injection parameters for isooctane

Injection mode	Pulsed splitless	
Injection volume	2 µL	
Injection port temperature	275 °C	
Pulse pressure and time	25.0 psi	0.25 min
Purge flow and time	50.0 mL/min	0.75 min
Gas saver flow and time	20.0 mL/min	3.00 min

DB-5ms Column and oven parameters for isooctane

GC column	DB-5ms (30 m × 0.25 mm id, 0.25 µm) (Part no.: 122-5532) with 5 m deactivated precolumn (0.25 mm id)	
Flow and mode	1.3 mL/min	Constant flow
Detector and outlet pressure	MSD	Vacuum
Oven temperature program	80 °C	1.00 min
20.00 °C/min	115 °C	1.00 min
10.00 °C/min	130 °C	0.00 min
25.00 °C/min	295 °C	0.25 min
1.00 °C/min	300 °C	0.00 min
30.00 °C/min	340 °C	0.00 min
Oven equilibrium time	0.50 min	
Total program time	18.43	
MSD Transfer line temp	325 °C	

Injection parameters for dichloromethane

Injection mode	Pulsed splitless	
Injection port temperature	275 °C	
Pulse pressure and time	25.0 psi	0.25 min
Purge flow and time	50.0 mL/min	0.75 min
Gas saver flow and time	20.0 mL/min	3.00 min

Table 3. GC Injection, Oven and MSD Parameters (continued)

DB-5ms Column and oven parameters for dichloromethane

GC column	DB-5ms (30 m × 0.25 mm id, 0.25 µm) (Part no.: 122-5532) with 5-m deactivated precolumn (0.25 mm id)	
Flow and mode	1.3 mL/min	Constant flow
Detector and outlet pressure	MSD	Vacuum
Oven temperature program	40 °C	0.48 min
33.00 °C/min	115 °C	1.00 min
10.00 °C/min	130 °C	0.00 min
25.00 °C/min	295 °C	0.25 min
1.00 °C/min	300 °C	0.00 min
30.00 °C/min	340 °C	0.00 min
Oven equilibrium time	0.50 min	
Total program time	18.43	
MSD Transfer line temp	325 °C	

Mass spectrometer parameters

Tune parameters	Autotune
Electron multiplier voltage	Autotune +400V
Solvent delay	5.00 min
Scan parameters	50–300 *m/z*
Quadrupole temperature	150 °C
Source temperature	230 to 250 °C

Miscellaneous parts

Septa	5182-0739	BTO septa (400 °C)
Liner	5181-3315	Deactivated 4 mm id double taper
GC Column ferrule	5181-3323	250 µm Vespel/graphite
MSD Interface ferrule	5062-3508	0.4 mm id preconditioned Vespel/graphite

Results

In general, PAH analysis requires that samples and standards be protected from light by use of amber vials, foil, etc., and should take place under "gold" lamps due to the potential for photochemical destruction by fluorescent lamps and/or sunlight. Figure 1 illustrates the importance of this issue raised in the Introduction. Although gold lamps were not available in this particular laboratory, as many precautions as possible were taken to avoid photochemical losses.

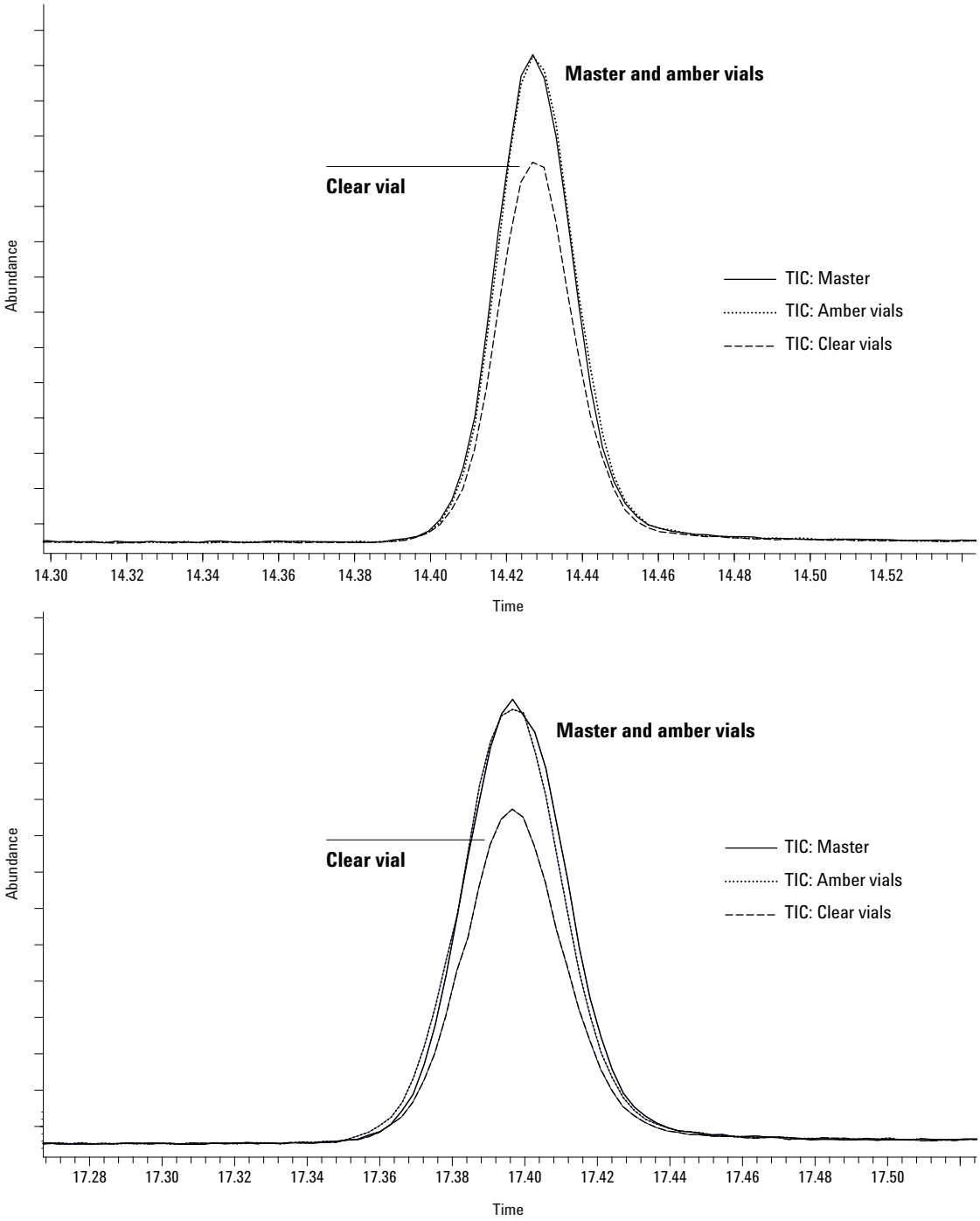

Figure 1. Illustration of photochemical degradation of selected PAHs. Total ion chromatograms (TICs) for benzo[a]pyrene (upper) and benzo[g,h,i]perylene (lower) in PAH standards stored in a clear vial, amber vial and from a master standard. The clear vial and amber vial were left in the laboratory in ambient light for several days then injected with a master standard that was stored at 4 °C until just prior to injection. The master and amber vial TICs for the analytes are essentially identical and overlap. The analyte TIC for the clear vial is visibly reduced in intensity. The losses are significant - on the order of 20% and 25%, respectively.

Gas Chromatography

A first concern is chromatography. Figure 2 shows a total ion chromatogram for selected PAHs under locked conditions. D_{10}-phenanthrene, a surrogate present in both samples and standards was chosen as the "locking compound" and was locked to elute at 9.750 minutes. Notice the run time is quite short; benzo[g,h,i]perylene elutes in less then 17.5 minutes. Besides allowing higher oven temperatures at injection, isooctane has several other features that make it superior to DCM as a solvent. At a high injector temperature, which is preferred for PAH analysis, DCM expands to 2.5 times the volume of isooctane and can overfill the liner if not (carefully) injected using pressure pulsing or ramped flow injection. Isooctane is a better "keeper" and storage solvent than DCM which is highly volatile and requires almost immediate re-sealing after injection. DCM rapidly "extracts" a pierced septum on the vial to create a "silicone" series in subsequent injections of the sample unless a pure Teflon septum is used (which is usually impractical). Evaporation of DCM by nitrogen blowdown is labor intensive as opposed to the tube heater approach used here.

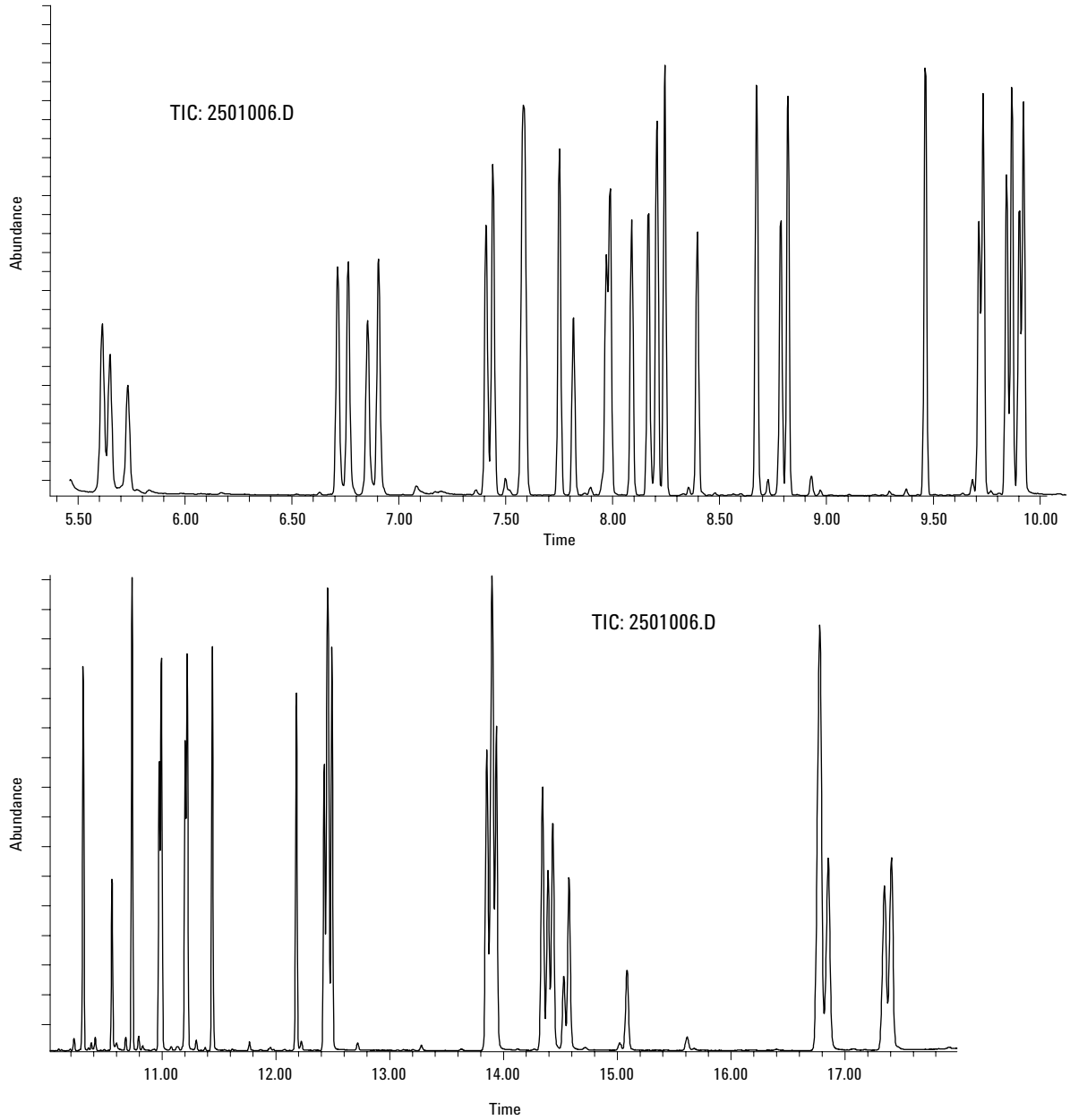

Figure 2. Total ion chromatogram of more than 50 PAHs injected from isooctane.

However, injecting from DCM is widely performed so a GC method with similar run time and program was created using the fast ramping available with either the 240-V oven or the oven insert for the standard oven (Agilent part no. G2646-60500). There are some minor shifts in retention times but the locking compound and overall runtime are maintained. The injection port temperature used here (275 °C) represents a compromise for the DCM injection. As mentioned earlier, a higher temperature can be used with isooctane, which is attractive for the higher ring PAHs and port cleanliness. The locked PAH retention times and nominal quantitation and qualifying ions are listed in Table 4 for both the isooctane and dichloromethane solvent GC programs.

Table 4. The PAH compound list, locked retention time for the isooctane and dichloromethane GC programs, and nominal values of relevant MS ions. The RTL compound is d_{10}-phenanthrene and locked at 9.750 under both programs. Compound RTs are ±0.006 min maximum. The first MS ion for each compound is the quantitation ion.

Compound	Isooctane program	DCM Program	Quantitation ions (*m/z*)
D_8-naphthalene	5.513	5.770	136, 137
$^{13}C_6$-naphthalene	5.560	5.800	134, 133, 132, 135
Naphthalene	5.556	5.800	128, 127, 129
Benzo[b]thiophene	5.645	5.870	134, 135, 136, 89
D_{10}-2-methylnaphthalene	6.623	6.740	152, 150
2-methylnaphthalene	6.673	6.790	142, 141
D_{10}-1-methylnaphthalene	6.763	6.870	152, 150
1-methylnaphthalene	6.816	6.920	142, 141
D_{10}-biphenyl	7.318	7.390	164, 162
Biphenyl	7.350	7.410	154, 153
D_{12}-2,6-dimethylnaphthalene	7.497	7.560	168, 150
1-ethylnaphthalene	7.486	7.550	141, 156
1,2-dimethylnaphthalene	7.658	7.710	156, 141
D_{18}-hexamethylbenzene	7.726	7.770	162, 180
D_{10}-acenaphthylene	7.877	7.920	160, 158
Acenaphthylene	7.895	7.940	152, 151
D_{12}-1,8-dimethylnaphthalene	7.515	8.040	168, 150
D_{10}-acenaphthene	8.078	8.110	164, 162
Acenaphthene	8.117	8.150	153, 154
4-methylbiphenyl	8.153	8.180	168, 167
D_8-dibenzofuran	8.303	8.330	176, 146
2,3,5-trimethylnaphthalene	8.582	8.600	170, 155
D_{10}-fluorene	8.694	8.710	176, 174
Fluorene	8.729	8.730	166, 165
Dibromooctafluorobiphenyl	9.060	9.070	296, 227, 278
1-methylfluorene	9.371	9.380	165, 180
D_8-dibenzothiophene	9.622	9.620	192, 193
Dibenzothiophene	9.643	9.640	184, 139
D_{10}-phenanthrene	9.751	9.750	188, 189
$^{13}C_6$-phenanthrene	9.770	9.770	184, 182, 183, 185
Phenanthrene	9.775	9.770	178, 176
D_{10}-anthracene	9.811	9.810	188, 189
Anthracene	9.829	9.830	178, 176
2-methylphenanthrene	10.309	10.310	192, 191, 189
9-methylanthracene	10.563	10.560	192, 191, 189
3,6-dimethylphenanthrene	10.735	10.740	206, 191, 189
D_{10}-fluoranthene	10.976	10.970	212, 211
Fluoranthene	10.993	10.990	202, 200

Table 4. Continued

Compound	Isooctane program	DCM Program	Quantitation ions (*m/z*)
D_{10}-pyrene	11.201	11.200	212, 211
Pyrene	11.223	11.220	202, 200
1-methylpyrene	11.440	11.440	216, 215, 213, 217
Benzo[b]naptho[2,1-d]thiophene	12.179	12.180	234, 235, 232
D_{12}-benz[a]anthracene	12.423	12.410	240, 236
Benz[a]anthracene	12.451	12.450	228, 226
D_{12}-chrysene	12.459	12.450	240, 236
$^{13}C_6$-chrysene	12.490	12.490	234, 232, 233, 235
Chrysene	12.491	12.490	228, 226
D_{12}-benzo[b]fluoranthene	13.849	13.830	264, 263, 265
Benzo[b]fluoranthene	13.888	13.870	252, 250, 253
D_{12}-benzo[k]fluoranthene	13.899	13.880	264, 263, 265
Benzo[k]fluoranthene	13.934	13.910	252, 250, 253
D_{12}-benzo[a]pyrene	14.390	14.370	264, 265, 263
Benzo[e]pyrene	14.343	14.310	252, 250, 253
Benzo[a]pyrene	14.432	14.410	252, 250, 253
D_{12}-perylene	14.529	14.510	264, 265
Perylene	14.576	14.550	252, 250, 253
3-methylcholanthrene	15.088	15.060	268, 252
D_{12}-indeno[1,2,3-c,d]pyrene	16.710	16.740	288, 289
Indeno[1,2,3-c,d]pyrene	16.775	16.740	276, 277, 274
D_{14}-dibenz[a,h]anthracene	16.764	16.730	292, 293
Dibenz[a,h]anthracene	16.847	16.810	278, 276, 274
D_{12}-benzo[g,h,i]perylene	17.338	17.310	288, 289
Benzo[g,h,i]perylene	17.402	17.370	276, 277, 274

There are several closely eluting PAHs which can convolute quantitation. For the 16 PAHs, there are four "critical pairs": phenanthrene and anthracene; benzo[a]anthracene and chrysene; benzo[b]fluoranthene and benzo[k]fluoranthene; and indeno[1,2,3-c,d]pyrene and dibenzo[a,h]anthracene. The first three of these critical pairs involve isomers, so a conflict between the common quantitation ions (for example, the most intense ions) is the issue, while the last pair involves a conflict between the quantitation ion and a confirmation ion. GC programs can be used to completely resolve these components but this requires slow oven ramps and consequently long method runs times (approximately 30 to 40 minutes). The method developed here aims at a compromise between resolution and run time. The resulting resolution under this method for the 4 critical pairs at 1 ng injected are shown in Figures 3, 4, 5, and 6. Note that the required resolution for accurate quantitation is always a function of the analyte amounts and related to column capacity. It is possible to overwhelm a nearby peak when large analyte concentration disparities exist [5]. As long as analyte concentrations remain below a few nanograms, the resolution should be sufficient. For this method, only the integration of the last critical pair, especially the surrogate, d_{12}-indeno[1,2,3-c,d]pyrene, needs to be carefully policed. If issues arise, quantitating on peak height may provide some improvement. Increased sensitivity available through RTL-GC/MS selected-ion-monitoring is easy to implement and will improve detection limits [6].

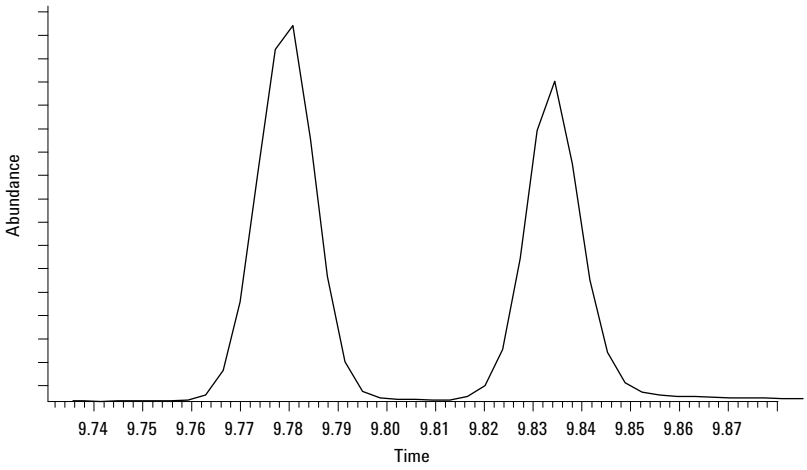

Figure 3. Total ion chromatogram showing resolution of phenanthrene and anthracene at 1 ng each. Calculated resolution for the valley for the common 178 *m/z* ion = 99%.

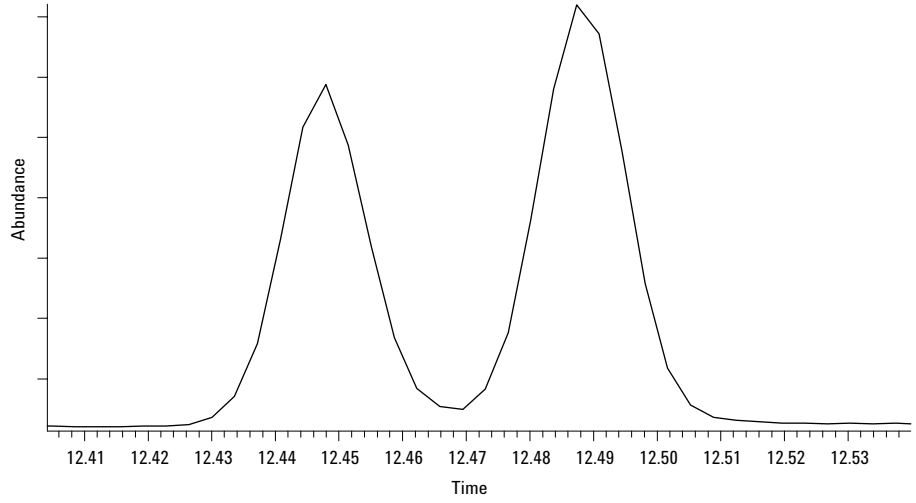

Figure 4. Total ion chromatogram showing resolution of benz[a]anthracene and chrysene at 1 ng each. Calculated resolution for the valley for the common 228 *m/z* ion = 95%.

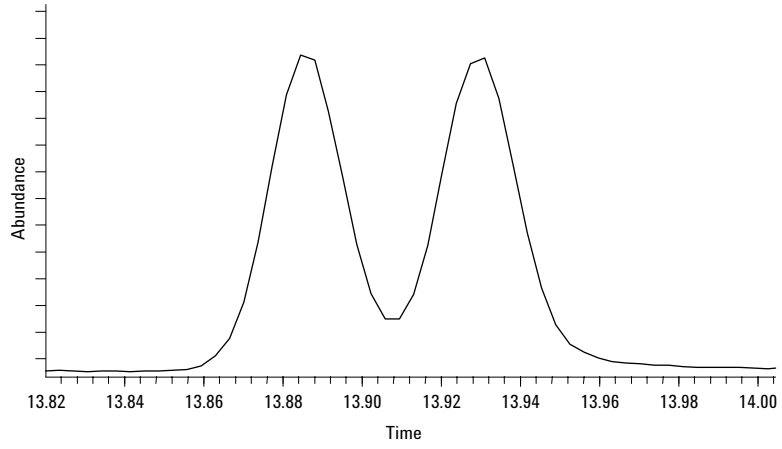

Figure 5. Total ion chromatogram showing resolution of benzo[b]fluoranthene and benzo[k]fluoranthene at 1 ng each. Calculated resolution for the valley for the common 252 *m/z* ion = 84%.

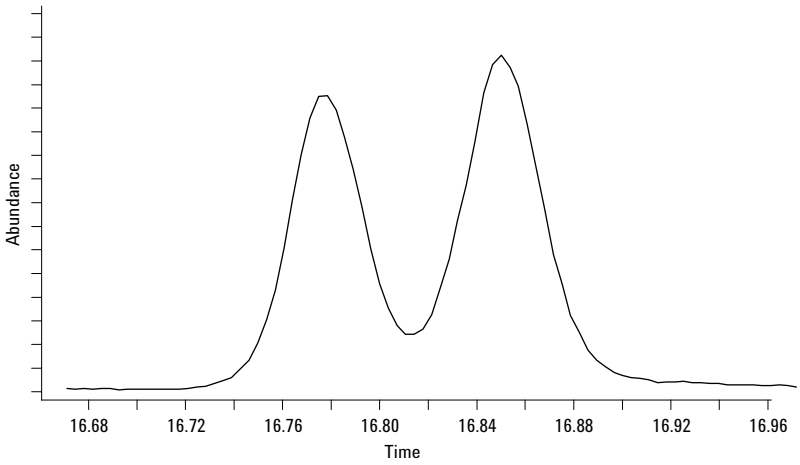

Figure 6. Total ion chromatogram showing resolution of indeno[1,2,3-cd]pyrene and dibenzanthracene at 1 ng each. Calculated resolution for the valley for the total ion current = 80%.

ODS-SPE Spike and Recovery Experiments

Table 5 shows the results for five ODS-SPE experiments at 50 ppt using the tube heater concentrator. There is good sensitivity in scan and the SPE surrogate recoveries averaged 77% (±3%). Accuracy was also very good with the average deviation from the expected value of 5%. Even naphthalene showed a high average recovery (70%) with good precision for both the surrogate (±6%) and the native (±2%). Anthracene was the only outlier showing slightly elevated values due to disproportionate loss of the d_{10}-surrogate. The data seems to suggest there may be a slight systematic error for a few of the analytes most likely due to spiking at these low levels.

Detection limits can be improved through RTL-GC/MS with selected-ion-monitoring which is easy to implement through the newly developed AutoSIM software [6].

Table 5. Results for five ODS-C18 SPE spike and recovery experiments at 50 ppt. Deuterated surrogates appear in the table just prior to the analyte they correct. The average deuterated surrogate recoveries are given under the "Average Recovery" heading as percent recovery and the relative standard deviation (RSD) in the surrogate is given under "RSD". For the native PAHs, the average amount of the five determinations is given next to the analyte as ng/L or ppt in the "Average" column followed by the RSD for the native value (precision). The "Average deviation" gives the average absolute deviation from the true value of 50 ppt as percent and indicates the degree of accuracy.

Surrogate compound	Average recovery (%)	RSD (%)	Native compound	Average (ng/L)	RSD (%)	Average deviation (%)
d_8-naphthalene	70	3	Naphthalene	53	2	6
d_{10}-acenaphthylene	80	4	Acenaphthylene	51	2	2
d_{10}-acenaphthene	77	5	Acenaphthene	53	3	5
d_{10}-fluorene	78	5	Fluorene	54	3	8
d_{10}-phenanthrene	79	5	Phenanthrene	53	1	5
d_{10}-anthracene	71	5	Anthracene	57	4	13
d_{10}-fluoranthene	81	4	Fluoranthene	52	2	5
d_{10}-pyrene	81	5	Pyrene	52	2	4
d_{12}-benz[a]anthracene	79	6	Benz[a]anthracene	52	3	4
d_{12}-chrysene	77	6	Chrysene	52	1	4
d_{12}-benzo[b]fluoranthene	80	7	Benzo[b]fluoranthene	52	1	5
d_{12}-benzo[k]fluoranthene	79	10	Benzo[k]fluoranthene	52	2	5
d_{12}-benzo[a]pyrene	74	7	Benzo[a]pyrene	52	5	4
d_{12}-indeno[1,2,3-c,d]pyrene	77	5	Indeno[1,2,3-c,d]pyrene	50	4	1
d_{14}-dibenz[a,h]anthracene	77	8	Dibenz[a,h]anthracene	48	4	4
d_{12}-benzo[g,h,i]perylene	77	8	Benzo[g,h,i]perylene	50	4	0.3

Summary

The retention-time locked GC/MS method for PAHs presented here provides sufficient separation for good quantitation in under 18 minutes. More than 60 PAHs and their common surrogates are characterized under RTL conditions to assist in unknown PAH identification.

AccuBond[II] ODS-C18 cartridges demonstrate reproducibility and accuracy better than 5% for extraction of the 16 PAHS of the U.S. EPA at 50 ppt in water (0.05 µg/L). Using a tube heater for concentration and solvent exchange to isooctane provides a better injection and storage solvent than dichloromethane, allows more rapid processing with less effort than nitrogen blowdown with the opportunity to recover vaporized solvent.

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Acknowledgements

The author gratefully acknowledges Angelica Reese and Cameron George for their assistance with column procurement. He is also indebted to Phil Wylie for his editorial comments and assistance.

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Appendix 1.

Concentrating Dichloromethane Using a Tube Heater

Harry Prest, Senior Applications Chemist, Agilent Technologies

CAUTION This method is ONLY to be applied to dichloromethane and is to be executed by skilled chemists! Other organic solvents are very flammable and must NOT be used.

This operation MUST be performed in a hood with all flames extinguished!

Agilent Technologies shall not be liable for any consequences or damages in connection with this method or material.

Motivation

Dichloromethane is a widely used solvent. SPE, size-exclusion chromatography (SEC) and open column chromatography (silica, alumina or Florsil) fractions can often be completely dichloromethane. Concentrating dichloromethane and retaining the more volatile compounds is always problematic. A variety of approaches are possible such as rotovap or nitrogen blowdown or others. Most methods require considerable expertise and attention to perform well. The method described here is simple, rapid, rugged, inexpensive, requires very little operator intervention, allows for convenient solvent exchange, and can be modified to collect the vaporized dichloromethane. Further, a number of samples can be rapidly concentrated at a single time. This method has been successfully applied in the author's laboratory at the University of California Santa Cruz to 50 mL volumes of dichloromethane from SEC. Recoveries are very good even for typically difficult compounds such as naphthalene, the hexachlorocyclohexanes, hexachlorobenzene and others.

Summary

This appendix describes the procedure for concentrating dichloromethane and exchanging into isooctane using a tube heater. One milliliter of isooctane and two Teflon boiling chips are added to approximately 10 mL (±1 mL) of dichloromethane (DCM) in a 16 mm × 125 mm test tube. The tube is placed in the aluminum tube heater manifold (at 77 °C) and allowed to boil until the only the isooctane "keeper" remains, approximately 20 to 30 mins. Transfer to GC vial with isooctane as routinely performed.

Materials

Test tubes 16 × 125 mm or 16 × 100 mm (check fit of tubes to heater manifold holes prior to use)

Blanked isooctane and dichloromethane

Solvent rinsed Teflon Boiling chips

Aluminum manifold for Kontes Tube Heater pre-drilled for 16 mm tubes

Glass "chimneys" to fit tubes

Procedure

1. Extinguish all flames in the laboratory or objects that may create a spark. Place the tube heater in the hood and pre-heat the manifold to 77 °C ±3 °C.
2. Add ~1 mL of isooctane and two solvent rinsed boiling chips to the test tube containing the DCM fraction. In this case, about 10 mL of DCM.
3. Place tube in the pre-heated aluminum tube heater manifold (77 °C ±3 °C). Place glass "chimney" around the tube. This MUST be done in a fume hood.
4. Allow tube to boil. Do not remove from heater until condensed. When a very small amount of boiling is all that remains, remove tube from heater. There will be about ½ mL of isooctane left.
5. After cooling, transfer with isooctane to GC vial and adjust volume.

Remarks

This method is tailored to eliminate some acetone and the majority of DCM and exchange into isooctane "keeper". It is not possible to eliminate all the DCM or acetone. If the tubes are removed too early and some DCM remains, early eluting analytes will show some chromatographic indications that this is the case such as eluting slightly earlier than expected versus standards in pure isooctane, etc.

Modifying the glass chimney to a condenser design will allow collection of the DCM with very high efficiency and has the added benefit of minimizing cross contamination. In fact, micro-scale organic glassware can be easily modified for this purpose.

Validation

Using the GC/MS parameters described in the accompanying application note, the tube heater concentration method was validated using 16 PAHs and their deuterated surrogates. Ten milliliters of DCM was spiked with 250 ng of deuterated PAH surrogates and 50 ng of native PAHs. After tube heater concentration and exchange into isooctane, transfer was made into amber GC vials and the volume adjusted to 1 mL. Dibromooctafluorobiphenyl was used as an injection internal standard. Analyte concentrations were corrected by their corresponding surrogate. At these concentrations, recoveries were essentially 100% for all PAHs and greater than 95% for naphthalene.

Table A1 cites the reproducibility and accuracy (as average absolute deviation from 50 ng) for trials on 2 different days. In general, accuracy and reproducibility is better than 5% and essentially limited by integration error.

Table A1. Surrogate corrected accuracy and reproducibility for 16 PAHs at 50 ng for trials on 2 separate days. Relative standard deviation (RSD) and accuracy is the average absolute deviation from 50 ng expressed as percent. The number of trials is given by n.

	Trial no. 1		Trial no. 2	
Compound	Percent accuracy (n=5)	Percent RSD (n=5)	Percent accuracy (n=6)	Percent RSD (n=6)
Naphthalene	1.8	2.3	1.8	1.6
Acenaphthylene	2.6	3.4	1.0	1.2
Acenaphthene	1.4	1.8	1.3	0.7
Fluorene	1.2	1.3	1.6	1.5
Phenanthrene	2.4	2.4	2.2	2.3
Anthracene	4.4	5.0	2.3	1.9
Fluoranthene	1.6	2.5	1.1	1.3
Pyrene	1.9	2.6	0.8	1.0
Benz[a]anthracene	4.9	1.8	3.1	1.0
Chrysene	2.8	3.0	1.3	1.7
Benzo[b]fluoranthene	3.2	3.8	1.9	2.6
Benzo[k]fluoranthene	3.0	2.5	1.7	2.4
Benzo[a]pyrene	5.8	5.5	2.2	2.7
Indeno[1,2,3-c,d]pyrene	2.0	2.2	4.6	5.7
Dibenz[a,h]anthracene	3.0	4.3	4.1	3.0
Benzo[g,h,i]perylene	3.7	3.0	1.6	2.4

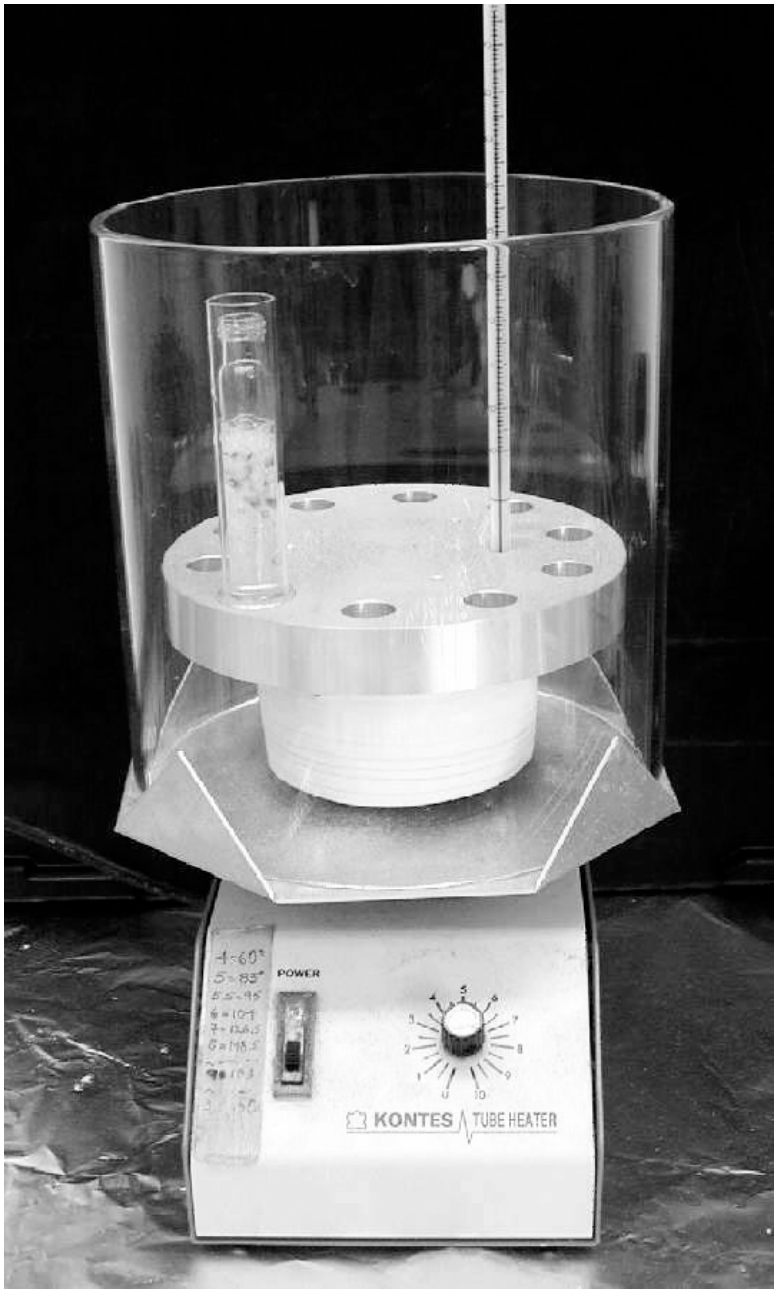

Figure A1. Tube heater apparatus.

Printed in the USA
July 12, 2002
5988-7150EN

Agilent Technologies

Solid-Phase Extraction and Gas Chromatography/Mass Spectrometry Analysis of Selected Phenols

Application

Environmental

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Abstract

Solid-phase extraction offers a simplified approach to the concentration of compounds present at trace levels in water. Polymeric resins, such as polystyrene-divinylbenzene, offer advantages over the commonly used octadecyl and similar silica-substrate solid-phase extraction adsorbents, especially for more polar compounds. Recently Agilent Technologies has expanded its offering of solid-phase extraction products to include a polystyrene-divinylbenzene solid-phase extraction material specifically for environmental applications, the AccuBond^{II} ENV cartridge. Using the retention time locked gas chromatography/mass spectrometry analytical method previously described [1], this note makes an initial demonstration of the accuracy and precision that can be achieved for selected phenols at 10 ppb in water using this polystyrene-divinylbenzene solid-phase extraction material. The solid-phase extraction procedure is rapid, uses reduced drying times, and requires only two surrogates. The cartridge design has been optimized to provide increased recoveries for phenol, which typically has low and irreproducible recoveries. Recoveries for phenol exceeded 70% and other phenols were greater than 90%. Precision was better than 5% and accuracy, as indicated by average absolute deviation as percent, was better than 8% for all phenols except 2-cyclohexyl-2,4-dinitrophenol. Sample delivery rate is high (20 to 25 mL/min) so a 1-liter sample can be extracted in less than an hour.

Introduction

Solid-phase extraction (SPE) has evolved to be a powerful tool for isolation and concentration of trace analytes in a variety of sample matrices. SPE has grown to replace liquid/liquid extraction due to the minimal use of solvent, the simplicity and flexibility of the approach, and the increased selectivity for analytes available. Beginning in and throughout the last decade, a large number of SPE applications were developed for compounds in matrices of environmental interest. The major focus of these applications was the collection and concentration of trace analytes from water. Most of the analytes were non-polar and strongly hydrophobic in nature such as polychlorinated biphenyls (PCBs), the organochlorine pesticides, polynuclear aromatic hydrocarbons (PAHs), for example, as these were relatively easy candidates for the technique and of widespread concern. More polar compounds like the phenols offer particular challenges.

SPE exploits the similarity in physicochemical properties of a class of analytes, their interaction with the SPE material, and their differences from the matrix. The phenols encompass a wide range in polarities and solubilities as shown in Table 1.

The pKa values indicate that the dinitrophenol and the tetra- and penta-chloro phenols are fairly acidic and therefore are predominately dissociated in water at near-neutral pHs. Acid-base equilibrium considerations require that the water sample be acidified to at least 2 pH units below that of the lowest pKa value(s) to generate phenols primarily in their non-ionized form. Octanol-water partition constants (K_{ow}) and water solubilities of the undissociated compounds range over a factor of more than several thousand. The high aqueous solubilities and low K_{ow} s of phenol and the monosubstituted phenols make these the most difficult phenols to capture and retain.

Table 1. Physicochemical Properties of Some Phenols

Compound	$\log_{10} K_{ow}$	pKa	Solubility$_{(aq)}$ g/L
Phenol	1.46	9.89	0.0884
4-chlorophenol	2.4	9.18	.027
4-methylphenol	1.96	10.26	.02
3-methylphenol	1.98	10	.022
4-nitrophenol	1.91	7.08	.013
2,4-dichlorophenol	3.2	7.68	.0045
2,4-dimethylphenol	2.35	10.6	.0088
2,4-dinitrophenol	1.67	4.09	.00034
2,4,6-trichlorophenol	3.69	7.42	.00043
2,3,4,6-tetrachlorophenol	4.45	5.38	.00018
Pentachlorophenol	5.05	4.92	0.000014

Polymeric resins were used early in the history of solid-phase extraction. These early materials needed extensive cleanup prior to use to avoid interferences obscuring analytes of interest. New generations of these polymers such as polystyrene-divinylbenzene (PS-DVB) have much lower backgrounds due to improvements in manufacturing processes. The use of PS-DVB polymers as an absorbent material has been demonstrated to provide improved recoveries for phenolic compounds as compared to the traditional and more commonly applied C18 material [2]. The details provided here ensure that analysts will observe less breakthrough of phenol, greatly improving overall recoveries.

The objective of this work was to develop a simple approach to SPE extraction and gas chromatography/mass spectrometry (GC/MS) analysis for selected phenols and perform a preliminary demonstration of accuracy and precision. A previous application note describes the retention-time locked GC/MS method in detail [1].

Experimental

The phenols were obtained from Ultra Scientific (North Kingstown, RI) and AccuStandard (New Haven, CT) as mixtures. Dilutions were made in acetone and in dichloromethane (Burdick and Jackson Grade, VWR Scientific, San Francisco, CA) for surrogates or spiking and standards, respectively. Sodium sulfate (analytical grade, VWR Scientific, San Francisco, CA) was kilned at 500 °C and stored in a desiccator.

Empty 6-mL cartridges and frits were obtained from Agilent Technologies Inc. (Wilmington, DE) for use as drying cartridges. AccuBond^{II} ENV PS-DVB cartridges containing 1000 milligrams of PS-DVB sorbent in a 6-mL cartridge were obtained from Agilent Technologies Inc. (Wilmington, DE). A summary of the equipment and consumables is given in Table 2.

Table 2. Equipment and Consumables Summary

Description	Part Number
Silanized amber vials	5183-4496
Vial crimp caps	5181-1210
AccuBond^{II} ENV PS-DVB polymeric resin as 1000 mg / 6-mL cartridge, box of 30	188-3060
Empty SPE Cartridges Reservoirs, 6 mL, box of 50	700-4006
Frits for 6 mL cartridges reservoirs, 100/pk	700-4031
Stopcock valves, 10/pk	5185-5758
SPE Manifold, 10-port	5185-5754
SPE Manifold, 20-port	5185-7565

Spike and Recovery Experiments

For the initial demonstration of the accuracy and precision of the approach, 1.0 L of deionized RO water was spiked with 21 phenols at 10 pbb each. Deuterated phenol, 2,4-dibromophenol, and 2,4,6-tribromophenol were added at 10 ppb as recovery surrogates. Three “calibrators” were also made at that time by adding the spike and surrogates to a silanized vial containing some dichloromethane (DCM) as a keeper. The solution was mixed and the pH lowered to ≤ 2 with 5N HCl.

The PS-DVB SPE cartridge was conditioned by sequentially rinsing with 9 to 12 mL of DCM, 9 to 12 mL of methanol, and 9 to 12 mL of 0.05N HCl. At no time after the initial addition of DCM was the column allowed to run dry.

The 1-L water sample was then pulled through the SPE cartridge at a flow between 20 and 25 mL/min such that the sample was processed in less than 1 hour. The SPE cartridge was dried briefly by drawing clean laboratory air through the cartridge for about 2 minutes while tapping the cartridge body to dislodge bound water. The SPE cartridge was then eluted with 9 mL of DCM. The DCM eluant was dried using a cartridge filled with anhydrous sodium sulfate.

The dried DCM eluant was evaporated under dry, filtered nitrogen and transferred to a silanized amber vial. At this point, the volumes of the sample and the three calibrators were brought to approximately 0.9 mL and 100 µL of a solution containing 2,5-dibromotoluene and 2,2',5,5'-tetrabromobiphenyl at 0.05 µg/µL in DCM was added as internal (injection) standards. A solvent blank, the three calibrators and the sample were then analyzed using an Agilent 6890 Plus GC and 5973N MSD according to operating parameters given in a previous note [1].

Results and Discussion

Corrected and uncorrected results of inter-day replicates for selected phenols are shown in Table 3. Phenol values were corrected to the deuterated phenol while all other compounds were corrected to 2,4-dibromophenol recoveries. With the exception of the 2-cyclohexyl-2,4-dinitrophenol, all RSD values and deviations are under 5% and 8%, respectively. The average RSD and absolute deviation for all the compounds are 4% and 6%, respectively. These indicate very good reproducibility and accuracy. An anomalously high value in the third trial seems to have inflated the deviation for 2-cyclohexyl-2,4-dinitrophenol.

Table 3. Spike and recovery results for accuracy and precision at 10 ppb using the AccuBond[II] ENV SPE cartridge. The average deviation is calculated as the relative average of the absolute deviations from 10 ppb and expressed as percentages. RSD represents the relative standard deviations.

Trial Number:	Trial #1		Trial #2		Trial #3		RSD	Average
Compound	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected	%	Deviation
Phenol	7.8	10.7	7.2	10.3	7.7	11.0	3%	7%
2-chlorophenol	9.8	9.3	9	8.9	10.3	9.3	3%	8%
2-methylphenol	9.9	9.4	9.1	9.0	10.5	9.5	3%	7%
3- & 4-methylphenol	9.8	9.3	9.2	9.1	10.4	9.4	2%	7%
2,4-dimethylphenol	9.9	9.4	9.4	9.3	10.5	9.5	1%	6%
2-nitrophenol	10.0	9.5	9.1	9.0	10.7	9.6	4%	6%
2,4-dichlorophenol	9.9	9.4	9.1	9.0	10.5	9.5	3%	7%
2,6-dichlorophenol	9.7	9.2	9.1	9.0	10.4	9.4	2%	8%
4-chloro-3-methylphenol	10.1	9.6	9.3	9.2	10.7	9.6	3%	5%
2,4-dibromophenol	10.5		10.1		11.1			
2,4,6-trichlorophenol	9.7	9.2	9.1	9.0	10.6	9.5	3%	7%
2,4,5-trichlorophenol	9.8	9.3	9.1	9.0	10.4	9.4	2%	8%
4-nitrophenol	10.0	9.5	9.8	9.7	11.5	10.4	4%	4%
2,3,4,5-tetrachlorophenol	9.7	9.2	9.3	9.2	10.7	9.6	3%	6%
2,3,5,6-tetrachlorophenol	9.9	9.4	9.1	9.0	10.4	9.4	2%	7%
2,3,4,6-tetrachlorophenol	9.8	9.3	9.3	9.2	10.6	9.5	2%	6%
2,4-dinitrophenol	10.6	10.1	9.8	9.7	11.9	10.7	5%	4%
2,4,6-tribromophenol	9.7	9.2	9.6	9.5	10.7	9.6	2%	5%
2-methyl-4,6-dinitrophenol	10.1	9.6	9.5	9.4	11.4	10.3	5%	4%
Pentachlorophenol	9.8	9.3	9.5	9.4	11.1	10.0	4%	4%
Dinoseb	10.2	9.7	9.4	9.3	11.5	10.4	5%	4%
2-cyclohexyl-4,6-dinitrophenol	11.2	10.7	10.6	10.5	14.4	13.0	12%	14%
2,2',5,5'-tetrabromobiphenyl	10.5	10.0	10.5	10.4	12.3	11.1	5%	5%

Typically, a 1-gram sorbent cartridge is considered an excessive use of material. However, work with 500-mg cartridges showed recoveries for phenol near and below 50%. Tandem cartridges revealed substantial phenol on the second cartridge. Increasing the polymer mass to 1 gram reduced breakthrough and consequently increased phenol recoveries. The methylphenols also demonstrated this behavior to a lesser degree and supported the change in sorbent bed mass.

Using a single surrogate to correct all the substituted phenols seems a tremendous simplification since the behavior and chemistries of the phenols differ widely. It is likely that this will become apparent at lower concentrations and most likely for the nitrophenols. Data does imply that the tetrabromobiphenyl (included in Table 3) may allow a better correction of the injection volume for the late eluters.

Conclusions

These preliminary results show that phenols can be extracted from aqueous samples accurately and precisely using AccuBond^{II} ENV PS-DVB polymeric resin. Coupled with a gas chromatographic analysis and retention time locking GC/MS [1], extraction, identification and quantitation of phenolic compounds can be done confidently, accurately and reproducibly. This method is a modification of U.S. EPA Method 528 [3]. The procedure here for the extraction of phenols from drinking water by polymeric SPE results in improved recoveries and greatly reduced drying times (2 minutes compared to 20 minutes), which increases sample throughput. The next steps in developing a full method would be an exploration of detection limits which will require extraction of replicates at lower concentrations. It is expected that the behavior of the more “active” compounds may suggest an expanded suite of surrogates.

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Printed in the USA
January 15, 2002
5988-5255EN

Improvements in the Agilent 6890/5973 GC/MSD System for Use with USEPA Method 8270

Application

Environmental

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Abstract

Method 8270 presents challenges due to the simultaneous measurement of acids, bases and neutrals over a concentration range that varies from lab to lab. Laboratories want GC/MS instruments that are linear and inert over a wide concentration range. Changes have been made to the 6890/5973 GC/MSD system in the inlet, column, and source areas based on feedback from our customers. System performance has been improved by maximizing linearity and minimizing activity.

Introduction

USEPA Method 8270 (including versions A, B, C and D) is used to determine the concentration of semivolatile organic compounds in extracts prepared from many types of solid waste matrices, soils, air sampling media and water. The January 1998, revision 4, 8270D lists 240 possible analytes that can be measured. Most laboratories analyze for a significantly smaller number of compounds, usually 70 to 100.

Regardless of the number of analytes, there is usually a mix of acids, bases and neutrals that must be measured concurrently. This mix presents a challenge for instrument design due to the interaction of the analytes with the instrument and consumables.

The calibration range required for the analysis varies depending on a particular laboratory's statement of work. Method 8270 does not specify a calibration range, yet traditionally a range of 20 to 160 ng (nanograms) has been used as a carryover from the USEPA Contract Lab Program (CLP). With the increased sensitivity of newer GC/MS systems, laboratories are moving toward lower minimum detection limits (MDLs) and pushing the 8270 calibration range down to 5 ng.

The 6890/5973 GC/MSD (Gas Chromatograph /Mass Selective Detector) system was designed to meet demand for these lower MDLs. To further enhance performance, two main areas of improvement were identified in communications with users.

The first was improving the linearity of the GC/MSD system at the high end of the calibration range where roll off or flattening out of the calibration was observed. The relative response factors (RFs) were lower than they should have been at higher concentrations. This was seen for phthalates and for PAHs (Polyaromatic Hydrocarbons).

The second area for improvement was recovery at the low end of the calibration range for active compounds. The most active compounds, the nitrophenols, showed lower RFs at the low end of the calibration range than what was expected on some systems. The most active of these, 2,4-dinitrophenol, showed RFs below method requirements on some systems.

A study was undertaken to address the high end linearity and the low end activity. The primary goals of the study were to meet the following 8270 requirements:

1. Minimum Average RF of 0.050 for the System Performance Check Compounds (SPCCs) (Method 8270D, section 7.3.4.2)
2. Maximum Relative Standard Deviation (RSD) of 30% for the Calibration Check Compounds (Method 8270D, section 7.3.5.2)
3. Maximum Mean Relative Standard Deviation of 15% across all compounds (Method 8000B, section 7.5.1.2.1)

Additional study goals to ensure maximum productivity for the user were:

1. Minimize activity in the entire GC/MSD system to maximize RFs for active compounds—this gives the user a greater margin for system degradation when analyzing dirty samples.
2. Maximize linearity in the GC/MSD system at the high end without losing significant sensitivity at the low end—this improves overall RSDs.
3. Preserve method resolution requirements for benzo[b]fluoranthene and benzo[k]fluoranthene when using thinner film columns for shorter analysis times.

Experiments were done to meet the study goals by dividing the system into three main sections : GC column, GC inlet, and MSD. Each of these areas is treated separately in a following section of this note.

Column

Column test system

To reduce the complexity of the chromatographic system and to provide the best possible sample introduction and detection, a COC/FID (Cool-On-Column/Flame Ionization Detector) system was used to test column performance. On-column injection eliminates any inlet activity while FID gives sensitive and essentially universal response for the analytes. The FID also provides directly comparable response information that can be used to validate analyte introduction between systems.

Test mix

To establish a test mix for evaluating the column and other components in the system, both anecdotal information and suggestions in the method were reviewed. Section 1.4 of 8270D points out the following compounds as potentially troublesome:

1.4.1 Benzidine may be subject to oxidative losses during solvent concentration and its chromatographic behavior is poor.

1.4.4 N-nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described.

1.4.6 Pentachlorophenol, 2,4-dinitrophenol, 4-nitrophenol, benzoic acid, 4,6-dinitro-2-methylphenol, 4-chloro-3-methylphenol, 2-nitroaniline, 3-nitroaniline, 4-chloroaniline, and benzyl alcohol are subject to erratic chromatographic behavior, especially if the GC system is contaminated with high boiling material.

Furthermore, the method cites several mixes of compounds for evaluating system performance. The DFTPP (decafluorotriphenylphosphine) mix adds 4,4'-DDT to the previously cited compounds. The system performance check compounds add N-nitroso-di-n-propylamine and hexachlorocyclopentadiene. Finally, the calibration check compounds add six more phenols as well as seven base/neutral compounds.

From anecdotal information, the phenols presented the greatest challenge. If the phenols cited are combined, the list is essentially all the phenols in EPA Method 604. While more test solute information was being collected, the phenols were run by COC/FID. Table 1 shows the RSD values for relative response factors from 5 to 160 ng on column. The last four compounds prove to be the most troublesome with 2,4-dinitrophenol being noticeably worse. Even so, the RSD values are all below 8%, indicating that COC/FID can be used to evaluate column performance. When done with unoptimized conditions/consumables in splitless sample introduction, the RSD values become unusable for these difficult compounds. With this information and the anecdotal performance data, a more comprehensive mix was devised.

Table 1. Cool-on-column FID, RSD of RFs from 5 to 160 ng on column

Solute	RSD
Phenol	3.0
2-Chlorophenol	3.1
2-Nitrophenol	3.2
2,4-Dimethylphenol	3.3
2,4-Dichlorophenol	3.1
4-Chloro-3-methylphenol	3.2
2,4,6-Trichlorophenol	3.3
2,4-Dinitrophenol	6.9
4-Nitrophenol	3.8
4,6-Dinitro-2-methylphenol	4.3
Pentachlorophenol	4.5

The "short mix" is comprised of the four phenols from above, several bases, several neutral compounds, and the internal standards at 40 ng/µL. The compounds were selected so that they were easily resolved and unambiguously detected by COC/FID. Figure 1 shows a sample chromatogram of the short mix on a 0.5 µm column.

Column testing

8270D states that a 30 m × 0.25 mm × 1 µm silicone coated capillary column be used in the analysis. However, the method also makes provisions for split injections, allowing a thinner film to be used. Because of the obvious time pressure to perform environmental analyses, thinner film columns are widely used. From customer inputs, film thickness ranged from 0.25 to 1 µm; consequently, 0.25, 0.5, and 1 µm film thickness columns were evaluated. Each of the columns had already passed the Agilent 5MS column checkout and were used as received. In addition, columns from another supplier were also tested, yielding similar results to the Agilent columns.

1 N-Nitrosodimethylamine
2 Aniline
3 2,4-Dinitrophenol
4 4-Nitrophenol
5 4,6-Dinitro-2-methylphenol
6 4-Aminobiphenyl
7 Pentachlorophenol
8 Benzidine
9 3,3-Dichlorobenzidine
10 Benzo[b]fluoranthene
11 Benzo[k]fluoranthene

ISTD 1 Dichlorobenzene-d4
ISTD 2 Naphthalene-d8
ISTD 3 Acenaphthene-d10
ISTD 4 Phenanthrene-d10
ISTD 5 Chrysene-d12
ISTD 6 Perylene-d12

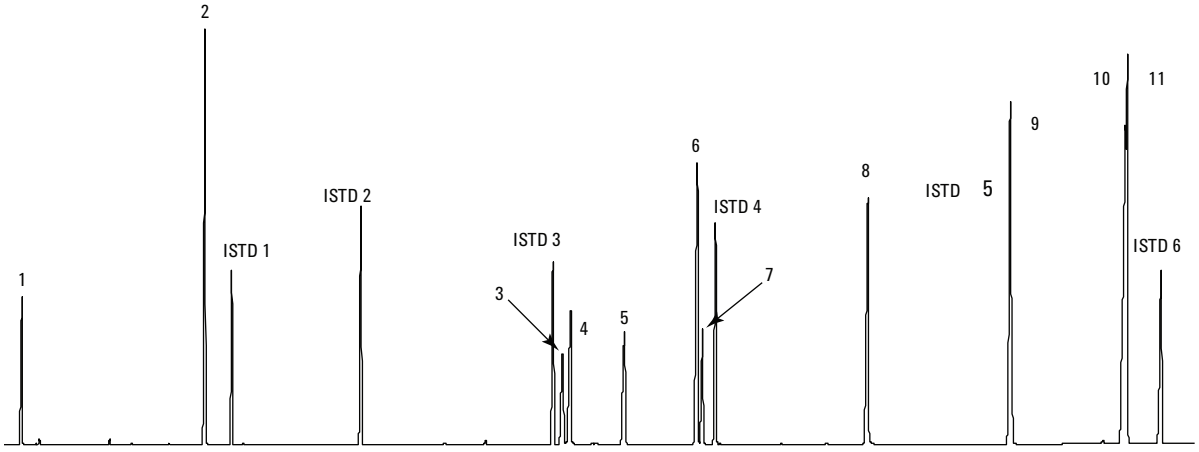

Figure 1. COC-FID Chromatogram and identification of short mix compounds.

The instrument parameters followed the 8270 method operating conditions as closely as possible. The COC inlet was run in oven track mode, the column flow was constant at 1.3 mL/min, and the FID was set to 300 °C. The oven program followed the 8270 method except that the program rate (10 °C/min) was adjusted for the different film thickness to resolve the test compounds.

Numerous columns from each film thickness were tested. It is important to remember that all of these columns passed the standard column testing protocol. An arbitrary metric was set for pass/fail criteria. This value was 10% RSD for the 2,4-dinitrophenol RFs from 5 to 160 ng on column. Of all the columns tested, only a fraction gave results below this metric. Film thickness was not a factor since the same fraction of columns passed for each film thickness. Some of the columns were so active that the column alone could cause the system to fail the method qualifying criteria. For this reason, it is imperative that a stringent test protocol be utilized for evaluating columns and that only specifically tested columns be used for 8270D. A comparison of a column that passed and one that failed the arbitrary criteria is shown in Table 2.

Table 2. COC-FID Results on a "Pass" and a "Fail" 0.5 µm Column, 5 to 160 ng

Solute	"Pass" RSD	Avg RF	"Fail" RSD	Avg RF
N-Nitrosodimethylamine	0.78	0.54	0.30	0.52
Aniline	1.2	1.45	0.59	1.43
2,4- Dinitrophenol	7.5	0.33	21.	0.28
4-Nitrophenol	0.49	0.52	2.9	0.50
4,6-Dinitro-2-methylphenol	4.7	0.40	14.	0.37
4-Aminobiphenyl	1.0	0.94	3.3	1.02
Pentachlorophenol	5.0	0.34	15.	0.26
Benzidine	2.6	0.74	2.8	0.72
3,3'-Dichlorobenzidine	7.9	0.57	0.58	0.62

Column selection

Since all the film thicknesses studied can meet the method objectives, the column selection is typically based on other analysis needs. As in all chromatographic systems, there is a balance between speed of analysis, resolution, and column capacity. The 0.25 µm film thickness columns offer the fastest analysis possible but with a compromise in resolution and capacity. Conversely, the 1.0 µm columns provide the best capacity but at a cost of time. Using the Agilent method translation tool, the 0.5 µm film column is only a factor of two slower than the thin film column while providing a twofold increase in capacity. The 0.5 µm film thickness column offers a good compromise between speed and capacity.

Inlet

There are many inlet related factors that affect 8270 performance. These include: split vs. splitless injection, syringes, injection volume, septa, inlet temperature, inlet seal, liners, using wool or not in the liner, and using a pulsed (flow programmed) vs a normal injection. Some of these parameters were studied to determine their contribution to low end activity and to high end linearity.

Split injection is allowed if the MSD has enough sensitivity. Split injections put less material on column, making it easier to meet resolution requirements on thinner film columns and at the same time improving peak shapes. However less material on column results in noticeable losses of active compounds due to column or MSD activity. High end linearity could be improved using split injections but the issue was solved as described in the source section of this note. Splitless injection is almost universally used and will be the focus of this inlet section.

A syringe experiment was not done as part of this study. Previous data show better reproducibility when using a 5 µL syringe in an ALS (Automatic Liquid Sampler). All injections were made using a 5 µL syringe with a tapered needle.

Injection volume was always 1 µL for the study. 8270 allows for 1 to 2 µL injection volumes, but previous data show worse reproducibility when using 2 µL injections. This is most likely due to expansion outside the liner and subsequent loss of analytes. Additionally, more residue is introduced with larger injection volume, negatively impacting instrument uptime.

Septa types were not studied and green septa were used. Inlet temperature was held at 250 °C. A new gold inlet seal was fitted with each liner, although changing the seal for a direct connect liner may not be necessary. Stainless steel (SS) seals were not used.

The inlet study focussed on liner types, carrier gas flow through the column during injection and the presence or absence of glass wool. The five different liner types that were used are described in Table 3. Two of the liners, the G1544-80700 and G1544-80730 are new designs. The column makes a direct connection into the liner bottom, similar to a capillary column connector.

Table 3. 2,4-Dinitrophenol Average RFs Using Various Inlet Liners, COC-FID

Splitless time			**0.2 min**		**0.2 min**		**0.75 min**		**0.75 min**	
Column flow			**1 mL/min**		**3 mL/min**		**1 mL/min**		**3 mL/min**	
Part number	**Liner**	**ng injected**	Avg RF	RSD	Avg RF	RSD	Avg RF	RSD	Avg RF	RSD
5062-3587	Single taper with glass wool	5	0.007	63	0.023	55	0.017	58	0.072	38
		160	0.122		0.198		0.187		0.228	
5181-3316	Single taper	5	0.092	37	0.136	21	0.105	33	0.125	22
		160	0.279		0.232		0.261		0.207	
5181-3315	Dual taper	5	0.203	14	0.215	11	0.201	15	0.216	12
		160	0.285		0.255		0.296		0.287	
G1544-80730	Single taper direct contact	5	0.287	5	0.269	7	0.272	6	0.229	9
		160	0.311		0.316		0.310		0.285	
G1544-80700	Dual taper direct contact	5	0.289	5	0.280	6	0.275	7	0.278	7
		160	0.331		0.330		0.327		0.328	
	COC	5	0.311	3	0.311	3	0.331	3	0.311	3
		160	0.331		0.331		0.331		0.331	

All liner experiments were performed using an FID to eliminate any affect an MSD would have on the results. RFs are not identical for MSD and FID due to inherent response differences. An approximate conversion is $RF_{fid} \times 0.7 = RF_{msd}$. Cool-on-column injection was done as a baseline for inlet performance.

The short mix was injected at the 5, 20, 80 and 160 ng levels, four replicates at each level. This series of 16 injections was made on each liner at each set of inlet conditions. There were four sets of inlet conditions. The splitless time was either 0.2 minute or 0.75 minute. The carrier flow through the column was either 1.0 mL/min or 3.0 mL/min held for the splitless time + 0.05 minute, then reduced to 1.2 mL/min. This is similar to a “pulsed splitless” injection, however flow programming gives the analyst control over the depressurization rate. The COC injections were made at a fixed column flow and the splitless time is not relevant.

Table 3 shows the results of these analyses. The average RFs for 2,4-dinitrophenol at the 5 ng and 160 ng levels are shown together with the RSD of all 16 RFs for each liner type/inlet conditions.

The 3587 liner shows the worst performance. 2,4-dinitrophenol has been eaten by the glass wool and low end activity is at its worst. Unfortunately most analysts use glass wool in the liner to prevent solids from contaminating the column.

The 3316 liner is the same as the 3587 but without the wool. Loss of 2,4-dinitrophenol can be attributed to contact with the gold inlet seal, the polyimide coating on the column outside and the stainless steel at both the top and bottom of the inlet. There could also be analyte contact with the stainless steel in the annular volume outside the liner.

The 3315 liner is the same as the 3316, but with a narrower opening at the top. This minimizes contact with the top of the inlet and there is an increase in 2,4-dinitrophenol response.

The new 80730 liner minimizes analyte contact with the polyimide on the column outside, the gold inlet seal and the inlet annular volume. Response for 2,4-dinitrophenol was significantly improved using this liner, even though it has a wide top similar to the 3316.

The new 80700 liner has the advantages of the 80730 and has a narrower top opening similar to the 3315. An additional increase is seen in 2,4-dinitrophenol response because analyte contact with inlet surfaces is minimized at both the top and bottom. Performance with this liner is nearly equal to that of COC and low end activity is minimized for the inlet only.

Figure 2 shows performance of the five liners and COC with one set of conditions that was used. The splitless time was 0.75 minute and the column flow was 3 mL/min during the injection. Each of the bars shows the average RF for 2,4-dinitrophenol of the four replicate injections at each level. The order is 5 ng at the top increasing to 160 ng at the bottom. Above each bar is listed the average RF and RSD across all 16 injections. The 3587 liner with the wool shows the worst performance and the new 80700 liner with the direct connect bottom and narrow top shows the best performance. The COC data show column performance isolated from a hot splitless inlet. There is a slight drop-off in COC RFs comparing 5 ng to higher levels. Data for the other three sets of experimental conditions show similar trends and are not presented.

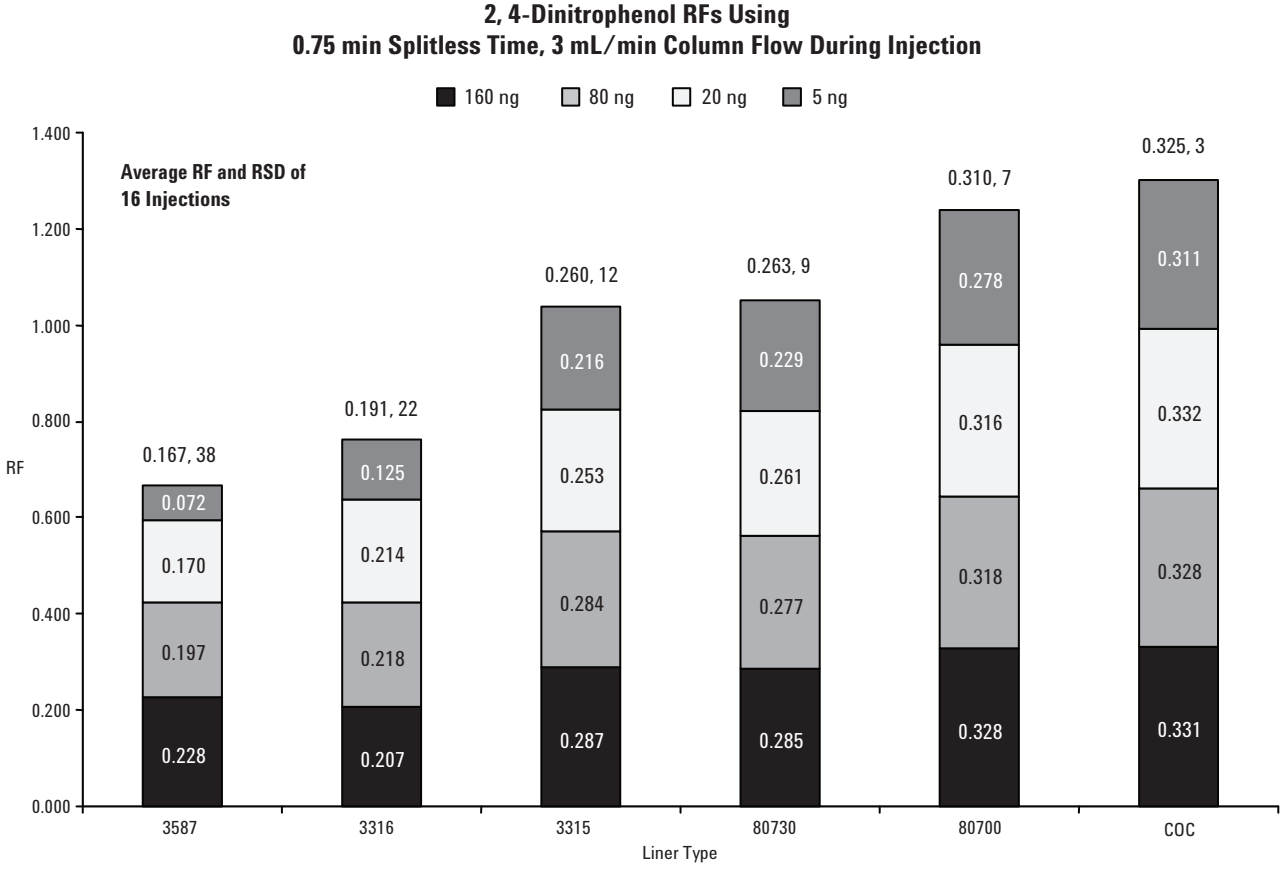

Figure 2. 2,4-Dinitrophenol RFs using five different liners.

Figure 3 shows performance of the 3587 liner at the four sets of experimental conditions. These data are shown as liners with wool are widely used. Comparing the first two bars shows the difference between a column flow of 1 mL/min and 3 mL/min during injection, both with a 0.2 minutes splitless time. Higher flow sweeps the inlet faster minimizing contact with the wool resulting in better 2,4-dinitrophenol performance. The higher column flow also means a higher inlet pressure. Previous work has shown that higher inlet pressures can keep the expanded solvent vapor contained in the liner. This holds true comparing the third and fourth bars with a 0.75 minute splitless time. The longer splitless time also results in a more complete transfer of 2,4-dinitrophenol onto the column at a fixed flow. In all cases performance suffers compared to COC.

In addition to 2,4-dinitrophenol, RFs and RSD were tracked for the other analytes in the short mix. Similar improvements were seen for the other active compounds although the liner effects were not as dramatic due to better performance of these compounds initially. No adverse effects were seen for the neutral or basic compounds when the acidic compounds improved.

Another factor that was monitored was ISTD (internal standard) reproducibility. Using the new direct connect liners showed variability in ISTD areas on some test systems greater than that using the standard liners. These systems still met the -50%/+100% 8270 ISTD criteria. Using a column flow of 3 mL/min during the injection period minimized this ISTD variability.

The high end linearity issue is not caused by the inlet although inlet parameters can affect it. The low end activity issue is directly related to inlet activity, including liner, seal, wool and stainless steel. Activity can be minimized by using pressure programmed flow and optimized splitless time. The liner and the presence of wool have the largest affect on low end activity. The liner must be chosen based on sample type, allowing for a trade-off of activity vs dirtiness of extract.

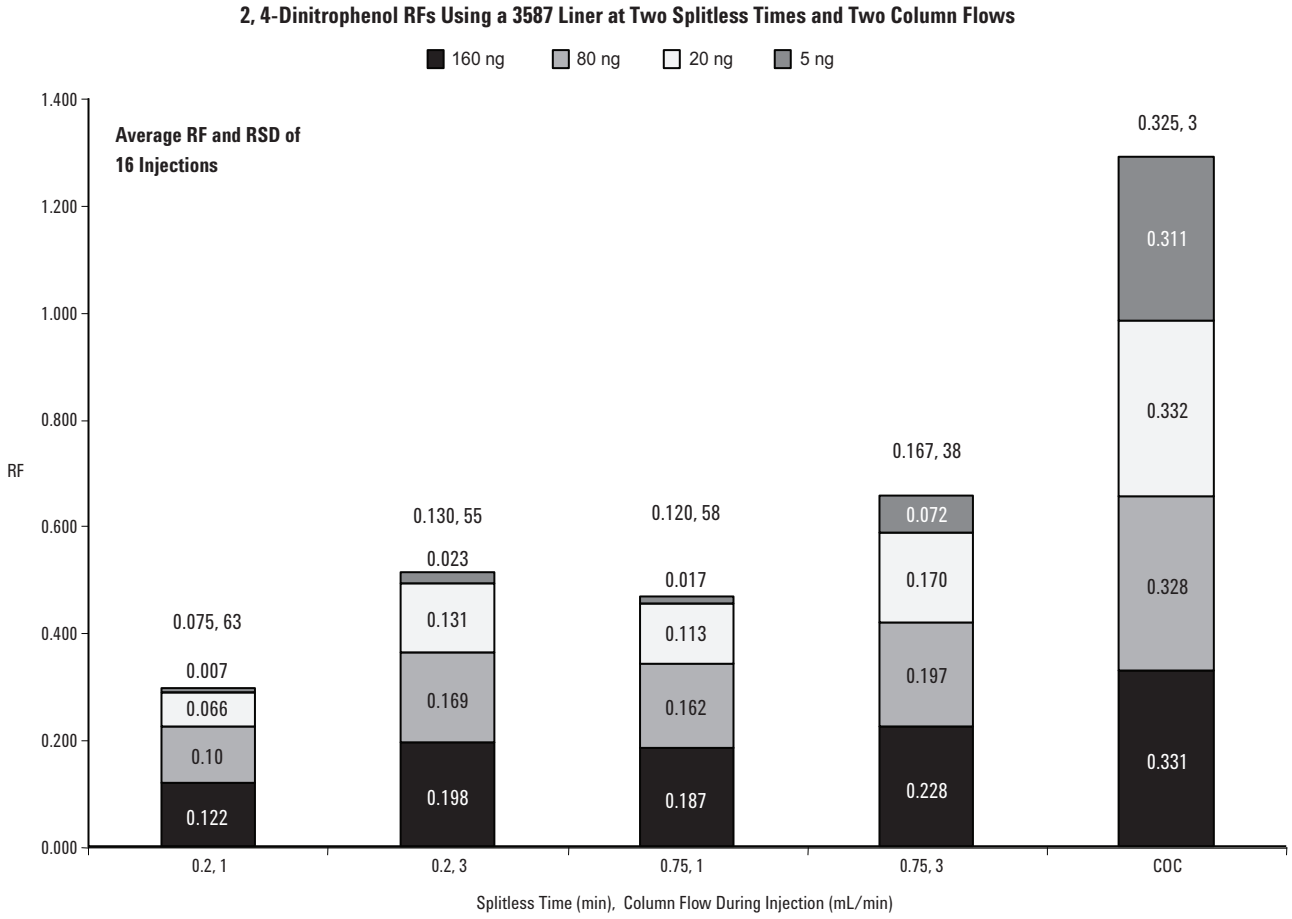

Figure 3. 2,4-Dinitrophenol RFs using a 3587 liner.

Source

The position of the column in the ion source, ion source materials, dimensions of the ion source, and parameters used for operation all affect the response of the system in this method.

In general, the column should be positioned beyond the end of the interface but not too far into the ion source. If the column tip is positioned inside of the interface guide tube, compounds are exposed to the hot metal surface of the interface and may decompose. On the other hand, if a significant length of column is exposed inside the ion source, the polyimide coating of the column can take on a static charge due to the ions and electrons in the ion source, and this charge interferes with the ejection of ions from the ion source chamber. In practice, a 2 to 3 mm extension of the column out of the interface has been found to yield the best results. This position may be set by one of two methods:

1 Put the column nut and ferrule on the column; open the analyzer door; push the column through the interface until 2 to 3 mm is sticking out of the end; then tighten down on the column nut; or

2 Put the column nut and ferrule on the column; open the analyzer door; push the column through the interface until it is just beyond the end of the interface. Tighten the nut only finger tight. Hold the MSD analyzer door closed, and then slide the column in until it just bottoms out (stops). You have now hit the left side of the source. Mark the column with typewriter correction fluid in the oven next to the nut. You can release the MSD analyzer door. Back the column out 12.5 mm (the source i.d.). Tighten the column nut. There should be 2 to 3 mm of column visible at the MSD end of the transfer line. You have now positioned the column just inside the source.

Method 1 above has the advantage that you can see what is happening, but a disadvantage is that it is difficult to measure the 2 to 3 mm column length inside the vacuum manifold. Method 2 has a few more steps but gives reproducible results, if followed exactly.

The material used for the standard MSD ion source may decompose some analytes under some conditions, especially when the source temperature is high. The Ultra source (patent applied for) has been found to reduce low end activity under the conditions typically used for this method. Table 4 shows a comparison of RFs for 2,4-dinitrophenol using different source materials. It also shows that the new Ultra source can be abrasively cleaned with minimal loss in performance.

The high end linearity is a function of the density of ions produced in the ion source. Reducing the ion source pressure improves the high end linearity. Therefore, increasing the size of the holes in the ion source improves the high end linearity, attended by some loss in sensitivity. To improve high end linearity, the hole in the drawout lens is made 6 mm in diameter rather than the standard 3 mm diameter. The change in dimension allows for a better match between the instrument's linear working range and the requirements of the method.

Another way of improving the high end linearity is to alter the operating parameters used in the method. A combination of a lower emission current (20 µA) and a high repeller setting (25 V) was determined to improve high end linearity so that the RSD of the analytes with strong response were single digit values. These analytes are the PAHs and phthalates. The emission current of 20 µA is set by the analyst. The revised tuning macros automatically set the repeller voltage to 25 V.

Table 4. 2,4-Dinitrophenol Average RFs Using Various Ion Sources

	COC-MSD Avg RFs of 2,4-dinitrophenol, n=4				
ng Injected	**5**	**20**	**80**	**160**	**Avg**
Ultra source	0.121	0.185	0.229	0.244	0.194
Ultra source air baked at 150 °C	0.120	0.185	0.228	0.244	0.194
Ultra source cleaned with 400 grit SC paper	0.119	0.181	0.220	0.231	0.188
Ultra source cleaned with metal polish	0.107	0.169	0.209	0.221	0.177
Standard source B	0.036	0.073	0.132	0.152	0.098
Standard source A	0.025	0.036	0.063	0.086	0.052

Data

As a result of this study, the G2860A 8270 Semi-Volatiles Applications Kit has been developed. The kit provides modified and/or pretested components to improve system performance for USEPA Method 8270. The kit includes an Ultra source, specially tested column, inlet liners and tune macros. The data in Table 5 are an average result of four Ultra source/column combinations. These can be considered typical of a 6890/5973 system with the applications kit installed.

The data in Table 5 are from calibrations at 5, 10, 20, 50, 80, 120 and 160 ng. This extended range exceeds the typical range of 20 to160 ng. The data meet the 8270 criteria listed in the Introduction section of this application note. The minimum average RF is well above the required 0.050 for all of the SPCCs. The RSDs for all the CCCs are significantly less than 30% required. The mean RSD of 7% across all compounds easily meets the minimum criteria of 15%.

Table 5. Typical results from a 6890/5973 GC/MSD System with the G2860A Applications Kit Installed, 5 to 160 ng, 1 µL Splitless Injection

	Avg RF	RSD		Avg RF	RSD
ISTDs					
1,4-Dichlorobenzene-d4		8	2-Chloronaphthalene	1.003	5
Naphthalene-d8		7	2-Nitroaniline	0.482	10
Acenaphthene-d10		7	Acenaphthylene	1.512	5
Phenanthrene-d10		8	Dimethylphthalate	1.187	4
Chrysene-d12		9	2,6-Dinitrotoluene	0.272	6
Perylene-d12		9	Acenaphthene (CCC)	0.958	5
			3-Nitroaniline	0.265	8
Analytes			2,4-Dinitrophenol (SPCC)	0.130	25
Pyridine	1.436	6	Dibenzofuran	1.421	4
N-Nitrosodimethylamine	0.799	6	2,4-Dinitrotoluene	0.364	9
2-Fluorophenol	1.189	4	4-Nitrophenol (SPCC)	0.205	11
Aniline	1.576	6	Fluorene	1.152	5
Phenol-d5	1.639	6	4-Chlorophenyl-phenylether	0.566	6
Phenol (CCC)	1.783	4	Diethylphthalate	1.177	5
bis(2-chloroethyl) ether	1.280	5	4-Nitroaniline	0.223	9
2-Chlorophenol	1.293	4	4,6-Dinitro-2-methylphenol	0.135	16
1,3-Dichlorobenzene	1.320	3	Diphenylamine (CCC)	0.518	6
1,4-Dichlorobenzene (CCC)	1.371	3	2,4,6-Tribromophenol	0.109	8
1,2-Dichlorobenzene	1.275	3	Azobenzene	0.177	5
Benzyl alcohol	0.895	7	4-Bromophenyl-phenylether	0.206	5
bis(2-chloroisopropyl)ether	2.273	9	Hexachlorobenzene	0.198	4
2-Methylphenol	1.356	7	Pentachlorophenol (CCC)	0.123	9
Hexachloroethane	0.615	3	Phenanthrene	1.064	4
N-Nitroso-di-n-propylamine (SPCC)	1.508	5	Anthracene	1.017	4
4-Methylphenol	1.243	7	Carbazole	0.734	7
Nitrobenzene-d5	0.489	3	Di-n-butylphthalate	1.248	7
Nitrobenzene	0.452	3	Fluoranthene (CCC)	1.184	5
Isophorone	0.770	3	Pyrene	1.344	5
2-Nitrophenol (CCC)	0.188	7	Benzidine	0.295	9
2,4-Dimethylphenol	0.309	8	Terphenyl-d14	0.963	5
bis(2-Chloroethoxy)methane	0.407	4	Butylbenzylphthalate	0.707	8
Benzoic acid	0.154	39	3,3'-Dichlorobenzidine	0.322	8
2,4-Dichlorophenol (CCC)	0.282	7	Benzo[a]anthracene	1.213	5
1,2,4-Trichlorobenzene	0.289	4	Chrysene	1.168	3
Naphthalene	0.919	4	bis(2-Ethylhexyl)phthalate	0.906	4
4-Chloroaniline	0.340	9	Di-n-octylphthalate (CCC)	1.650	9
Hexachlorobutadiene (CCC)	0.191	5	Benzo[b]fluoranthene	1.197	9
4-Chloro-3-methylphenol (CCC)	0.341	6	Benzo[k]fluoranthene	1.108	8
2-Methylnaphthalene	0.606	4	Benzo[a]pyrene (CCC)	0.995	7
Hexachlorocyclopentadiene (SPCC)	0.267	11	Indeno[1,2,3-cd]pyrene	0.807	8
2,4,6-Trichlorophenol (CCC)	0.370	8	Dibenz[a,h]anthracene	0.689	9
2,4,5-Trichlorophenol	0.368	6	Benzo[g,h,i]perylene	0.741	8
2-Fluorobiphenyl	1.222	4	Average of analyte RSDs		7

Continued

The additional study goals were also met. Method resolution requirements for benzo[b]fluoranthene and benzo[k]fluoranthene can be met when using thinner film columns depending on inlet parameters used. Linearity has been maximized at the high end without losing significant sensitivity at the low end. This improves overall RSDs. Activity in the entire GC/MSD system has been reduced thereby maximizing RFs for active compounds such as the nitrophenols. This gives the user a greater margin for system degradation when analyzing dirty samples.

These system improvements ensure maximum productivity for the analyst using an Agilent Technologies 6890/5973 GC/MSD for USEPA Method 8270.

Instrument Operating Parameters

Two sets of recommended instrument operating parameters are listed in Table 6 and Table 7. These are starting conditions and may have to be optimized.

The ramped flow and splitless times in Table 6 result in less material on column, better peak shape and resolution of benzo[b]fluoranthene and benzo[k]fluoranthene using a 0.5 µm column as provided in the G2860A 8270 Semi-Volatiles Applications Kit. However, less material on column may result in lower response factors for active compounds.

The ramped flow and splitless times in Table 7 result in more material on column, resulting in worse peak shape and benzo[b]fluoranthene and benzo[k]fluoranthene are not resolved. However, more material on column may result in higher response factors for active compounds.

The 0.5 µm film thickness column is a compromise of speed versus resolution. A 1.0 µm film thickness column is recommended in 8270 for best resolution and best peak shape at higher analyte concentrations. Using a 1.0 µm film thickness column also results in the longest run times. A 0.25 µm film thickness column will give shorter run times, but capacity suffers and consequently so does peak shape. Some laboratories meet method resolution requirements using split injections on a 0.25 µm column.

Many users have had success keeping this method running by clipping the front end of the column on a regular basis, daily if needed. The first compounds to suffer degradation from not clipping the column are the phenols.

The 5181-3316 liner is also a compromise. The absence of wool helps to preserve active analytes but potentially subjects the column to degradation from dirty samples. Adding a wisp of wool will help protect the column but active analytes will decompose. The new direct connect liners are the best choice for clean samples or for minimizing inlet activity.

The method operating parameters given here should only be considered a good starting point. Optimization of the parameters by the analyst are dependent on the analytes and calibration ranges required by the individual laboratory's statement of work.

Table 6. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890		
Inlet Liner	5181-3316, single taper, 4 mm i.d., deactivated		
Inlet	EPC Split/splitless		
Mode	Splitless, 1 µL		
Inlet temp	250 °C		
Pressure	9.24 psi		
Purge flow	30 mL/min		
Purge time	0.35 min		
Gas saver	Off		
Oven			
Oven ramp	°C/min	Next °C	Hold min
Initial		40	1.00
Ramp 1	15	100	0.00
Ramp 2	20	240	0.00
Ramp 3	10	310	6.00
Total run time	25 min		
Equilibration time	1.0 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP-5MS, 19091S-133, specially tested		
Length	30 m		
Diameter	0.250 mm		
Film thickness	0.5 µm		
Mode	Ramped flow		
Flow	mL/min	mL/min	Hold min
Initial		1.2	0.00
Ramp 1	99	2.0	0.35
Ramp 2	10	1.2	0.00
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
MSD	Agilent Technologies 5973 with Ultra Source		
Solvent delay	3.2 min		
EM voltage	DFTPP tune - 75 volts		
Low mass	35 amu		
High mass	500 amu		
Threshold	50		
Sampling	2		
Scans/sec	3.25		
Quad temp	150 °C		
Source temp	230 °C		
Transfer line temp	310 °C		
Repeller voltage	25 V as set by new tuning macro		
Emission current	20 µA set by the analyst		

Table 7. *Alternate* Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 6890		
Inlet Liner	5181-3316, single taper, 4 mm i.d., deactivated		
Inlet	EPC Split/splitless		
Mode	Splitless, 1 µL		
Inlet temp	250 °C		
Pressure	23.14 psi		
Purge flow	30 mL/min		
Purge time	0.50 min		
Gas saver	Off		
Oven			
Oven ramp	°C/min	Next °C	Hold min
Initial		40	1.00
Ramp 1	15	100	0.00
Ramp 2	20	240	0.00
Ramp 3	10	310	6.00
Total run time	25 min		
Equilibration time	1.0 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP-5MS, 19091S-133, specially tested		
Length	30 m		
Diameter	0.250 mm		
Film thickness	0.5 µm		
Mode	Ramped flow		
Flow	mL/min	mL/min	Hold min
Initial		3.0	0.55
Ramp 1	10	1.2	0.00
Inlet	Front		
Outlet	MSD		
Outlet pressure	Vacuum		
MSD	Agilent Technologies 5973 with Ultra Source		
Solvent delay	3.2 min		
EM voltage	DFTPP tune - 200 volts		
Low mass	35 amu		
High mass	500 amu		
Threshold	50		
Sampling	2		
Scans/sec	3.25		
Quad temp	150 °C		
Source temp	230 °C		
Transfer line temp	310 °C		
Repeller voltage	25 V as set by new tuning macro		
Emission current	20 µA set by the analyst		

Conclusion

In this paper, we have demonstrated a process for examining the performance of various components on a complex analytical method composed of many compounds. The challenges of analyzing different classes of compounds in the shortest time while meeting the method requirements are difficult. This study has led to the development of a 25-minute 8270D method suitable for an extended calibration range of 5 to 160 ng. The G2860A 8270 Semi-Volatiles Applications Kit provides the components necessary to convert an existing 6890/5973 to perform this analysis.

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Printed in the USA
November 7, 2001
5988-3072EN

Agilent Technologies

A New Approach to the Analysis of Phthalate Esters by GC/MS

Application

Gas Chromatography/Mass Spectrometry

March 2001

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Abstract

A new instrumental method for the determination of 29 phthalate esters, including six recently banned from baby toys by the European Union, using positive chemical ionization and retention-time locking is described. Positive chemical ionization provides a high degree of selective ionization for the phthalates, primarily producing spectra in which the protonated molecule (M+1) is the base peak. This provides easy discrimination among the phthalates on the basis of their molecular weight, while retention-time locking increases confidence in the identification of the various isomers.

In this approach, both pure compounds and technical mixtures are considered. Although this work focuses on the more commonly used 1,2-substituted esters, the 1,3-isomers and 1,4-isomers are also characterized.

The combination of positive chemical ionization and retention-time locking makes the method rugged, durable and applicable to a wide variety of matrices.

Introduction

The widespread use and manufacture of plastics have made the phthalate esters one of the most ubiquitous classes of compounds in our everyday environment. These “plasticizers” increase polymer flexibility due to their function as intermolecular “lubricants”. Because they are additives and not reagents, they are not chemically bound in the polymer and are available to leach from the matrix. Phthalates are also components of cosmetics, detergents, building products (flooring, sheeting, films), lubricating oils, PCB substitutes, carriers in pesticide formulations and solvents. Consequently, the potential for human exposure is very high. Toxicological studies have linked some of these compounds to liver and kidney damage, and to possible testicular or reproductive-tract birth defect problems, characterizing them as endocrine disruptors. Scientists at the U.S. Centers for Disease Control have, for the first time, documented human exposure to phthalates by determinations of the monoester metabolites in human urine [1]. Their work leads to the conclusion that “phthalate exposure is both higher and more common than previously suspected.”

Of particular concern were the significantly higher concentrations of the dibutyl phthalate metabolite in urine of women of childbearing age (20-40 years) than in other portions of the population.

The presence of phthalate esters in polyvinyl chloride (PVC) toys has generated the most

controversy. While regulators in Greece have completely banned soft PVC toys, Austria, Denmark, Finland, France, Germany, Norway and Sweden have unilaterally banned phthalates in PVC toys for children under three years old. In December of 1999, the European Union (EU), concerned with a "serious and immediate risk" to children, placed an emergency ban on six of the phthalate esters in soft PVC toys and childcare products meant to be placed in the mouths of children under the age of three [2]. None of the six banned phthalates may exceed 0.1% by weight.

These heightened concerns suggest the need for an improved method of detecting and characterizing phthalate esters which is applicable to a wide variety of matrices. This application note describes such an analytical method.

Phthalate Structure and Mass Spectra

The three primary structures of phthalates are shown in Figure 1. Although there are three possible positions for the ester linkages, the most commonly used phthalates are based on the 1,2-benzenedicarboxylic acid structure (top). There are an infinite number of possible alkyl side chains, (R) and an infinite number of combinations of the side groups (R and R'). For example, the diisononyl phthalate consists of an array of compounds due to the isomeric branched-chain alkyl groups on both side chains.

For phthalate esters with saturated alkyl side chains (without oxygen), the most intense peak in the electron impact (EI) ionization mass spectrum at 70 eV is always at *m/z* 149 due to the rapid formation and stability of the ion shown in Figure 2. (The only exception is R=R'=CH_3 where the base peak is at *m/z* 163).

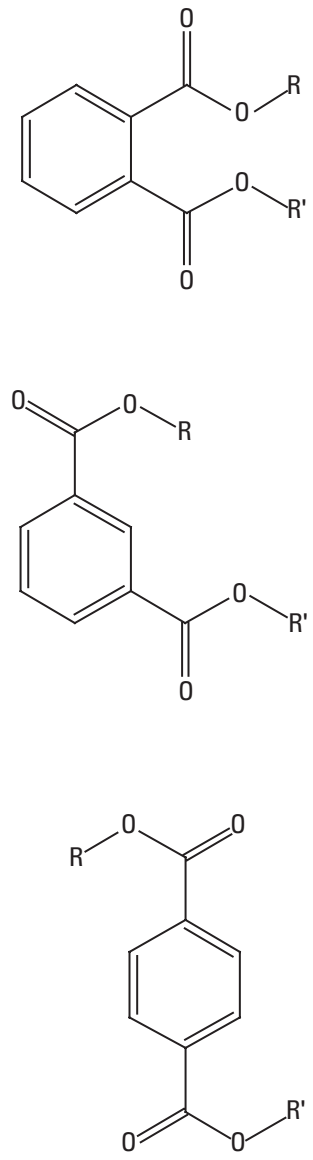

Figure 1. Phthalic ester (top) or the 1,2-benzenedicarboxylic acid ester, isophthalic ester (middle) or the 1,3-benzenedicarboxylic acid ester, and terephthalic ester (bottom) or the 1,4-benzenedicarboxylic acid ester. R and R' represent alkyl side chains which may be branched and contain oxygen.

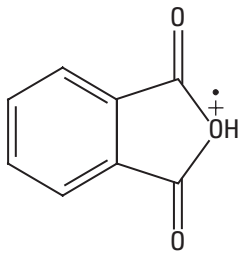

Figure 2. The most abundant ion in the mass spectra of the phthalate esters with saturated alkyl side chains; *m/z* 149. The exception is for dimethyl phthalate where both R and R' are CH_3 and so the H on the oxygen is replaced by CH_3 and consequently *m/z* 163 becomes the base peak.

Invariably, the molecular ion is very weak or altogether absent; other fragments that provide information on the phthalate identity are also of very low abundance. As an example, consider the EI mass spectrum of dibutyl phthalate, one of the six banned by the EU, and bis(4-methyl-2-pentyl) phthalate in Figure 3. Identifying fragments have relative intensities of less than 10%. Gas chromatography provides some separation of the phthalates, but with the array of possible isomers and essentially a single identifying ion (i.e., *m/z* 149), distinguishing the individual phthalates of concern is difficult. More confident identification of the phthalates is possible using chemical ionization mass spectrometry in conjunction with retention-time locking (RTL).

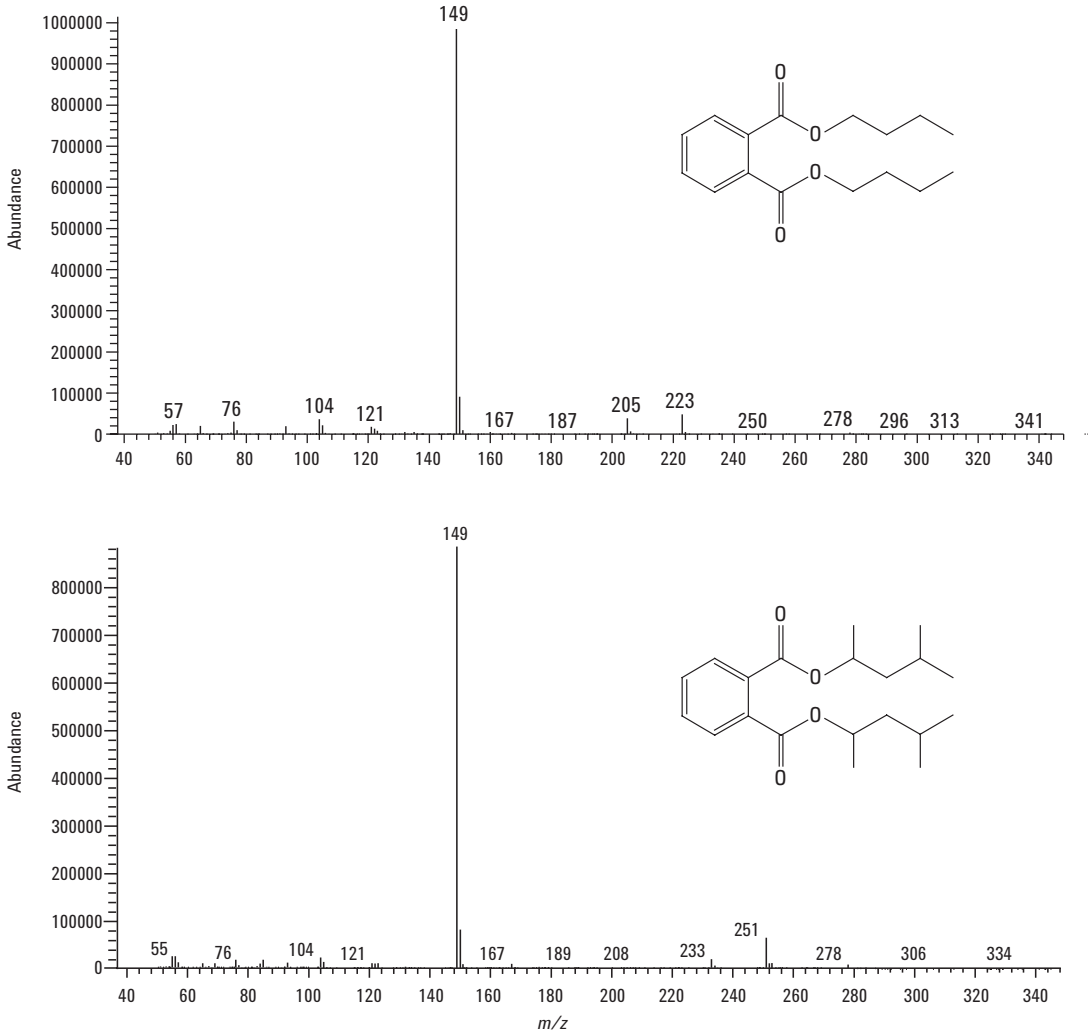

Figure 3. Electron impact ionization mass spectra of di-n-butyl phthalate (upper panel) and bis(4-methyl-2-pentyl) phthalate (lower panel) from *m/z* 50 to 350 at 70 eV. Notice the lack of intense fragments and molecular ions. The molecular weights are 278 and 334 g/mole, respectively.

Retention-time locking allows compound retention times achieved on any one Agilent 6890 gas chromatograph (GC) to be replicated to within a few seconds on any other Agilent 6890 gas chromatograph (GC) applying the same GC method [3-5]. RTL is a powerful approach to compound identification. RTL allows the creation of compound acquisition methods and quantitation databases that can be reproduced in any laboratory, anywhere, because a compound can have a universally fixed and reproducible retention time. It is important that RTL be applied in conjunction with the appropriate detection scheme and sample reparation methods.

Chemical ionization provides a more selective form of ionization than electron impact [6]. By judicious choice of the reagent gases, the degree of compound fragmentation can be controlled to a certain extent. In positive chemical ionization, methane reagent gas usually provides more fragmentation than gases of higher proton affinity such as ammonia. Less fragmentation would be helpful in identifying the phthalates. Instead of all phthalates generating a single, similar ion, positive ionization can provide phthalate ester molecular weights.

Experimental

Phthalate esters were obtained from Ultra Scientific (North Kingstown, RI), AccuStandard (New Haven, CT), and ChemServices (West Chester, PA) as neat compounds and mixtures. Dilutions were made in isooctane (Burdick and Jackson Grade, VWR Scientific).

The configuration and operating parameters of the Agilent 6890Plus GC (standard 120V or "faster ramping" 220V), 7683 Automatic Liquid Sampler and 5973N MSD with CI option used for acquiring the data are given in the following tables. PCI reagent gas purities were 99.99% or higher.

Injection Parameters

Injection Mode	Pulsed Splitless	
Injection Port Temperature	300°C	
Pulse Pressure & Time	25.0 psi	1.00 min
Purge Flow & Time	20.0 mL/min	3.00 min
Gas Saver Flow & Time	20.0 mL/min	3.00 min

Oven Parameters

Temperature Program	80°C	1.00 min
50.00°C/min	200°C	0.00 min
15.00°C/min	350°C	2.00 min
Oven Equilibrium Time	0.25 min	
MSD Transfer Line Temp	325°C	

Column Parameters

GC column (122-5532)	DB-5MS 30 m;	
	0.25 mm i.d.; 0.25 µm film	
Initial Flow & Mode	1.2 mL/min	Constant Flow
Detector & Outlet Pressure	MSD	Vacuum

Mass Spectrometer Parameters

Tune Parameters	PCI Autotune (NH3)
Electron Multiplier Voltage	Autotune + 400V
Solvent Delay	4.00 min
Scan Parameters	194 - 550 *m/z*
Quadrupole Temperature	150°C
Source Temperature	250°C
Ammonia Gas Flow (MFC setting)	0.5 mL/min (10%)

Miscellaneous Parts

Septa	5182-0739	BTO septa (400°C)
Liner	5062-3587	Deactivated 4 mm i.d. single taper
GC column ferrule	5181-3323	250 µm Vespel
MSD interface ferrule	5082-3508	0.4 mm i.d. graphitized Vespel

Results and Discussion

As expected using methane as the reagent gas, the PCI mass spectra of the phthalates show ions corresponding to the protonated molecule $[M+H]^+$ and adducts $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$. Because of the relatively vigorous fragmentation produced by methane, the spectra of the dialkyl phthalate esters still resemble that produced in EI. In most cases, the fragment at *m/z* 149 is the base peak, however ions at *m/z* M+1, M+29 and M+41 are relatively intense with $[M+H]^+$ from 10% to 30% (Figure 5). The dialkyl phthalate spectra also show a fragment corresponding to loss of one of the alkyloxy side chains to produce an ion shown in Figure 4. This is the most intense fragment for the dimethyl and diethyl phthalates and for the dibutyl and dipentyl (diamyl) phthalates, about 75% of the 149 base peak. As the length of ester alkyl chain increases, the intensity of this fragment decreases. (Apparently, in the dialkyl isophthalates, loss of the alkyl side chain not accompanied by the oxygen may be a preferred route.)

Although positive chemical ionization with methane provides more information than EI on phthalate identity, the methane reagent is still rather unselective in ionization and will produce more chemical noise in the background, complicating identification in complex matrices.

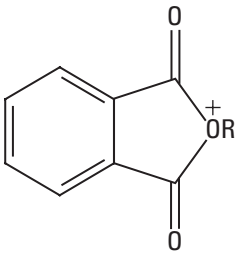

Figure 4. One of the most intense fragments in the methane PCI spectra of the phthalate esters is formed by loss of one of the alkyloxy side groups.

Applying ammonia as the reagent gas in PCI to reduce chemical noise and enhance identification of the phthalates is a more useful approach. The relatively gentle ionization produces protonation of the dialkyl phthalates, with *m/z* M+1 the base peak in their spectra. When combined with retention-time locking, identification of phthalates becomes further simplified. Compare the spectra of the di-n-butyl phthalate acquired using methane versus ammonia as the reagent gas (Figure 5). The protonated molecule is the single dominant peak in the ammonia PCI mass spectrum of the di-n-butyl phthalate, and the adduct at *m/z* 296 ($[M+NH_4]^+$) is relatively small.

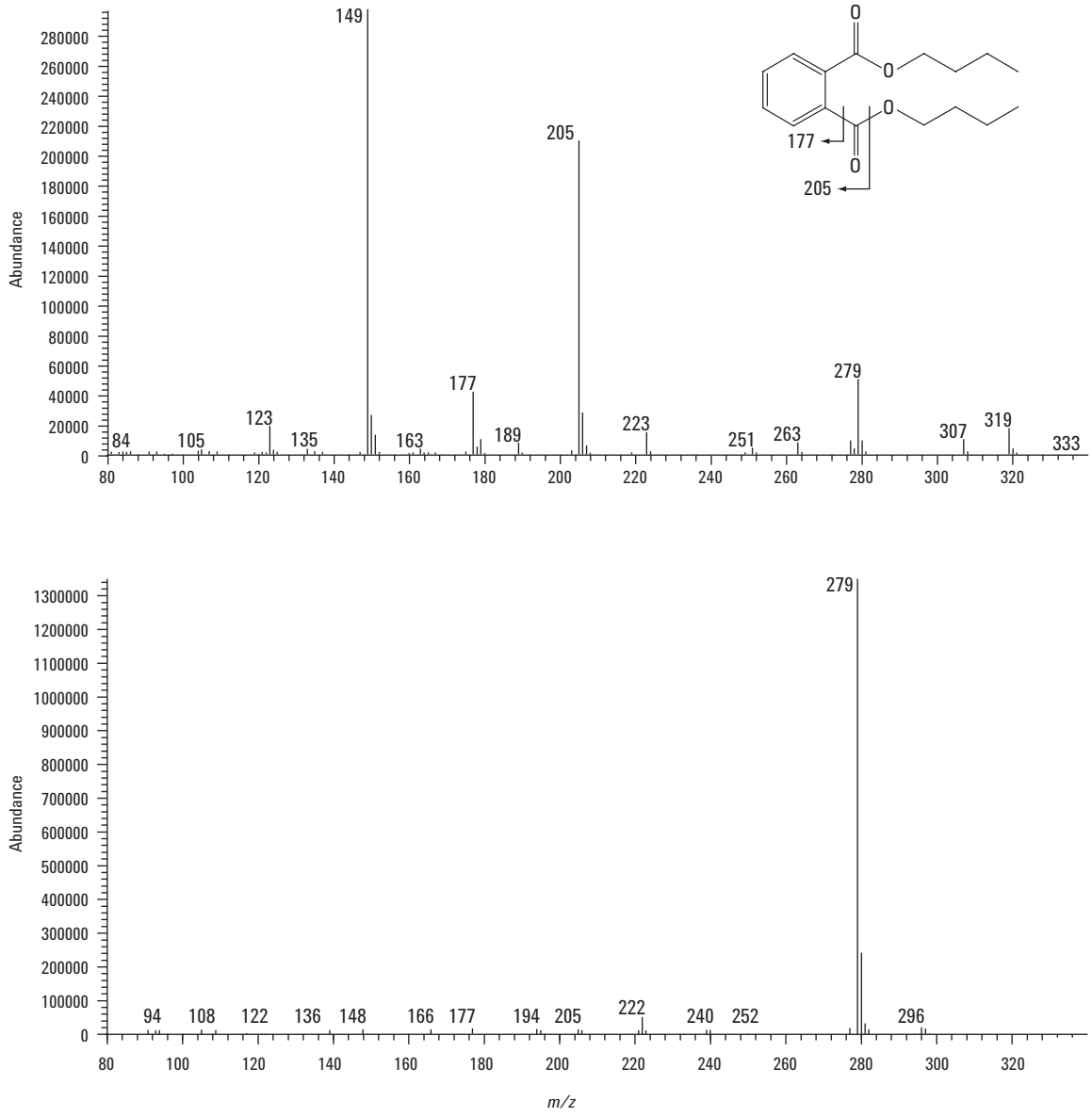

Figure 5. PCI methane (upper panel) and ammonia (lower panel) mass spectra of di-n-butyl phthalate. The PCI methane mass spectrum shows substantial fragmentation but relative to the EI spectrum in Figure 3, high abundance for the higher *m/z* ions such as the protonated molecule at *m/z* 279. The ion at *m/z* 205 is generated by loss of an oxybutyl fragment; a process described in Figure 4. The PCI-ammonia mass spectrum consists almost completely of the protonated molecule.

This implies an easy method for identification. Whereas the EI spectra of the phthalates most frequently result in a base peak at *m/z* 149, the dialkyl phthalate PCI-ammonia spectra have base peaks at *m/z* = M+1. All dialkyl phthalates molecular formulas can be expressed as

$$C_8H_6O_4(CH_2)_y\,(CH_2)_x.$$

These phthalates have (nominal) molecular masses given by

$$M = 166 + (x + y)\cdot 14, \text{ or}$$
$$M = 166 + (w)\cdot 28,$$

where x and y are the side chain lengths, and the second formula applies to symmetrical side chains (i.e., x = y = w). For example, di-n-butyl phthalate has x = y = 4, and therefore a (nominal) molecular mass of 278 which produces *m/z* 279 as the base peak. Interestingly, the PCI-ammonia spectra of the dialkyl isophthalates and terephthalates appear to have base peaks at *m/z* M+18 due to $[M+NH_4]^+$. Because of the greater steric access to the ester linkages, adduct formation may be preferred.

Table 1 gives the phthalate names, CAS numbers, molecular formula, nominal molecular mass, base peak in the PCI-ammonia spectrum and the RTL elution times. These retention times are "locked" relative to diphenyl phthalate, which has been chosen as the RTL locking compound and locked to elute at 9.450 min. Notice that the branched chain isomers elute prior to their straight chain forms on this column phase.

Table 1. Phthalate compound names, Chemical Abstracts Services numbers (CAS), molecular weights (M. Wt.), molecular formulas, nominal base peak in the PCI-ammonia spectrum and retention time (RT) in minutes. Retention times are locked relative to diphenyl phthalate (9.450 min). Retention time ranges are given for the isoalkyl phthalate technical mixtures. Phthalates banned by the EU are indicated by an asterix*. Benzyl benzoate is included since it is used as a surrogate in U.S. Environmental Protection Agency Method 8061.

Name	CAS	M. Wt.	Molecular Formula	Base Peak	RT (min)
dimethyl phthalate	131-11-3	194	$C_8H_4O_4(CH_3)_2$	195	4.32
dimethyl isophthalate	1459-93-4	194	$C_8H_4O_4(CH_3)_2$	212	4.54
diethyl phthalate	84-66-2	222	$C_8H_4O_4(C_2H_5)_2$	223	4.81
diethyl terephthalate	636-09-9	222	$C_8H_4O_4(C_2H_5)_2$	240	5.06
benzyl benzoate	120-51-4	212	$C_{14}H_{12}O_2$	230	5.62
diisobutyl phthalate	84-69-5	278	$C_8H_4O_4(C_4H_9)_2$	279	5.95
di-n-butyl phthalate*	84-74-2	278	$C_8H_4O_4(C_4H_9)_2$	279	6.40
bis(2-methoxyethyl) phthalate	117-82-8	282	$C_8H_4O_4(C_2H_4OCH_3)_2$	283	6.57
diamyl phthalate	131-18-0	306	$C_8H_4O_4(C_5H_{11})_2$	307	6.94
bis(2-ethoxyethyl) phthalate	605-54-9	310	$C_8H_4O_4(C_2H_4OC_2H_5)_2$	311	7.13
butyl benzyl phthalate*	85-68-7	312	$C_8H_4O_4(C_4H_9)(CH_2C_6H_5)$	313	8.42
diphenyl phthalate	84-62-8	318	$C_8H_4O_4(C_6H_5)_2$	319	9.45
diphenyl isophthalate	744-45-6	318	$C_8H_4O_4(C_6H_5)_2$	319	10.30
dicyclohexyl phthalate	84-61-7	330	$C_8H_4O_4(C_6H_{11})_2$	331	9.32
bis(4-methyl-2-pentyl) phthalate	146-50-9	334	$C_8H_4O_4(CH_3C_5H_{10})_2$	335	6.93
diisohexyl phthalates	146-50-9	334	$C_8H_4O_4(C_6H_{13})_2$	335	7.55 - 8.28
dihexyl phthalate	84-75-3	334	$C_8H_4O_4(C_6H_{13})_2$	335	8.34
dibenzyl phthalate	523-31-9	346	$C_8H_4O_4(CH_2C_6H_5)_2$	347	10.51
hexyl-2-ethylhexyl phthalate	75673-16-4	362	$C_8H_4O_4(C_2H_5C_6H_{12})(C_6H_{13})$	363	8.84
bis(2-n-butoxyethyl) phthalate	117-83-9	366	$C_8H_4O_4(C_2H_4OC_4H_9)_2$	367	8.98
bis(2-ethylhexyl) phthalate*	117-81-7	390	$C_8H_4O_4(C_2H_5C_6H_{12})_2$	391	9.32
di-n-octyl phthalate*	117-84-0	390	$C_8H_4O_4(C_8H_{17})_2$	391	10.28
dioctyl isophthalate	137-89-3	390	$C_8H_4O_4(C_8H_{17})_2$	408	10.84
diisononyl phthalates*	28553-12-0	418	$C_8H_4O_4(CH_3C_8H_{17})_2$	419	9.40 - 11.10
dinonyl phthalate	84-76-4	418	$C_8H_4O_4(C_9H_{19})_2$	419	11.19
diisodecyl phthalates*	26761-40-0	446	$C_8H_4O_4(CH_3C_9H_{18})_2$	447	10.16 - 11.86
didecyl phthalate	84-77-5	446	$C_8H_4O_4(C_{10}H_{21})_2$	447	12.05
diundecyl phthalate	3648-20-2	474	$C_8H_4O_4(C_{11}H_{23})_2$	475	12.87
didodecyl phthalate	2432-90-8	502	$C_8H_4O_4(C_{12}H_{25})_2$	503	13.65
ditridecyl phthalate	119-06-2	530	$C_8H_4O_4(C_{13}H_{27})_2$	531	12.01 - 13.81

Technical formulations of the isoalkyl phthalates tended to contain substantial amounts of the straight chain isomer, which may convolute quantitation as well as peaks that may be construed as originating from nonequivalent side chains i.e., $x \neq y$ in equation 1). These impurities can be detected as M±14 around the mass of the nominal isomer. For example, technical grade diisononyl phthalate contains compounds that generate ions at *m/z* 391 (minor), 405, 433, and 447 in addition to the nominal diisononyl phthalate compound at *m/z* 419. The "gentle" ionization of ammonia reagent gas, the elution times and the study of the spectra of other pure isomers, such as the dinonyl phthalate, suggest that these fragments are not formed by the PCI process but are due to these different alkyl side chain impurities (Figure 6). To demonstrate the utility of the PCI-ammonia compared to conventional EI analysis, consider the chromatograms presented in Figure 7. The EI spectra of the phthalates produce *m/z* 149 as the base peak for all the phthalates present; distinguishing ions are minor constituents (<10% relative intensity), making identification complicated. However, by examining the appropriate PCI-ammonia ions, the various phthalates are easily distinguished.

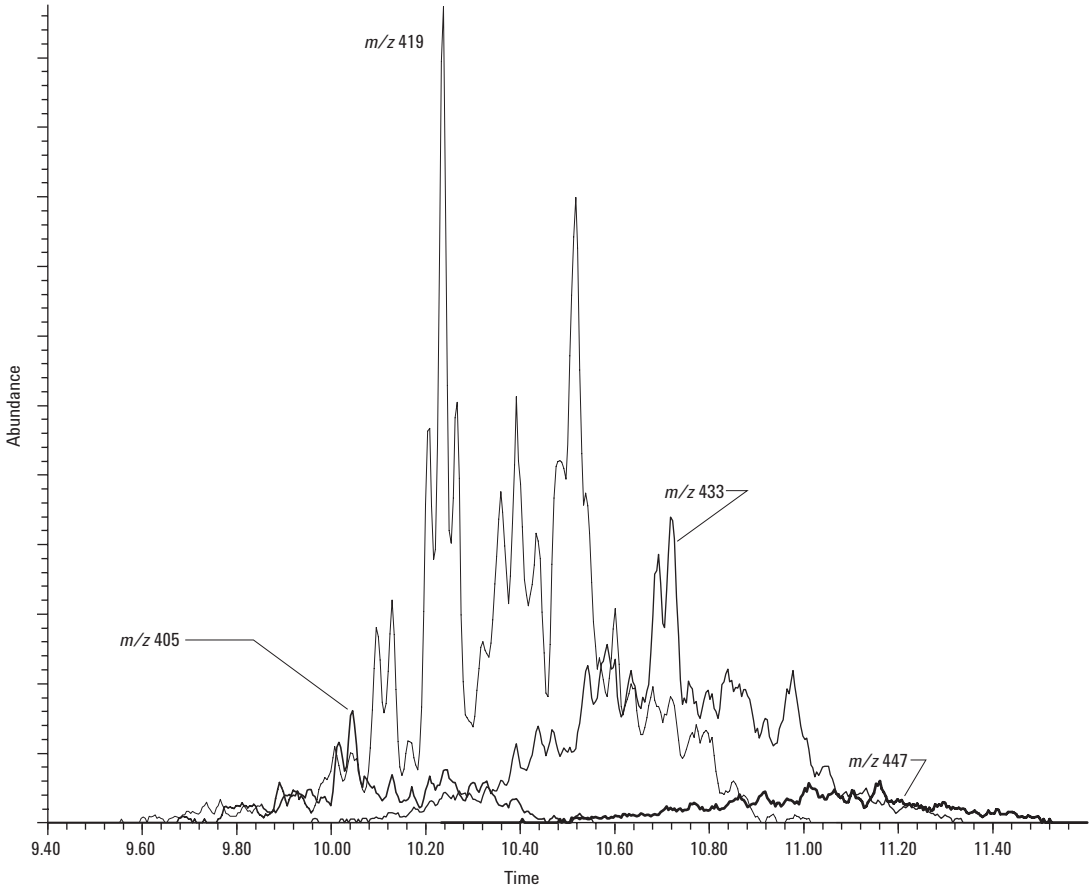

Figure 6. PCI-Ammonia extracted ion chromatogram of technical diisononyl phthalate. The diisononyl appears as the major component at *m/z* 419 while ions at *m/z* 405, 433, and 447 indicate alkyl chains shorter by one CH_2 unit and longer by one and two CH_2 units respectively.

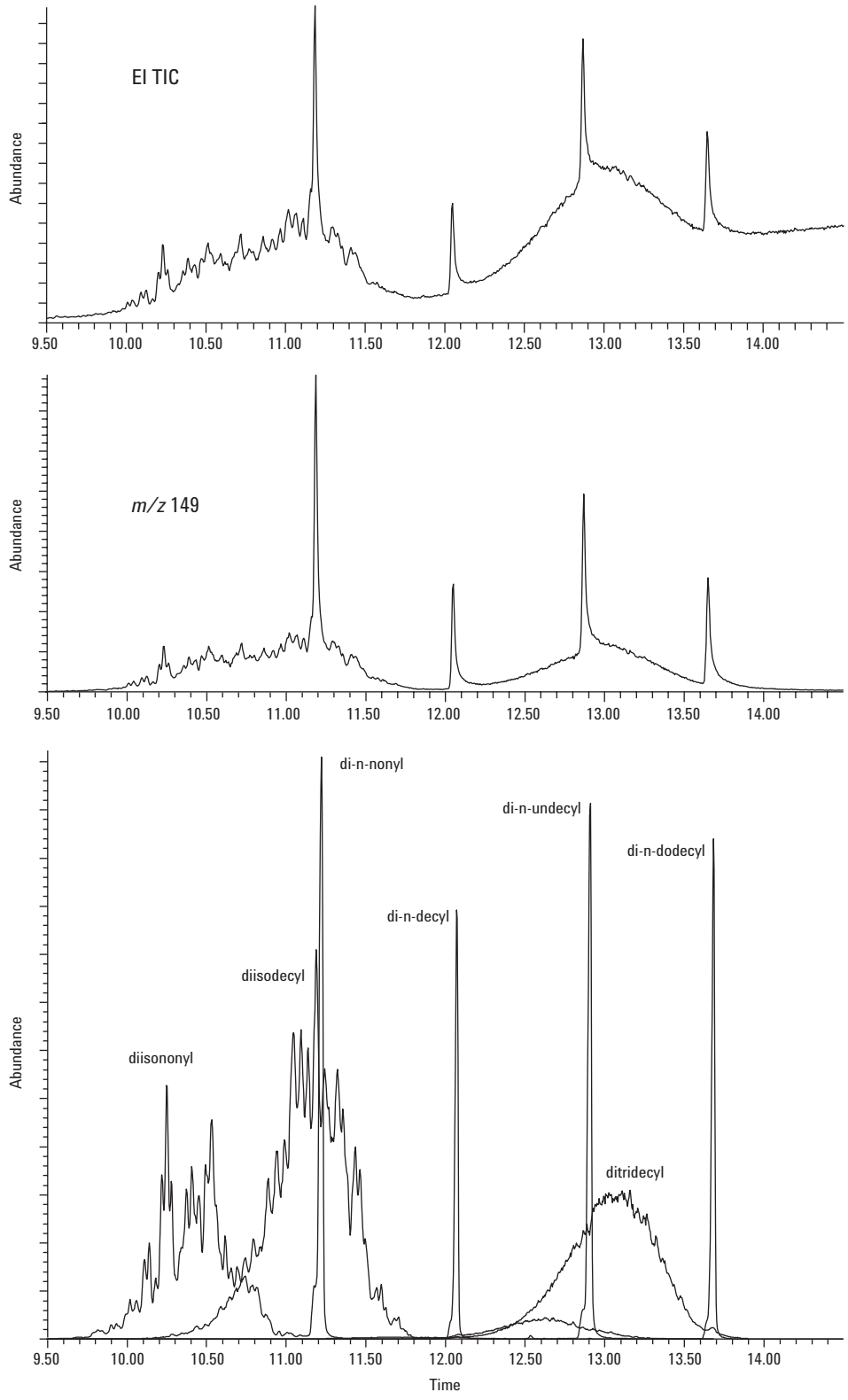

Figure 7. Chromatograms of dinonyl, diisononyl, didecyl, diisodecyl, diundecyl, didodecyl, ditridecyl phthalate esters in EI (upper panel), EI as an extracted ion chromatogram at *m/z* 149 (middle panel), and PCI-extracted ion chromatogram with ions selected for the individual phthalate classes as given in Table 1. The EI information is insufficient to identify coeluting phthalates. For example, the dinonyl and diundecyl phthalates are "buried under" the signals from the isodecyl and ditridecyl phthalates.

Conclusions

Applying GC - electron impact (EI) mass spectrometry to the determination of phthalates requires full chromatographic separation. The EI spectra of the phthalates are distinguished only by ions of very low intensity. In EI, the phthalates produce a single common ion (*m/z* 149) as the most intense spectral peak, regardless of the alkyl side chain substitution. Applying tandem mass spectrometry (i.e., EI/MS/MS) gains nothing, because there is a common parent ion, and therefore any daughter ions would also be non-unique. However, the combination of positive chemical ionization with retention-time locking allows even complex mixtures of phthalates to be characterized. Ammonia reagent gas produces the protonated molecule as the base peak, which immediately allows the phthalates to be distinguished on the basis of their substitution. PCI is also an advantage in complex matrices, where the non selective ionization of EI produces a high chemical background. This method should therefore be suitable for use in phthalate determinations in environmental media, plastics, cosmetics and many other matrices.

"Locking" the retention time enhances confidence in the characterization of the various phthalate isomers on the basis of their definitive retention time. This is especially helpful for determinations using selected ion monitoring (SIM), since SIM groups need not be edited after column maintenance [4]. The data in Table 1 facilitate the development of a SIM method. The extension of the method to phthalates which elute at higher temperatures (>350°C) is also easily accomplished.

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Printed in the U.S.A.
March 7, 2001
5988-2244EN

Agilent Technologies

Analysis of Nitroaromatics and Nitro-Polycyclic Aromatic Hydrocarbons by Capillary Gas Chromatography with the Agilent 6890 Micro-ECD

Application

Gas Chromatography

March 1997

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Abstract

A new electron capture detector (ECD) for the Agilent 6890 Series gas chromatograph (GC) allows very sensitive detection of nitroaromatic compounds at low picogram levels with a linear response over three orders of magnitude.

This application note describes the performance of the new 6890 Series Micro-ECD when analyzing two types of nitro-aromatic compounds—explosives and nitrated polycyclic aromatic hydrocarbons (nitro-PAHs).

Introduction

Electron capture detection is most often used for the sensitive and selective detection of halogenated compounds. However, other compound classes also have electron capturing properties and can, therefore, be detected at low levels using an electron capture detector (ECD). Compounds containing a nitro-function—particularly nitroaromatics—are strong electron-capturing molecules. The ECD provides a very sensitive tool for trace analysis of these solutes.

This application note demonstrates that the 6890 Series Micro-ECD provides an extremely sensitive alternative to the typical NPD or MS detection[1, 2] for nitro-PAHs and explosives.

Experimental

The analyses were performed on an 6890 Series GC. Injection was automated splitless using an Agilent 7673 automatic sampler. The instrument configuration and analytical conditions used for the analysis of the nitro-PAHs and explosives are summarized in table 1.

Results and Discussion

The sensitivity of the ECD depends on the makeup flow rate. The 6890 Micro-ECD optimized the argon/5% methane (Ar/CH_4) makeup gas flow rate for the analysis of nitro-PAHs. Nitropyrene was used as test solute. The makeup flow rate was varied from 10 to 80 mL/min; at each setting, five runs were made.

Figure 1 shows the mean peak areas plotted versus the makeup flow rate. The optimum flow rate was obtained between 20 to 30 mL/min. At lower flow rates, the peak area decreased and the detector became less stable, shown in the increasing standard deviation on peak area. At higher flow

Table 1. Instrumental Configuration and Analytical Conditions

Chromatographic System	
Gas chromatograph	6890 Series
Inlet	Split/splitless
Detector	Micro-ECD
Automatic sampler	7673 Series
Liner	Single taper deactivated (part number 5181-3316)
Data handling	ChemStation (DOS Series)
Column	30 m x 0.25 mm id x 0.25 µm HP-5 MS (part number 19091S-433)
Experimental Conditions	
Inlet temperature	250 °C
Injection volume	1 µL
Injection mode	Splitless
Purge time	0.75 min
Purge flow	50 mL/min
Carrier gas	Hydrogen
Head pressure	58 kPa at 50 °C
Carrier gas mode	Constant flow
Flow, velocity	1.4 mL/min, 40 cm/s
Oven temperature	50 °C, 1 min initial, 20 °C/min to 320 °C, 0.5 min hold
Detector temperature	320 °C
Detector gases	Argon/5% methane: 20 mL/min

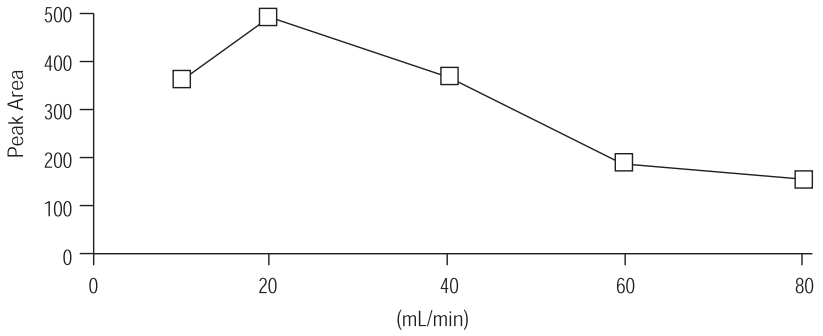

Figure 1. Peak area of 1-nitropyrene versus argon/5% methane makeup gas flow rate.

rates, the detector was stable (exhibiting a small standard deviation), but sensitivity drastically decreased.

Nitrogen is an alternative makeup gas for electron capture detection. It can usually be used interchangeably with Ar/CH_4; similar results for the effect of makeup gas flow rate are expected.

Next, the linearity of the detector response was measured. Using nitropyrene as the test solute, standard solutions of 1, 10, 50, 100 and 1,000 ppb were analyzed. The calibration curve for this compound, as shown in figure 2, exhibits a very good correlation coefficient (r = 0.99996).

Nitrated Polycyclic Aromatic Hydrocarbons

Nitro-PAHs are an important class of environmental pollutants.[3] Polycyclic aromatic compounds are formed during incomplete combustion of organic material. In the presence of nitrogen oxides (NO_x), the neutral PAHs (such as naphthalene or pyrene) are converted into nitro-PAHs.[3–5]

The nitro-PAHs have much higher mutagenic and carcinogenic activity than the neutral PAHs, but their extremely low concentration (measured as pg/m^3) in environmental samples, particularly air particulates, makes them difficult to monitor. Very sensitive detection is needed.

Using the optimized GC conditions, a mixture of 11 nitro-PAHs, each having a concentration of 40 pg/mL (40 ppb), was analyzed. The chromatogram for this analysis is shown in figure 3. Good peak shapes were obtained for all compounds. The detection limit, which varied from 0.1 to 1 pg for the different PAHs, is at least one order of magnitude lower than that obtained by nitrogen-phosphorus detection (NPD), mass spectrometry (MS), or MS-MS.[2] It can, therefore, be concluded that the 6890 Micro-ECD offers greater sensitivity for the detection of these nitro-PAHs than other methods.

Explosives

Explosives can be present as residues at chemical waste sites or on materials close to an explosion. Sensitive and fast methods are needed for analyzing and monitoring these compounds for environmental remediation or forensic evidence.

Although explosives are often analyzed by high pressure liquid chromatography (EPA method 8330), capillary gas chromatography (CGC) can provide a good alternative for most solutes using NPD or MS. Some of the nitro-aromatics are included in the target compound lists of EPA methods 8090 and 8270 (CGC-MS).

Explosives such as TNT (2,4,6-trinitrotoluene) contain one or more nitro-functions. CGC-ECD can provide a very sensitive and fast screening method for detecting these compounds.

The chromatogram in figure 4 shows the results of a standard mixture of explosives using the analytical conditions in table 1. The concentration of the test solutes was 100 pg/mL

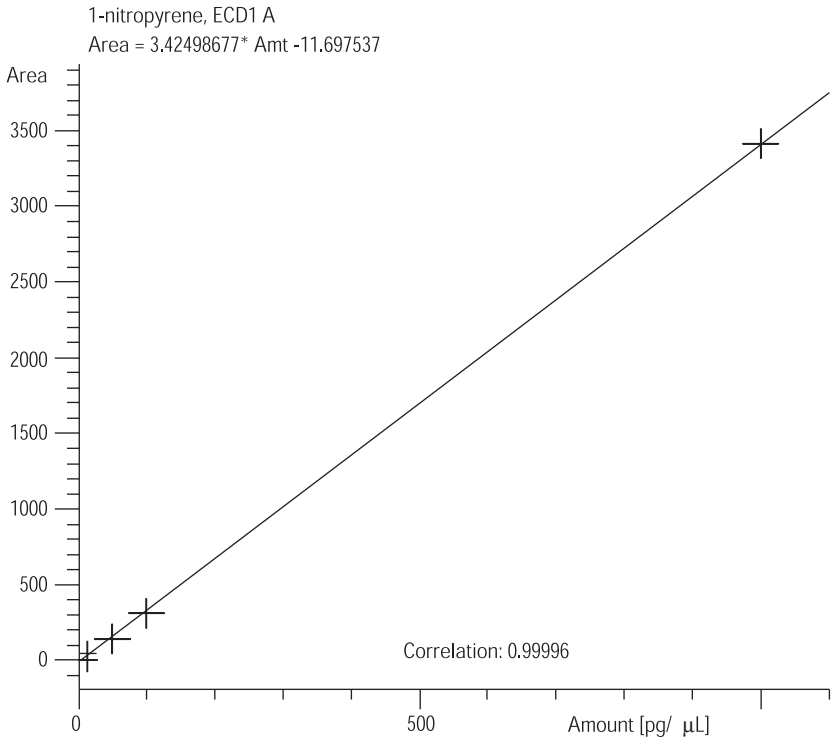

Figure 2. Calibration curve for 1-nitropyrene from 1 to 1,000 ppb

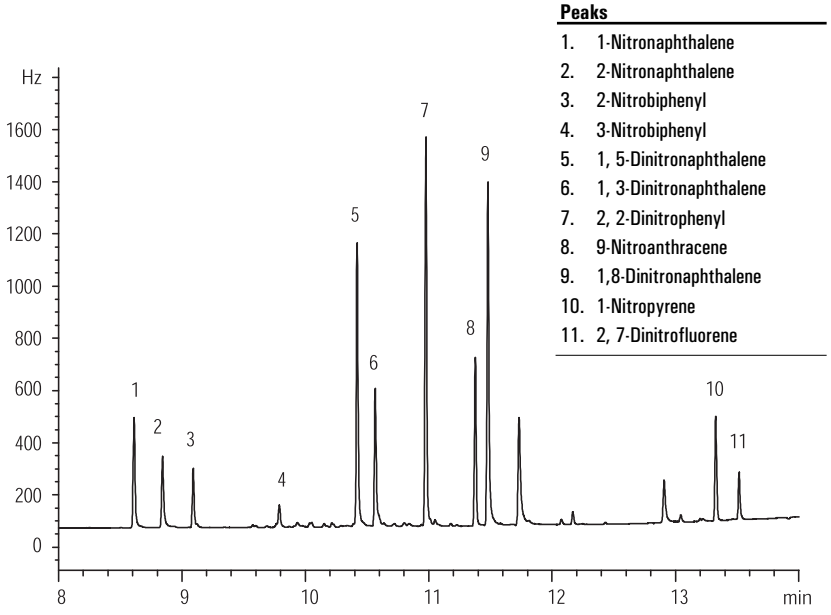

Figure 3. CGC-ECD analysis of nitrated polycyclic aromatic hydrocarbons (solute concentration: 40 ppb)

(100 ppb), except for 1,2-dinitrobenzene, which was present as an impurity. As the chromatogram shows, the different nitro-, dinitro-, trinitro-, and amino-nitro-compounds are well separated and elute with good peak shape.

The ECD response is dependent on the number of nitro-groups. For the mono-nitroaromatics, the detection limit is around 10 pg, while for the di- and tri-nitroaromatics the detection limit is below 1 pg. This example confirms that CGC-ECD can be used as a fast screening method for the analysis of this category of explosives.

Conclusion

The Agilent 6890 Series Micro-ECD allows very sensitive detection of nitroaromatic compounds. The detector was successfully used for the analysis of nitrated polycyclic aromatic hydrocarbons and explosives. Detection limits below 1 pg were obtained, and the detector was found to give a linear response over three orders of magnitude.

Peaks	
1. Nitrobenzene	7. 1,2-Dinitrobenzene (impurity)
2. 2-Nitrotoluene	8. 2,4-Dinitrotoluene
3. 3-Nitrotoluene	9. 1,3,5-Trinitrobenzene
4. 4-Nitrotoluene	10. 2,4,6-Trinitrotoluene
5. 1,3-Dinitrobenzene	11. 4-amino-2,6-Dinitrotoluene
6. 2,6-Dinitrotoluene	12. 2-amino-4,6-Dinitrotoluene

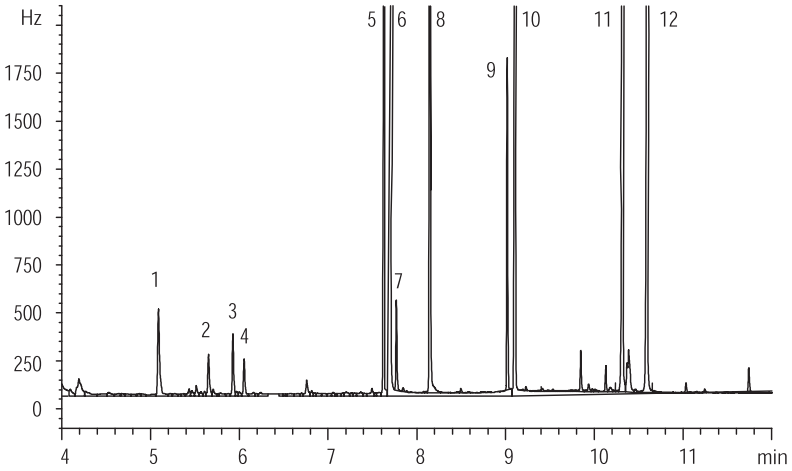

Figure 4. CGC-ECD analysis of explosives (solute concentration: 100 ppb)

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Printed in the USA 3/2000
5965-8015E

application

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LC/DAD/MS Analysis of Carbonyl (2,4-Dinitrophenyl)hydrazones

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Abstract

A simple and sensitive LC/MS method has been developed for the analysis of carbonyl compounds derivatized with (2,4-dinitrophenyl) hydrazine (DNPH) using the Agilent 1100 LC/MSD system. Detection is carried out simultaneously with the diode-array detector and the LC/MSD using negative ion atmospheric pressure chemical ionization (APCI).[1] The method was applied to 78 carbonyls including 1-alkanals (from formaldehyde to octadecanal), saturated and unsaturated aliphatic aldehydes and ketones, aromatic carbonyls (including hydroxy- and/or methoxy-substituted compounds), aliphatic dicarbonyls, and aliphatic carbonyl esters.

Introduction

The ability to identify carbonyls and to measure their concentrations at levels of parts per billion (ppb) or lower in complex mixtures is important in many areas, including biomedical research and environmental chemistry—especially air pollution. A well-established method utilizing UV detection of the DNPHs of both simple and multifunctional carbonyl compounds[2, 3] has been extended to include simultaneous MS detection using APCI in negative ion mode. Concentration of the compounds of interest from ambient air samples and subsequent derivatization is simplified by the use of C18 SPE cartridges impregnated with the derivatizing reagent. The combined methodology has been applied to several studies involving air pollution phenomena.[1]

Experimental

The system included an Agilent 1100 Series binary pump, vacuum degasser, autosampler, thermostatted column compartment, diode-array detector, and an LC/MSD. The LC/MSD was used with the APCI source. Complete system control and data evaluation were carried out using the Agilent ChemStation for LC/MS.

Sample Collection and Preparation

Carbonyl-DNPH standards were synthesized in our laboratory as described previously.[2, 3] Carbonyls were purchased from commercial suppliers (Aldrich Chemical Co., Lancaster Synthesis, Wiley Organics, Fluka Chemical Corp.) or were prepared as described in previous work.[2, 3]

Air samples were collected by drawing air at 1 liter/minute through C18 Sep-Pak cartridges (Waters Corporation) impregnated with (2,4-dinitrophenyl)hydrazine/phosphoric acid.[4] Collected carbonyl compounds were derivatized to (2,4-dinitrophenyl)hydrazones on the cartridge, and were then eluted with 2 mL of acetonitrile. The eluate was analyzed directly by LC/MS. The sample can be concentrated for the analysis of the higher molecular weight carbonyls, which are present at lower levels in ambient air.

Results and Discussion

DNPH derivatives are used to analyze carbonyl compounds by liquid chromatography to maximize detection of small, polar molecules, many of which cannot be analyzed using gas chromatography. The original LC/UV method for the analysis of DNPH derivatives of carbonyls was first improved by the use of a diode-array detector and HP particle beam LC/MS interface to provide positive identification of about 40 carbonyls at ppb levels in laboratory studies of air pollution chemistry[5] and in urban air.[6] The mass spectrometer provided extra dimensions of information to the already-rich data of the diode-array LC method, allowing the quantitation of coeluting analytes and the identification of unknowns for which standards were not initially available. However, the particle beam interface could not provide the detection limits necessary for measurement of carbonyls in ambient air, due to the significant percentage of water in the LC gradient required for the separation of the more complex mixtures.

To overcome this limitation, API-LC/MS was evaluated for this application. Both electrospray (ESI) and APCI in positive ion and negative ion modes were evaluated. APCI negative ion detection was found to provide the most sensitive and specific information about these compounds, giving 1–2 orders of magnitude better sensitivity than either ESI positive or negative ion or APCI positive ion detection.

Parameters for the acquisition of mass spectral data were automatically optimized by carrying out multiple injections of a standard mixture of 13 carbonyl-DNPH derivatives, using the system's Flow Injection Analysis Series capability. A fragmentor setting was chosen to obtain maximum $[M–H]^-$ for all 13 compounds present in the test mixture. Further optimization of the fragmentor voltage for specific compounds could be carried out to obtain distinct fragments, as the fragmentor voltage is time-programmable during acquisition. Those compounds which required a high percentage of acetonitrile for elution (eluting after 33 minutes) were found to have much better response with a corona current of 10 µA versus 4 µA for the smaller, early-eluting analytes. The scan range can be lowered to 50 amu if significant fragment ions below 125 amu are generated by in-source collision-induced dissociation (CID); the chemical noise, especially in the TIC, is lower when starting the scan at 125 amu.

Mobile phases containing acetonitrile often do not give optimal response in APCI compared to methanol/water eluents, and acetonitrile seems to form carbon on the corona needle more quickly than methanol. However, for this analysis, adequate separation of carbonyls in a reasonable analysis time could not be achieved using methanol/water instead of acetonitrile/water, even trying a variety of columns. Nonetheless, maintenance of the APCI spray chamber after extended use with high flow rates of acetonitrile/water only required cleaning of the corona needle and spray shield with mild abrasive cloth and solvent.

Early work with this method was carried out using a similar column but with dimensions of 4.6 mm i.d. × 150 cm at a flow rate of 1.4 mL/min, with results comparable to those obtained on the 3mm i.d. column. An additional gradient has also been developed utilizing THF as a mobile phase modifier. This gradient method is capable of better separation of C3 and C4 carbonyl compounds which co-elute using the acetonitrile/water gradient.

Tables 1–6 (shown on pages 9–14) list the first 78 carbonyl compounds that have been analyzed with this method, along with chromatographic and spectral details. The method has been used for more than 140 carbonyl compounds, including several with molecular weights of approximately 650 Da.

Figure 1 shows the 360 nm and MS total ion chromatograms of a mixture of the DNPH derivatives of 13 carbonyls. The amount injected per component is 60 ng (as carbonyl). The UV chromatogram is labeled with the identity of the peaks and the MS chromatogram with the mass of the base peak in the spectrum ($[M–H]^-$ anion).

Figure 2 shows extracted ion chromatograms from the data in Figure 1, illustrating how the specificity of the MS detector can help with coelution, sometimes even allowing quantitation of coeluting peaks.

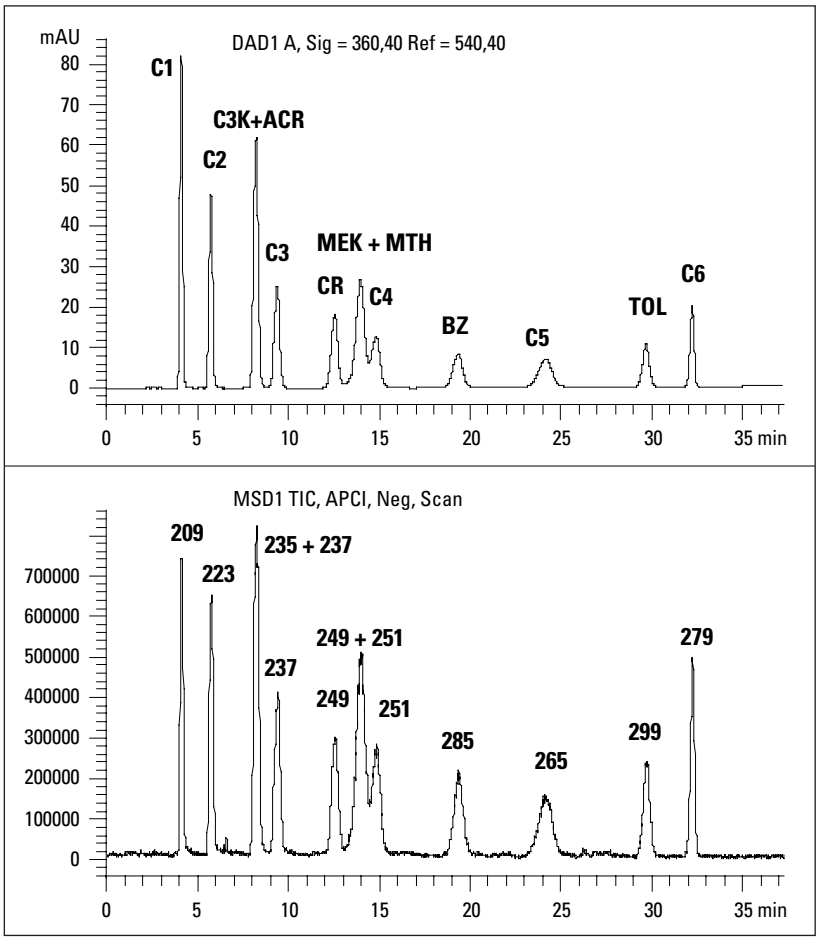

Figure 1. Liquid chromatography analysis of a mixture of the DNPH derivatives of 13 carbonyls by ultraviolet absorption at 360 nm (diode array detector, top) and by atmospheric pressure negative chemical ionization mass spectrometry (total ion current, bottom): C1, formaldehyde; C2, acetaldehyde; C3K, acetone; ACR, acrolein; C3, propanal; CR, crotonaldehyde; MEK, 2-butanone; MTH, methacrolein; C4, butanal; BZ, benzaldehyde; C5, pentanal; TOL, *m*-tolualdehyde; C6, hexanal.

Chromatographic Conditions

Column:	Nucleosil 100-5 C18 HD 5 µm, 3 × 250 mm
Guard column:	Phenomenex Security Guard C18, 3 mm i.d. × 4 mm
Mobile phase:	A = water B = acetonitrile
Gradient:	Start with 49% B at 26 min 49% B at 40 min 100% B
Post-time:	5 minutes
Flow rate:	1.0 ml/min
Column temp:	38°C
Injection vol:	20 µl
Diode-array detector:	Signal: 360, 40; 385, 40; 430, 40 nm Reference: 540, 40 nm

MS Conditions

Source:	APCI
Ionization mode:	Negative
Vcap:	1500 V
Corona current:	10 µA
Nebulizer:	60 psig
Drying gas flow:	4 l/min
Drying gas temp:	350°C
Vaporizer temp:	500°C
Scan:	125–600 amu,
Threshold:	150 counts
Gain:	5
Step size:	0.1 amu
Peak width:	0.1 min
Time filter:	On
Fragmentor:	50 V

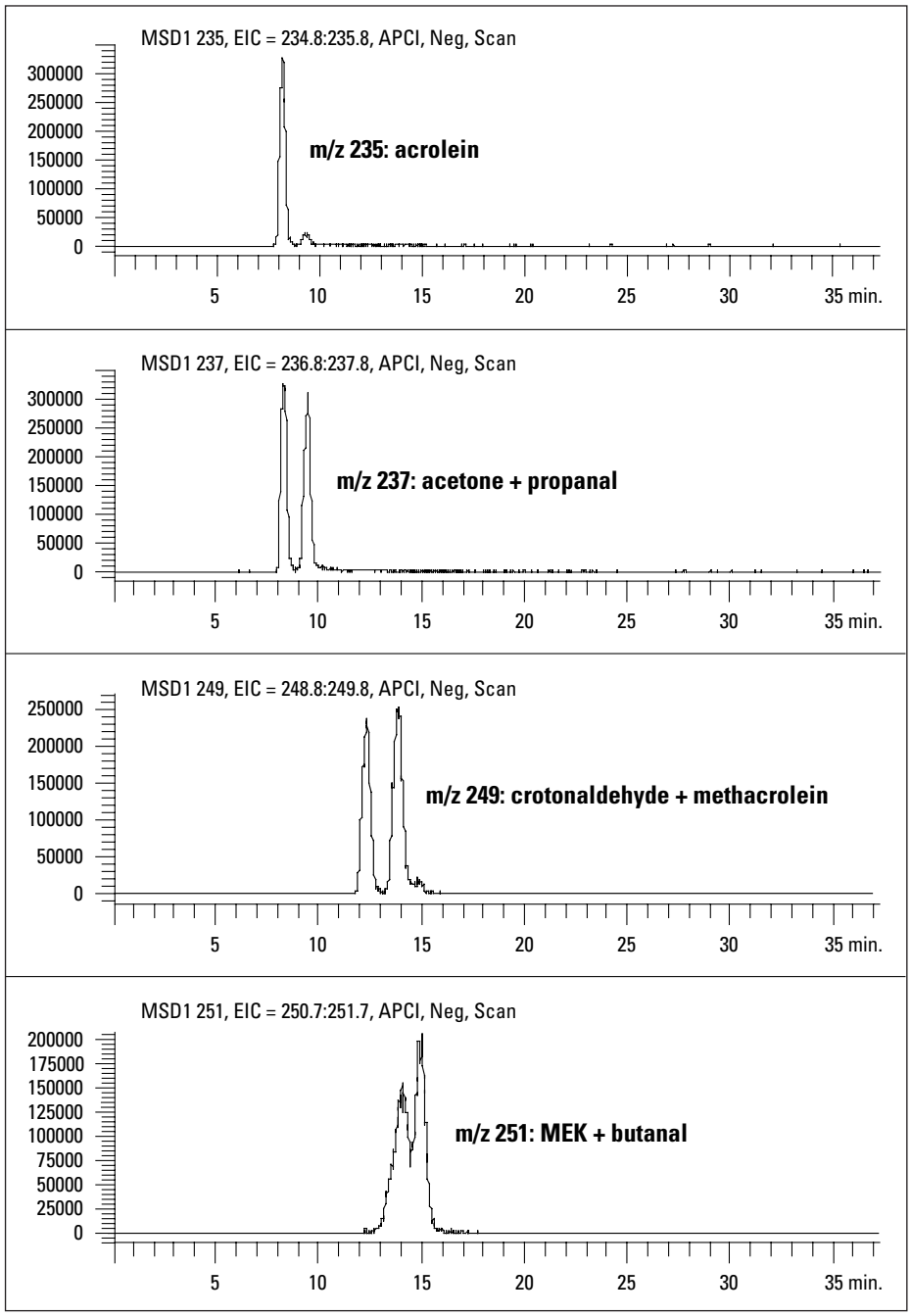

Figure 2. Extracted ion chromatograms for the region of Figure 1 containing acrolein (*m/z* 235), acetone and propanal (*m/z* 237), crotonaldehyde and methacrolein (*m/z* 249), and MEK (2-butanone) and butanal (*m/z* 251).

Figure 3 shows mass spectra of two carbonyl DNPHs from the data in Figure 1: formaldehyde DNPH and hexanal DNPH. Using conditions optimized for best detection of the $[M–H]^-$ ion, these spectra show little fragmentation even with the high vaporizer temperature (500°C) found to be optimal for the method.

Figure 4a shows the MS total ion chromatogram of a sample taken from a study of the reaction of the unsaturated ketone 4-hexen-3-one with ozone in a laboratory smog chamber. The LC/MS analysis allows the identification of unreacted 4-hexen-3-one, and of the carbonyl reaction products acetaldehyde, 2-oxobutanal, formaldehyde, glyoxal, and cyclohexanone (the latter a product of oxidation of cyclohexane, added to scavenge any OH radical which may form as a side product of the ozone-unsaturated ketone reaction). Figure 4b shows the mass spectra of the DNPH derivatives of cyclohexanone and of the dicarbonyl compound 2-oxobutanal. The spectra contain the ion *m/z* 182, which is characteristic of many carbonyl DNPHs and can be used to help locate and identify carbonyl DNPHs in complex mixtures.

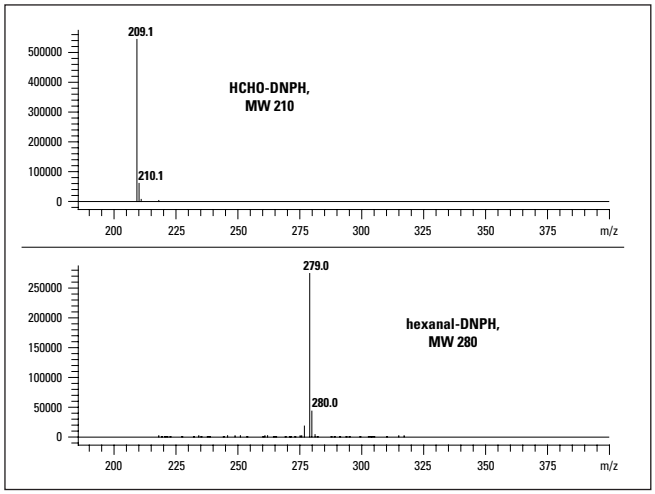

Figure 3. Atmospheric pressure negative chemical ionization mass spectra of analytes in Figure 1: (top) formaldehyde DNPH, (bottom) hexanal DNPH.

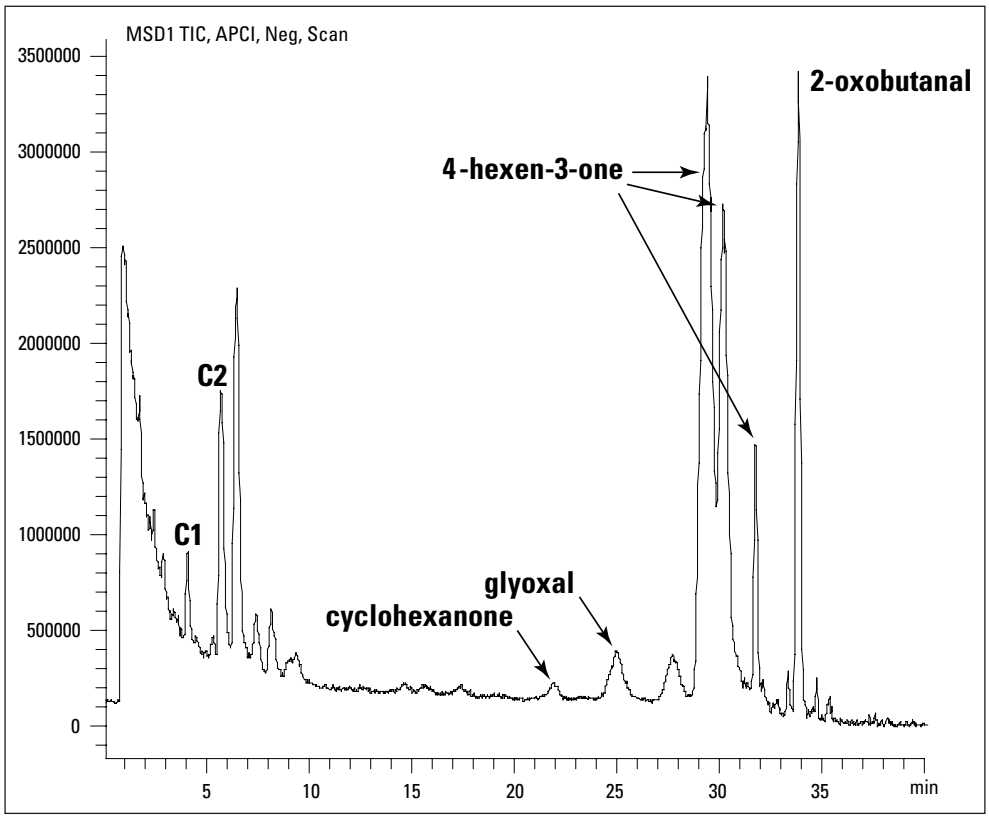

Figure 4a. Atmospheric pressure negative chemical ionization mass spectrometry analysis of the carbonyl products of the reaction of ppb levels of ozone with 4-hexen-3-one in the presence of cyclohexane: (a) total ion current chromatogram with DNPH derivatives of unreacted 4-hexen-3-one (three peaks due to syn/anti isomers of DNPH) and of the reaction products formaldehyde (C1), acetaldehyde (C2), cyclohexanone, glyoxal, and 2-oxobutanal.

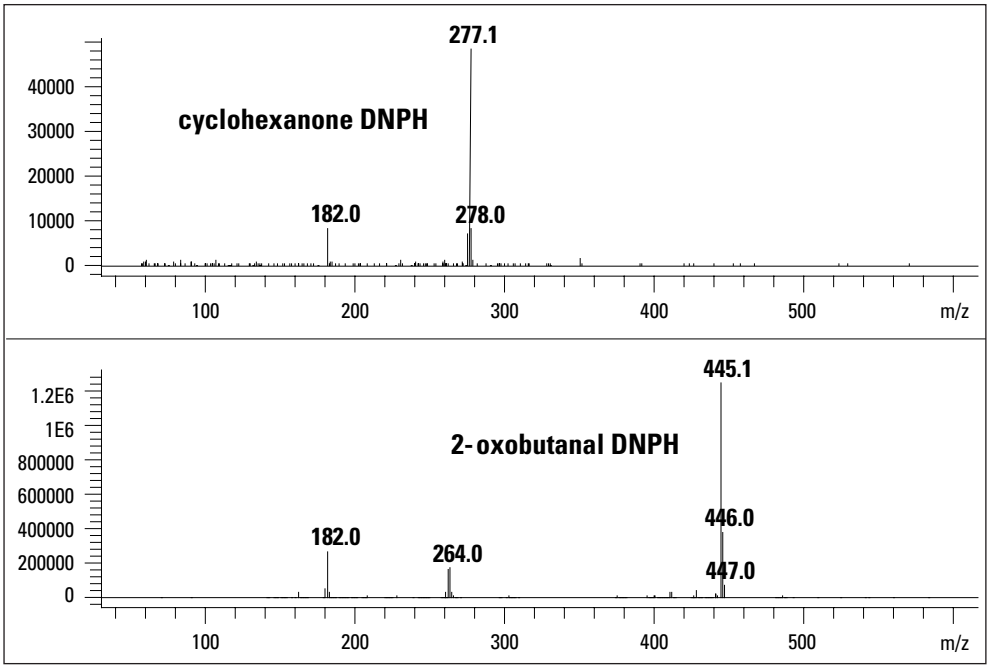

Figure 4b. Atmospheric pressure negative chemical ionization mass spectra of DNPHs of cyclohexanone and 2-oxobutanal.

Figure 5a shows the UV and MS chromatograms from the LC/MS analysis of an ambient air sample collected during early morning peak traffic in Porto Alegre, Brazil, where the mixture of vehicle fuels is unique in the world.[7] The MS data is shown using the base peak chromatogram (BPC), a very useful tool for helping to filter noise from the MS data. The BPC reconstructs an MS chromatogram using only the most intense ion (the base peak) from each spectrum, rather than adding up the abundances of all ions in each spectrum as does the total ion chromatogram (TIC).

Figure 5b shows an expanded view of the region of the UV and MS chromatograms, in which the C6 to C18 straight-chain alkanals elute. In Figure 5c, the extracted ion chromatograms for specific compounds show the distinctive masses of the $[M-H]^-$ ions, which confirm and/or identify the peaks detected with the UV detector.

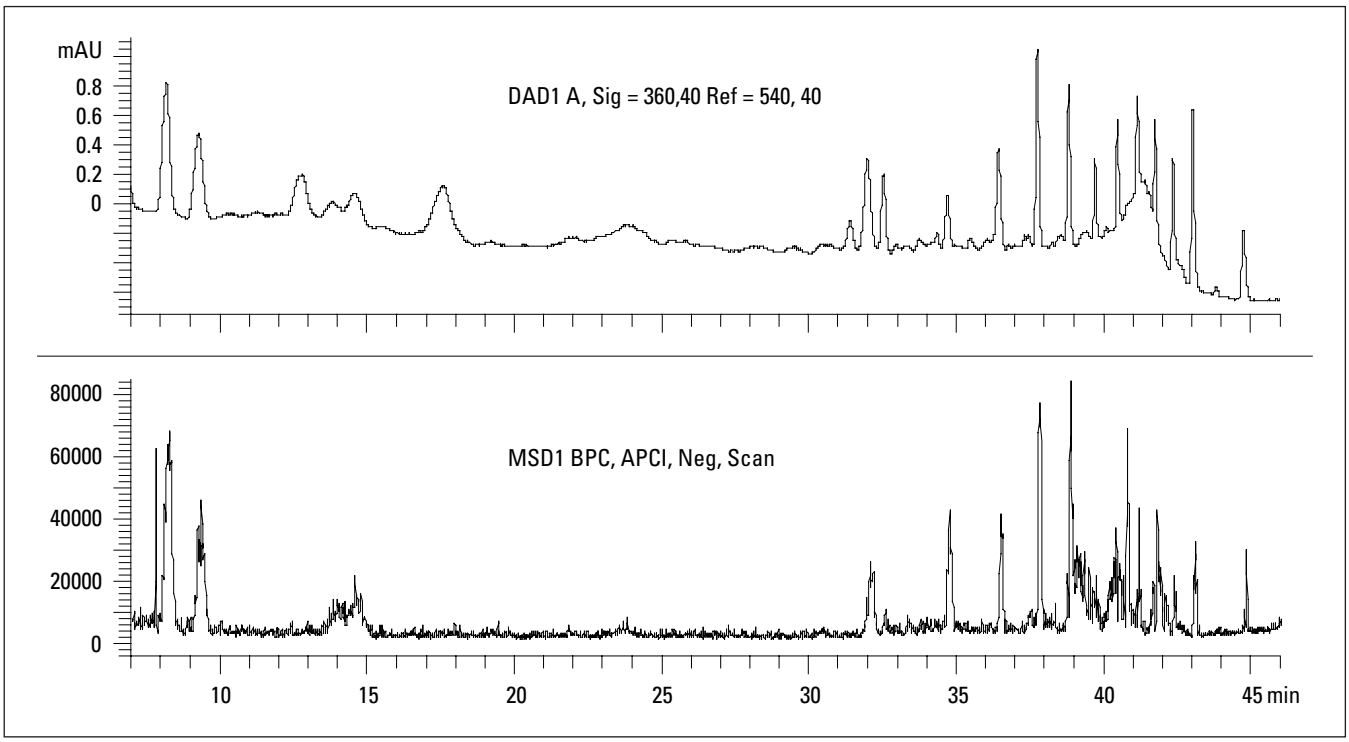

Figure 5a. LC/MS analysis of an ambient air sample collected in Porto Alegre, Brazil, during early morning peak traffic: (top) UV 360 nm chromatogram; (bottom) APCI negative ion base peak chromatogram (BPC).

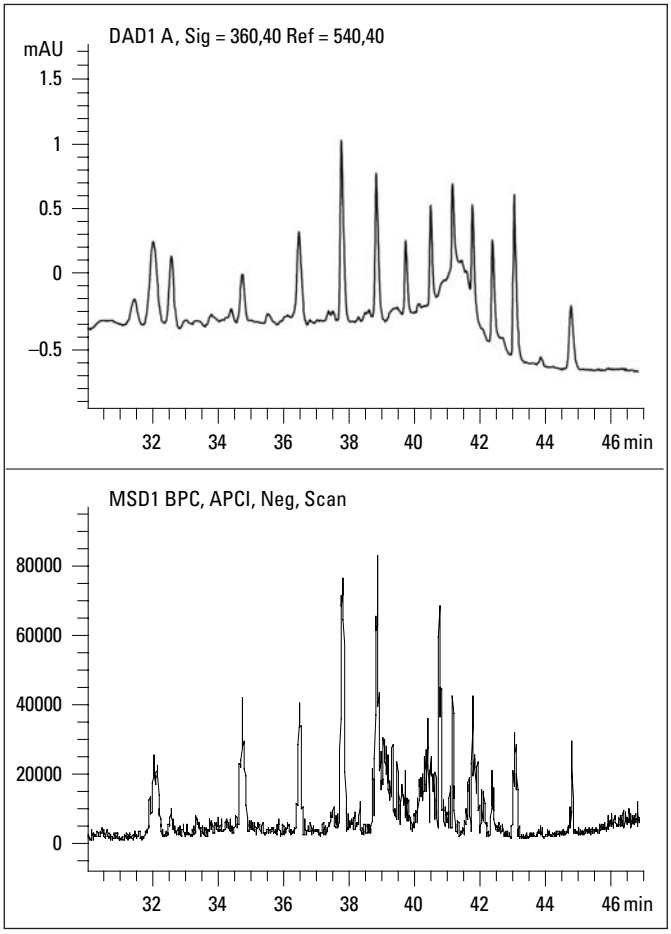

Figure 5b. Expanded region from C6 to C18 alkanals of the analysis in Figure 5b: (top) UV 360 nm chromatogram; (bottom) APCI negative ion base peak chromatogram (BPC).

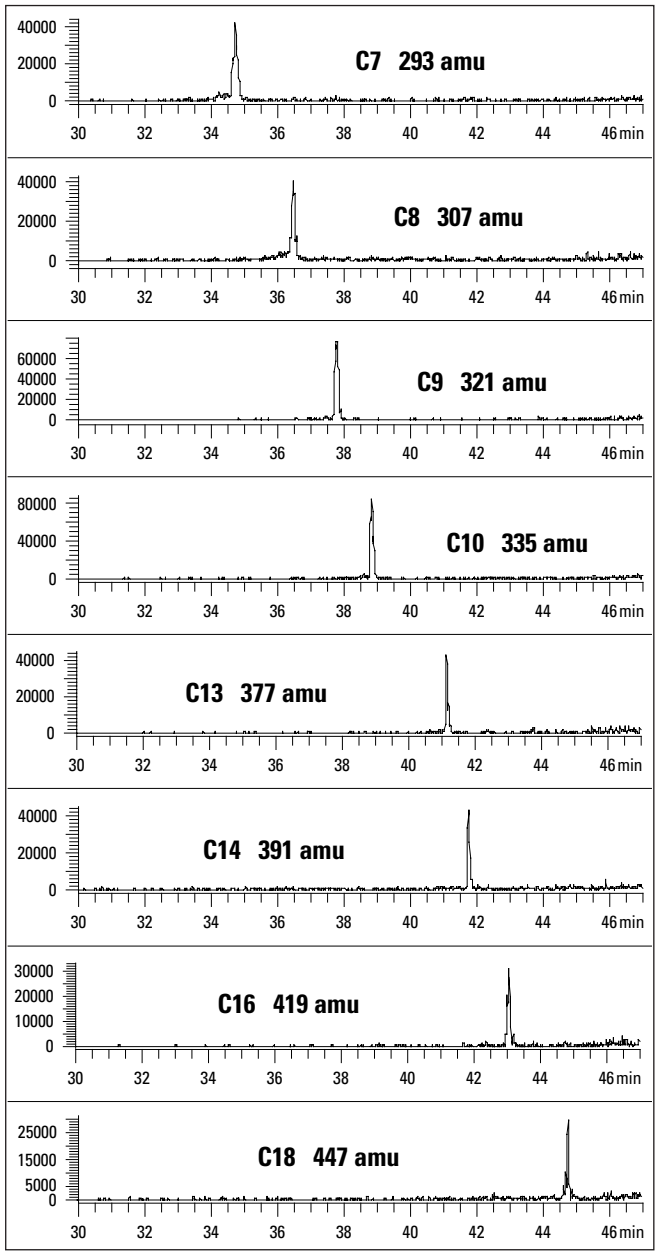

Figure 5c. Extracted ion chromatograms of the $[M–H]^-$ ion for 1-alkanal DNPH derivatives in Brazil air sample. Each EIC is labeled with the carbon number of the 1-alkanal DNPH derivative and the observed mass of the M–H ion.

Summary and Conclusions

This note describes the straightforward addition of API mass spectrometry to a well-established LC method for carbonyl analysis. The resulting APCI-LC/MS method is robust and sensitive, with application not only to simple aldehydes and ketones, but also to hydroxy carbonyls, dicarbonyls, carbonyl esters and keto acids as well. This development has improved a long-standing technique in environmental research, and is applicable to many other fields in which carbonyl-containing compounds are important but difficult to analyze with adequate selectivity, sensitivity, and/or confidence in identification.

Acknowledgments

The authors would like to thank ***Christine Miller*** of Agilent Technologies for review and helpful comments.

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Table 1. Summary of Data for the DNPH Derivatives of 1-Alkanals.

Carbonyl	Carbonyl-DNPH			
	RRT[a]	UVmax[b]	MW[c]	BP[de]
formaldehyde	1.00	355	210	209
acetaldehyde	1.40	364	224	223
propanal	2.30	365	238	237
butanal	3.65	366	252	251
pentanal	5.98	367	266	265
hexanal	7.92	366	280	279
heptanal	8.54	366	294	293
octanal	8.96	365	308	307
nonanal	9.28	363	322	321
decanal	9.52	362	336	335
undecanal	9.74	362	350	349
dodecanal	9.92	361	364	363
tridecanal	10.09	361	378	377
tetradecanal	10.24	361	392	391
pentadecanal	10.43	361	406	405
hexadecanal	10.62	361	420	419
heptadecanal	10.85	360	434	433
octadecanal	11.12	360	448	447

[a] RRT = retention time of carbonyl-DNPH relative to that of formaldehyde-DNPH (4.08 ± 0.02 min).

[b] UV max = wavelength of maximum absorption, nm, from 200–600 nm absorption spectrum recorded with diode array detector.

[c] MW = molecular weight of carbonyl-DNPH.

[d] BP = base peak (most abundant ion), *m/z,* in atmospheric pressure negative chemical ionization mass spectrum.

[e] No ions other than BP and ^{13}C contribution to BP (see text) were present in the spectra of the DNPH derivatives of 1-alkanals.

Table 2. Summary of Data for the DNPH Derivatives of Other Saturated Aliphatic Carbonyls.[a]

Carbonyl	Carbonyl-DNPH				
	RRT	UV max	MW	BP	Other Ions[b]
ALDEHYDES					
2-methylpropanal	3.69	363	252	251	none
3-methylbutanal	5.51	363	266	265	none
2-methylbutanal	5.70	363	266	265	263 (1)
2,2-dimethylpropanal	5.66	364	266	265	none
cyclohexylmethanal	8.09	366	292	291	none
KETONES					
acetone	2.01	368	238	237	none
acetone-d_6	1.98	367	244	243	237–242[c]
2-butanone	3.44	369	252	251	none
2-pentanone	5.51	371	266	265	none
3-pentanone	5.51	370	266	265	263 (2)
3-methyl-2-butanone	5.52	370	266	265	263 (2)
3,3-dimethyl-2-butanone	7.78	370	280	279	263 (1)
2,4-dimethyl-3-pentanone	8.27	370	294	293	277 (8)
cyclohexanone	5.36	373	278	277	275 (23)
2-methylcyclohexanone	7.94	371	292	291	289 (25)
nopinone[d]	8.30	372	318	317	315 (5)

[a] RRT, UV max, MW, and BP are defined in footnotes *a–d* of Table 1.

[b] *m/z;* Not including ^{13}C contribution to BP; see text. The percent abundance of the ion relative to that of BP is given in parentheses.

[c] Abundances relative to that of BP = 3% (*m/z* = 237), 4% (238), 6% (239), 13% (240), 24% (241), and 44% (242).

[d] 6,6-Dimethylbicyclo [3.1.1] heptan-2-one.

Table 3. Summary of Data for the DNPH Derivatives of Unsaturated Aliphatic Carbonyls.[a]

Carbonyl	Carbonyl-DNPH				
	RRT	UV max	MW	BP	Other Ions[b]
ALDEHYDES					
acrolein	2.01	380	236	235	none
crotonaldehyde[c]	3.08	382	250	249	none
methacrolein	3.44	381	250	249	none
2-ethylacrolein	5.55	379	264	263	none
trans-2-hexenal	7.60	382	278	277	275 (7)
2-methyl-2-pentenal	7.67	384	278	277	275 (3)
cis-4-heptenal	7.78[d]	365	292	291	289 (7)
	7.95[e] (2%)	366	292	291	289 (7)
trans-2-decenal	9.41	381	334	333	331 (6)
trans-2-undecenal	9.64	380	348	347	345 (5)
KETONES					
methyl vinyl ketone	2.68[e] (13%)	368	250	249	none
	2.87[e] (3%)	372	250	249	none
	3.06[d]	379	250	249	none
1-penten-3-one	4.76[d]	378	264	263	247 (8)
	4.99[e] (12%)	376	264	263	247 (9)
3-penten-2-one	4.43[e] (3%)	382	264	263	none
	4.83[d]	384	264	263	none
4-methyl-3-penten-2-one	6.94	386	278	277	263 (3)
4-hexen-3-one[c]	7.12[e] (4%)	385	278	277	none
	7.38[e] (35%)	357	278	277	none
	7.72[d]	357	278	277	none
6-methyl-5-hepten-2-one[f]	8.27	368	306	305	289 (4)
4-acetyl-1-methylcyclohexene	8.64	369	318	317	301 (4)

[a] RRT, UV max, MW, and BP are defined in footnotes *a*–*d* of Table 1.

[b] *m/z;* not including ^{13}C contribution to base peak; see text. The percent abundance of the ion relative to that of BP is given in parentheses.

[c] Predominantly the trans isomer.

[d] Largest peak.

[e] Smaller peak; percent of largest peak (peak height basis at 360 nm) given in parentheses.

[f] Two coeluting peaks.

Table 4. Summary of Data for the DNPH Derivatives of Aromatic Carbonyls.[a]

Carbonyl	Carbonyl-DNPH				
	RRT	UV max	MW	BP	Other Ions[b]
benzaldehyde	4.75	384	286	285	none
o-tolualdehyde	7.13	386	300	299	none
m-tolualdehyde	7.29	385	300	299	none
p-tolualdehyde	7.35	388	300	299	none
acetophenone	6.77	382	300	299	none
2,5-dimethylbenzaldehyde	8.15	389	314	313	none
2-hydroxybenzaldehyde (salicylaldehyde)	2.97	391	302	301	none
4-methoxybenzaldehyde (*p*-anisaldehyde)	4.98	398	316	315	none
3,4-dimethoxybenzaldehyde	3.07	398	346	345	none
4-hydroxy-3-methoxybenzaldehyde (vanillin)	1.75	402	332	331	329 (2), 315 (1)
4-hydroxy-3-methoxyacetophenone (acetovanillone)	2.37	393	346	345	343 (5), 329 (45), 313 (4), 298 (2)
3,5-dimethoxy-4-hydroxybenzaldehyde (syringaldehyde)	1.54	436	362	361	360 (40), 359 (1), 345 (1)
4-hydroxy-3-methoxycinnamaldehyde (coniferyl aldehyde)	2.67	415	358	357	356 (12), 355 (22), 325 (5), 310 (10)

[a] RRT, UV max, MW, and BP are defined in footnotes *a–d* of Table 1.

[b] *m/z;* not including ^{13}C contribution to BP; see text. The percent abundance of the ion relative to that of BP is given in parentheses.

Table 5. Summary of Data for the DNPH Derivatives of Dicarbonyls.[a]

Carbonyl	Carbonyl-DNPH					
	RRT	UV max	MW (mono)	MW (di)	BP	Other Ions[b]
glyoxal	6.09	415	238	418	417	237 (14), 238 (16)
methylglyoxal	7.90	432	252	432	431	251 (14), 249 (17)
2-oxobutanal[c]	8.31	410	266	446	445	263 (12)
2,3-butanedione	1.50[e] (1%)	362	266		265	none
	1.79[e] (2%)	369	266		265	none
	8.31[d]	403		446	445	265 (7), 263 (48)
succinic dialdehyde	0.81[e] (5%)	360	266		265	
	1.55[d f]	338[f]			247[f]	
	6.42[e] (12%)	368		446	445	263 (80)
glutaraldehyde	7.34	368	280	460	459	279 (10)
2,3-pentanedione	8.72	402	280	460	459	443 (8), 279 (15)
2,4-pentanedione	1.03	310	280	460	262	302 (14), 232 (6), 360 (7), 279 (0.1), 288 (5)
3,4-hexanedione	8.89	400	294	474	473	293 (5), 291 (12)
pinonaldehyde[g]	3.73[e] (9%)	368	348		347	none
	9.07[d]	368		528	527	345 (16)

[a] RRT, UV max, and BP are defined in footnotes *a–d* of Table 1. MW (mono) and MW (di) are the molecular weights of the mono-DNPH derivative and di-DNPH derivative, respectively.

[b] *m/z;* not including ^{13}C contribution to BP; see text. The percent abundance of the ion relative to that of BP is given in parentheses.

[c] Prepared by reaction of ozone with 1-penten-3-one, 2-ethylacrolein, and 4-hexen-3-one.

[d] Largest peak.

[e] Smaller peak; percent of largest peak (peak height basis at 360 nm) is given in parentheses.

[f] This compound is not the mono-DNPH derivative; see text.

[g] (2,2-Dimethyl-3-acetylcyclobutyl) ethanal, prepared by reaction of ozone with pinene.

Table 6. Summary of Data for the DNPH Derivatives of Other Carbonyls.[a]

Carbonyl	Carbonyl-DNPH				
	RRT	UV max	MW	BP	Other Ions[b]
methyl glyoxylate[c]	0.89[f] (60%)	355	268	267	none
	1.72[e]	357	268	267	none
ethyl glyoxylate	1.24[f] (65%)	356	282	281	none
	2.71[e]	359	282	281	none
2-oxoethyl acetate[d]	1.14[e]	360	282	281	249 (18)
	1.22[f] (13%)	356	282	281	249 (16)
methoxyacetone	1.59[e]	363	268	267	none
	2.25[f] (30%)	370	268	267	none
2-furaldehyde	2.14[e]	392	276	275	none
	3.00[f] (25%)	383	276	275	none

[a] RRT, UV max, MW, and BP are defined in footnotes *a–d* of Table 1.

[b] *m/z;* not including ^{13}C contribution to base peak; see text. The percent abundance of the ion relative to that of BP is given in parentheses.

[c] Prepared by reaction of ozone with methyl acrylate and with methyl *trans*-3-methoxyacrylate (MTMA).

[d] Prepared by reaction of ozone with MTMA and with *trans*-2-hexenyl acetate.

[e] Largest peak.

[f] Smaller peak; percent of largest peak (peak height basis at 360 nm) is given in parentheses.

**Printed in the U.S.A. January 2000
(23) 5968-8850E**

Measuring Hydrocarbon Oil Index according to ISO 9377-2 (DIN H53)

Environmental Application

Gas Chromatography

Bernhard Wüst

Abstract

Agilent Technologies developed this analytical method to comply with ISO 9377-2 and DIN H53 Standards. The method employs a simple instrument configuration consisting of a Gas Chromatograph (GC) equipped with a Split/Splitless (S/SL) inlet and Flame Ionisation Detector (FID), making the method both easy to implement and robust to operate. The combination of an Agilent GC system (see configuration above), selected capillary GC column, proprietary injection port liner, appropriate chemical standards as well as method installation and support by an Agilent Applications Specialist, provide a *complete solution* for the measurement of the Hydrocarbon Oil Index.

Introduction

The Hydrocarbon Oil Index (HOI) is defined as the total amount of compounds which can be extracted from the sample (potable water, surface water and waste water) with a non-polar solvent having a boiling point between 39°C and 69°C (replacing the use of halogenated solvents). In addition, the compounds must not absorb on Florisil and must elute between n-decane ($C_{10}H_{24}$) and n-tetracontane ($C_{40}H_{82}$) when analysed by GC using an apolar analytical column. Restrictions on the use of halogenated hydrocarbons as solvents for analytical applications combined with the increasing cost of and reduced availability of technical grade halogenated solvents as well as documented environmental considerations resulted in the change to a non-polar solvent for HOI determinations.

This analytical method complies with the ISO Standard 9377-2 for the determination of the Hydrocarbon Oil Index by Gas Chromatography. The method is suitable for HOI determinations in concentrations above 0.1 mg/L in drinking waters, surface waters, waste waters and waters from sewage treatment plants.

Agilent Technologies
Innovating the HP Way

Experimental

The Agilent 6890 and Agilent 6850 Gas Chromatographs are well suited to the HOI application. Both instruments use the same Split/Splitless inlet system and identical Flame Ionisation Detector. Discrimination effects on higher boiling compounds are the most critical aspect of the analysis and are prevented by the use of a special injection port liner which, in combination with the Agilent S/SL inlet system, produces robust and reliable data. Additional benefits such as reduced maintenance time and faster analytical cycle times are also available by using a S/SL inlet as compared to a Cool-on-Column inlet.

The Agilent GC ChemStation standard software is able to subtract background signals to produce a chromatogram with any column bleed removed. In addition, both the Agilent 6890 and 6850 are able to save column compensation signals in memory and subsequently subtract the column background from the sample data and provide a background-subtracted signal to the ChemStation or data integration system. Standard ChemStation software tools are used to control the GC and perform the data analysis required of the sample data. A custom report template is included with the HOI method and generates a report conforming to the ISO Standard (see figure 3).

Instrument Parameters

Oven temperature profile	Isothermal 35°C (1.5 min), ramp 5°C/min to 60°C hold 0 min, ramp 15°C/min to 350°C hold 5 min.
Inlet (S/SL)	375°C Splitless Mode
Detector (FID)	375°C
Analytical Column	Agilent Part Number 19095Z-221E (HP1, 15m x 0.53mm x 0,15 µm)
Inlet Liner	Agilent Part Number 5183-4647
Carrier Gas	Helium
Carrier Gas Flow Rate	7.4 ml/min Constant Flow Mode
Injection Mode	Automated (Agilent 7683) Fast Injection
Injection Volume	1.0 µl

Calibration Curve

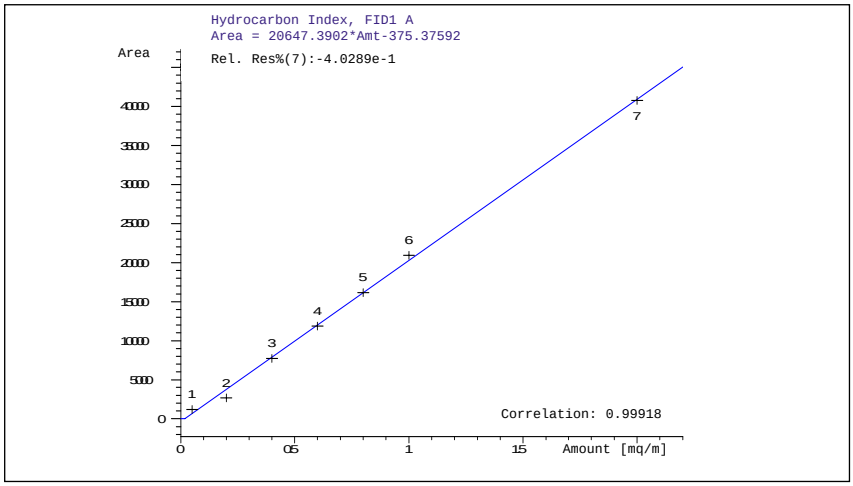


Figure 1: Oil Standards – Calibration Curve

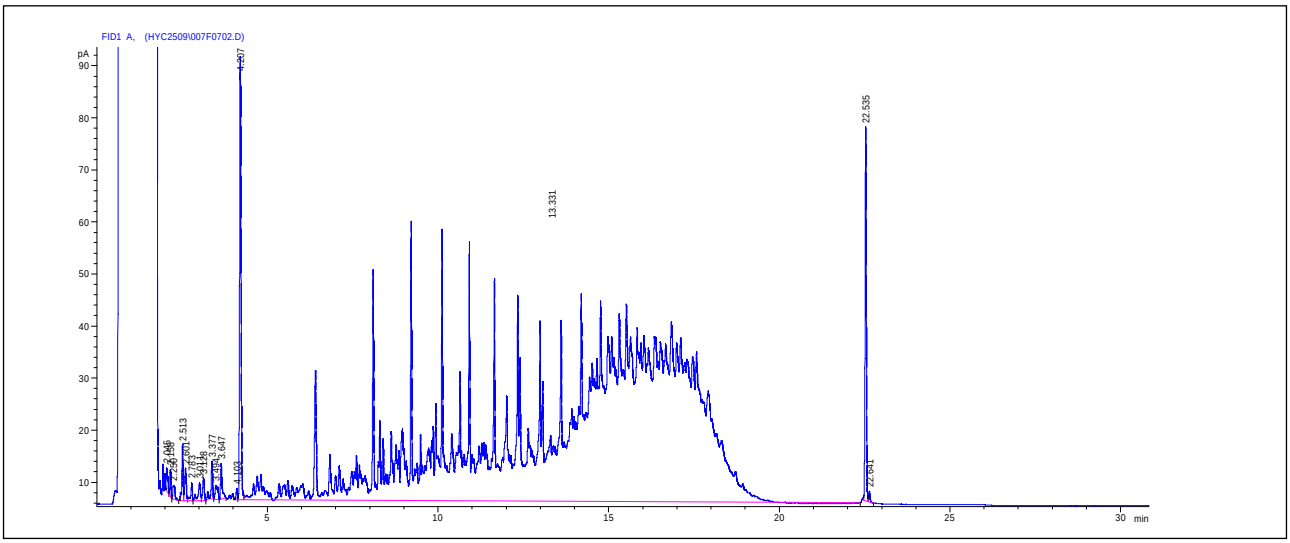


Figure 2: 0.6 mg/ml Oil Standard

Discrimination Test:

25 sequential injections of a standard containing tetracontane C-40 and decane C-10 were run to test for discrimination effects within the analytical system. The data obtained is presented in Figure 3 and demonstrates the robustness of the method and that the analytical system clearly passes the discrimination test specified in the ISO Standard.

Results and Dicussion

The Agilent 6890 and 6850 Gas Chromatographs equipped with a Split/Splitless inlet and Flame Ionisation Detector can be used for Hydrocarbon Oil Index determinations in accordance with ISO 9377-2 (DIN H53) standard. In addition, the analytical system is easy to operate and requires minimal maintenance. Together, the Agilent GC, analytical capillary column, special inlet liner, custom report template, selected chemical standards and Agilent applications expertise provide a complete analytical solution for Hydrocarbon Oil Index determinations.

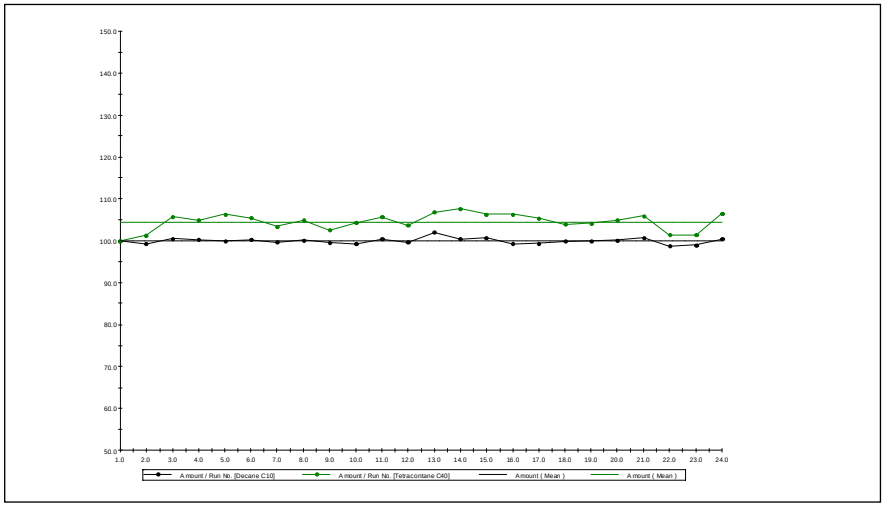
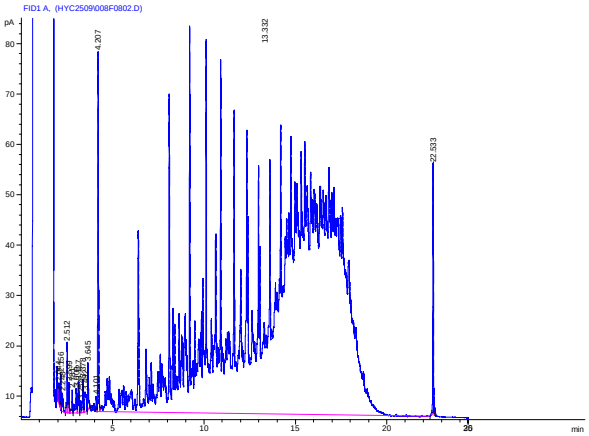


Figure 3: Discrimination Test

```
Data file   : C:\HPCHEM\1\DATA\HYC2509\008F0802.D
Sample Name : 1000 mg/l                                          1
================================================================
Injection Date    : 9/26/2000 3:23:50 AM       Seq Line : 8
Sample Name       : 1.0 mg/ml                  Vial No. : 8
Acq Operator      : berwuest                   Inj. No. : 2

                                               Inj. Vol. : -
Acq.Method        : HYCA04.M
Analysis Method   : C:\HPCHEM\1\METHODS\ISO93774.M
Last Changed      : Fri, 29. Sep. 2000, 10:04:10 am

Analysis of Hydrocarbon Index according to Iso 9377-4 and
DIN H53
```




```
================================================================================
                              Hydrocarbon Index Report
================================================================================
Calib. Data Modified      : Fri, 29. Sep. 2000,08:25:56 am
Multiplier                : 1.000000

        Hydrocarbon Index    1.052 mg/ml
        Low Boilers          9.061 mg/l
        High Boilers         not detectable  mg/l

================================================================================
                              Detailed Report
================================================================================
Name              StartTime EndTime Ret Time Total Area  Res.F    Amount
--------------------------------------------------------------------------------
Low Boilers          2.00    4.00     -        239.48    0.03784  9.06
Decane C10            -       -       4.21    213.73    0.03784  8.09
Hydrocarbon Index    4.31   22.37    13.33  15408.08    0.00006  1.05
Tetracontane C40      -       -      22.53    205.67    0.03247  7.94
High Boilers          -       -       -        -          -      n.d.
================================================================================
```

Figure 4: Report Printout

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Germany

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Publication Number 5988-0621EN

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application

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An approach to the determination of N-nitrosodimethylamine at part-per-quadrillion levels using Positive Chemical Ionization and Large-Volume Injection

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Introduction

N-nitrosodimethylamine (NDMA, Figure 1) is one of a series of nitroso compounds known to be carcinogenic. NDMA is found in nitrate-cured or smoked meats,[1] cheeses,[2] tobacco smoke,[3] cooked foods and in beverages such as beer[4] (both foreign and domestic[5–7]). The presence of NDMA in surface waters designated for use drinking water use is of particular concern and the U.S. Environmental Protection Agency (EPA) has promulgated a regulatory standard for these waters of 0.7 ng/l (700 ppq). When in 1998 NDMA was detected in California drinking water, the source was associated with the production and use of a rocket fuel component, unsymmetrical dimethylhydrazine. In response, the California Department of Health Services (DHS) announced an action level in drinking water of 2 ng/l (2 ppt). However, the best available methods in the literature provide *detection* limits on the order of 1–3 ng/l. EPA methods 625 and 1625 specify a detection limit for NDMA of 50 ppb—25,000 times the California DHS action level and 70,000 times the EPA regulatory standard. It follows that using existing methodologies, *any* detection of NDMA represents a violation.

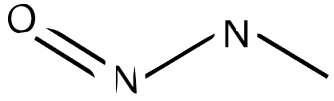

Figure 1. N-nitrosodimethylamine, $(CH_3)_2N_2O$, 74 g/mole, CAS Registry No. 62-75-9

Determining NDMA at ppt or ppq concentrations in water is an analytical challenge. The extraction methods that have been applied, such as liquid-liquid or solid-phase extraction,[8–10] produce concentration factors of 500 to 1000, but overall recoveries are generally low. The high polarity and volatility of NDMA contribute to lowered recoveries and extensive extract concentration by evaporation can lead to high losses.

To increase sensitivity and specificity, one prevalent detection scheme involves use of the chemiluminescent nitrogen detector. Electron impact mass spectrometry has also been used but the fragmentation pattern is not very favorable (Figure 2). While the molecular ion at 74 m/z may be a reliable quantitation ion, the confirming ions at 42 and 43 m/z are hardly unique and are easily compromised by fragments from interferences.

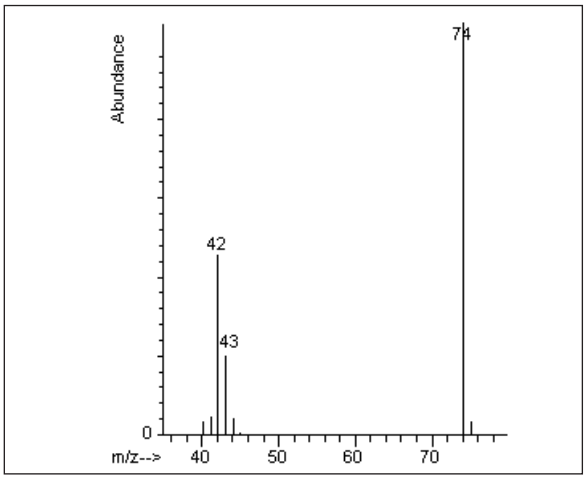

Figure 2. Electron impact ionization mass spectrum of NDMA

One approach to overcoming the unfavorable electron impact (EI) ionization mass spectrum of NDMA is to apply positive chemical ionization (PCI). PCI can provide enhanced analyte selectivity and sensitivity. Utilizing large-volume injection (LVI) should lower the concentration of NDMA that can be detected in an extracted sample. This note describes the combined application of these two techniques as a possible approach to determining NDMA at ppt and ppq concentrations.

Experimental

NDMA standards were made by serial dilution in 1-ml of dichloromethane from a 100 ng/µl standard (Ultra Scientific, Kingstown, RI; part number NS-100). Dichloromethane was chosen as the solvent, because this solvent is used in both liquid-liquid and solid-phase extraction techniques.

Instrumental Section

The 6980 Plus GC / 5973 MSD with chemical ionization option was operated in the selected-ion-monitoring mode (SIM) with ammonia reagent gas. An HP-210 GC column 50%-trifluoropropyl-50%-methyl-siloxane (30-m, .25 mm i.d., 0.5 µm film thickness, Part Number 19091C-733) was used with a 5-m, 0.32 mm i.d. uncoated retention gap (Part Number 19091-60600) joined by a press-fit connector (Part Number 5062-3555). A 100-µl syringe was used in the integrated automated liquid sampler 7683 injector for the 50-µl injections. GC oven conditions and mass selective detector settings are given in Tables 1 and 2.

Table 1. GC and Injector parameters

Oven Temperature Program	Temp	Time
Initial Temperature	45°C	3.00 min
Ramp 50°C / min	180°C	0.50 min
GC Oven Equilibrium Time		3.00 min
MSD Transfer Line		225°C
Inlet Mode		Split
Split Flow		50 ml / min
Gas Saver		Off
Column Flow (Helium carrier gas)		2.0 ml / min
Mode		Constant Flow
Outlet Pressure		Vacuum
Injection Volume		50 µl
Syringe Size		100 µl
Plunger Speed		Slow
Solvent Washes A, B	Methanol*	Dichloromethane

* A solvent that "wets" the glass bore improves syringe life.

Table 2. MSD parameters

Tune File *	PCINH3.U
Ammonia Reagent Gas Flow	10 %
EM Voltage	PCI CH_4 AutoTune + 400V
MS Quadrupole Temp	106°C
MS Source Temp	250°C
Acquisition Mode	SIM
Solvent Delay	5.25 min
SIM Ions	Dwell
75.1 amu	80 msec
92.1 amu	80 msec

* PCI Autotune parameters were used for these experiments. Autotune provides high sensitivity over a large mass range, but even greater sensitivity for these low molecular weight ions can be achieved by manual adjustment of the tuning parameters.

Large-Volume Injections

The APEX ProSep™ 800 Series XT Plus Preseparation System Inlet (APEX Technologies, Cincinnati, OH) was used as the inlet for large-volume injections.[11, 12] Injections were made into a fused-silica preseparation column packed with deactivated fused-silica wool in the top 3 to 7 cm of the column (available from APEX). The ProSep Precolumn Temperature Module and Flow Module parameters that were successful for this particular preseparation column are given in Tables 3 and 4. This is a very flexible device, and the parameters given can be further optimized to provide better performance for particular extracted matrices. For example, a higher final precolumn temperature than 180°C can be applied to remove high-boiling contaminants.

Table 3. ProSep Precolumn Temperature Program

	Target	Duration
Initial	45°C	0.05 min
250°C / min	180°C #	6.00 min

Higher bake-out temperatures are recommended for extracted samples.

Table 4. ProSep Precolumn Mode Program

	Mode	Duration
Initial	Split	0.05 min
1	Splitless	0.07 min*
2	GC Split **	2.50 min*

*These times should be appropriately optimized.

** It is recommended that ProSep Split be implemented instead of simply GC Split due to superior venting.

Results

The application of PCI with ammonia reagent gas to NMDA produces a simplified mass spectrum consisting only of protonated NDMA, $[NDMA+H]^+$, and the ammonium adduct, $[NDMA+NH_4]^+$, which correspond to 75 m/z and 92 m/z, respectively. PCI provides a three-fold advantage over the EI approach. First, the relatively non-unique 74, 43, 42 m/z ions of the EI have been replaced by higher-mass ions. Second, PCI provides increased sensitivity for NDMA and a reduction in low-mass, "background" ions which enhances the signal-to-noise ratio. Third, by manipulating the ammonia gas flow, the abundances of the 92 m/z and 75 m/z ions can be controlled. As the ammonia flow into the source is increased, the abundance of the $[NDMA+NH_4]^+$ adduct also increases, allowing the ratio of 92 m/z to 75 m/z to be controlled by the analyst. For example, at 0.4 ml/min of ammonia—a relatively low flow setting of the reagent gas mass flow controller (8% of the total 5-ml/min provided by the controller)—the ratio of the protonated form to adduct is biased toward the protonated form: $[NDMA+H]^+$ **:** $[NDMA+NH_4]^+ = 4$ **:** 3. At higher flows, the situation reverses and $[NDMA+NH_4]^+$ predominates, e.g., at 0.9 ml/min ammonia (18% flow setting) $[NDMA+H]^+$ **:** $[NDMA+NH_4]^+ = 1$ **:** 5. It is therefore possible to produce an intense confirming ion for quantitative applications. A good compromise between signal intensities and ion abundancies was achieved at a 0.5 ml/min ammonia flow setting. Figure 3 shows the 75 m/z and 92 m/z SIM signals for a 40-fg/µL standard for this flow. Under these conditions, $[NDMA+H]^+$ is 79% of $[NDMA+NH_4]^+$ according to the integrated signal areas.

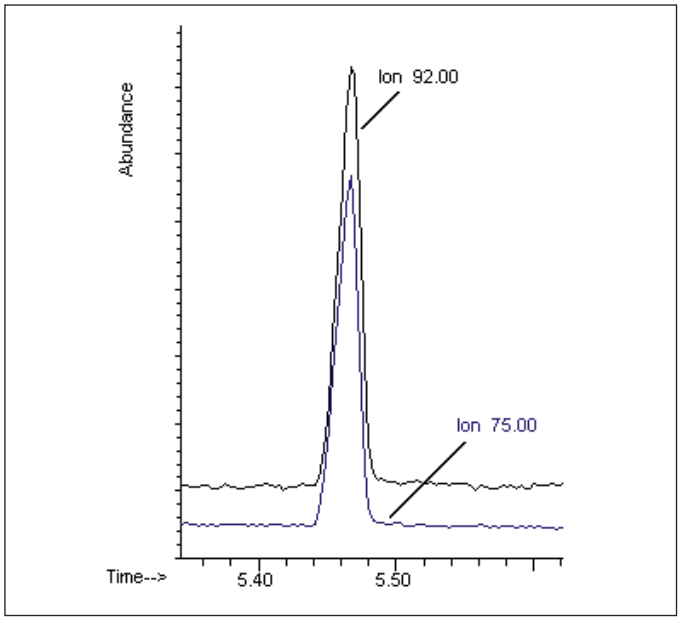

Figure 3. Extracted ion chromatogram for NDMA at 40-fg/µl using PCI-SIM with NH_3 reagent gas.

Figure 4 shows the results of a linear regression of the response of the 92 m/z ion for 50-µl injections of NDMA standards from 20-fg/µl to 4000-fg/µl. The regression fit was very good, $r^2 = 0.999$, considering the propagation of error in the dilutions. The relative standard deviation in the response factors was less than 6% and could be improved by using a perdeuterated or ^{15}N-labeled NDMA surrogate.

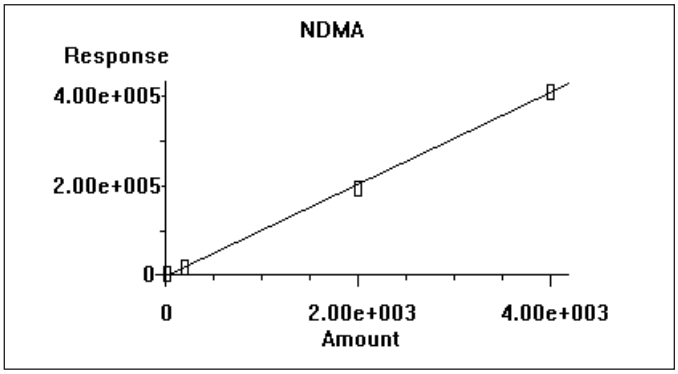

Figure 4. Linear regression of response of the 92 m/z ion versus NDMA concentration from 20-fg/µl to 4000 fg/µl, $r^2 = 0.999$.

Table 5 shows the excellent degree of reproducibility in the ratio of 75 m/z confirming ion to 92 m/z target ion over a wide range of concentrations. The absolute value of the ratio was 0.79, with a relative standard deviation of < 3%. This high precision is important to the degree of confidence in confirming and quantitating NDMA.

Table 5. Reproducibilities of the ratio of the integrated areas of 75 m/z : 92 m/z and the response of the 92 m/z target ion for 5 injections at 5 concentrations.

Concentration as fg NDMA / µl	RSD Ratio 75 mz / 92 m/z	RSD Response by 92 m/z area
20	2.9%	2.4%
40	2.2%	3.2%
200	0.7%	0.8%
2000	0.7%	1.7%
4000	0.3%	0.9%

Table 5 also shows the excellent reproducibility of the response of the 92 m/z ion for replicate 50-µl injections. Even at the 20-fg/µl concentration, precision is better than 3%.

Conclusions

Concentration and recovery factors for NDMA using present published methodologies suggest effective preconcentration of NDMA in samples to be on the order of 500, e.g., 60–70% recovery of NDMA in extraction of a 1-liter water sample. This implies that the low 20-fg/µl NDMA standard corresponds to a sample concentration of 40 pg/l, or 40 parts-per-quadrillion. Alternatively, to quantitate NDMA at 0.5 ppt in water, which is 4 times lower than the California DHS limit and slightly lower than the EPA regulated limit, quantitating at 20 fg/µl is equivalent to requiring the extraction of *only* 80 ml of water even if recoveries are still only 50%. Extracting small volumes presents a significant simplification of the process and offers savings in solvent and related materials, and in processing time.

With NDMA eluting in about 5 1/2 minutes, the analysis is fast, and the run-to-run cycle time is short—less than 13 minutes between injections. The method may be further optimized for even more rapid analysis.

The 5973 MSD provides very stable ratios for the confirming ion that can be optimized for quantitative purposes as described. In contrast to EI, in which many possible interfering fragment ions are possible that may distort the ratio of the target and confirming ion(s), PCI with ammonia is unlikely to cause fragmentation-induced interferences because of the relatively "gentle" nature of ammonia reagent gas. Interferences could occur involving compounds with molecular weights of 74 or 91 g/mole eluting at the same retention time but that is unlikely scenario.

The high degree of reproducibility in the injections, even at very low NDMA concentrations, demonstrates the robustness of large-volume injections using the APEX ProSep with the 6890/5973 MSD. It should be emphasized that the reproducibility of 2.4% for the replicate 50-µl injections of the 20-fg/µl standard reported here was for the *absolute* response. Use of an internal standard should further lower the deviation in response and improve quantitation.

Using this approach it should now be possible to satisfy the 2 ppt action level for NDMA set by the State of California and the 700 ppq regulatory standard promulgated by the U.S. EPA.

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(references continued . . .)

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Acknowledgments

Harry Prest is very grateful to Dr. William Steeber of the California Department of Health Services, Los Angeles, CA and John M. Hughes of the Gas Phase Chemists Council for their review of the manuscript.

Printed in the U.S.A. September 1999
(23) 5968-7799E

Revision 1.0 - December 1999

Improved Data Quality in the Automated HPLC Analysis of PNAs (PAHs)

Application Note

Enviromental

Angelika Gratzfeld-Hüsgen
Rainer Schuster

This note describes an improved method for analyzing polycyclic aromatic hydrocarbons (PNAs) by HPLC. During the investigations the major focus was on improving precision of retention times and peak areas by using vacuum degassing and stable, low ambient temperatures. Thorough degassing prevented quenching effects in fluorescence detection and improved retention-time stability. Thermostatting the column temperature slightly below ambient temperature improved resolution of critical peak pairs. With this method the retention-time stability was within 0.05 – 0.2 % RSD. The RSD for the area in the low ng range was in general below 2 %. An improved diode-array detection system allowed PNAs to be detected at ppb levels and their identity at less than 1 mAUFS confirmed automatically by UV-Visible spectral library search.

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Introduction

Hydrocarbons with multiple-ring structures are collectively referred to as polynuclear aromatic hydrocarbons, commonly abbreviated as PNAs or PAHs (figure 1).

This class of compounds are suspected to be carcinogenic or mutagenic. This has lead to legislative restrictions on their release into the environment. They are mainly formed due to incomplete combustion of organic material, such as fossil fuels.

Analysis by HPLC with UV-Visible diode-array and/or fluorescence detection has become a well-established method for the determination of PNAs in soil, water, sludge air and food. However, despite the common use of this method, degassing techniques and temperature stability still cause problems with qualitative and quantitative evaluation.

The best separation of PNAs is achieved at or slightly below ambient temperatures. Without column cooling this often results in unstable retention times caused by ambient temperature fluctuation. To get optimum sensitivity in fluorescence detection, excitation and emission wavelength switching is required. This implicates not only good resolution of the compounds but also stable retention times.

Traces of oxygen in the mobile phase deteriorate fluorescence sensitivity because of quenching effects. Helium degassing and other conventional techniques however will not give highest sensitivity compared to the vacuum system used in this study.

For samples with higher contamination levels a UV-Visible diode-array detector was used to get spectral information for identification and peak-purity control. A major improvement in the sensitivity of this detector allowed spectra to be taken at ppb levels with identification at less than 1 mAUFS.

Current evaluation software for diode-array detectors focuses on interactive spectral evaluation. This is very time consuming, requires sophisticated operators and is impractical for routine analysis.

Traditionally standard columns of about 4 mm id are used for PNA analysis, requiring high solvent amounts with high purchase and disposal costs. Although narrow-bore columns with 2 mm id are available, ideally suited for use at lower flow rates and give higher mass sensitivity they are still not widely used. Further, much of the currently-available routine HPLC equipment does not give reliable results for gradient operation at low flow rates.

In this study we investigated the Agilent 1100 Series HPLC system to determine to what extent this new system could solve some or all of the problems described.

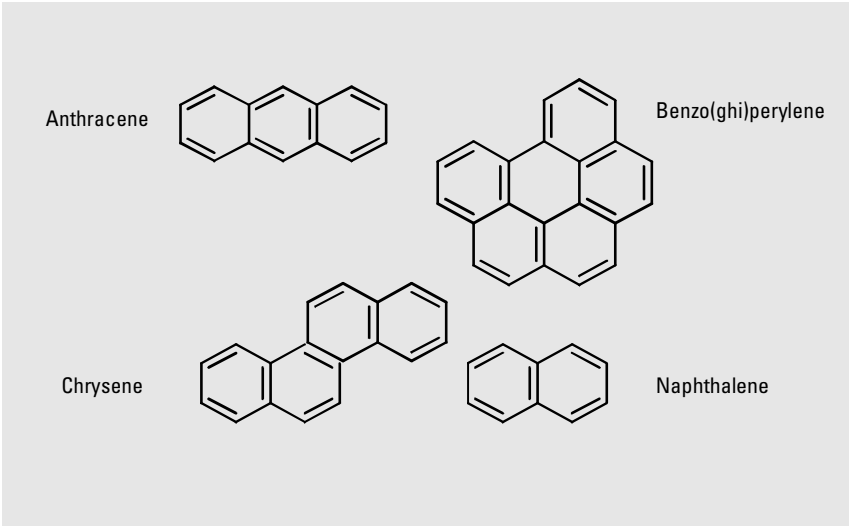


Figure 1
Examples of PNAs (PAHs)

Experimental

The system comprised an Agilent 1100 Series quaternary gradient pump, vacuum degasser, autosampler, thermostatted column compartment, diode-array detector and HP 1046A fluorescence detector. Complete system control and data evaluation was done on the Agilent ChemStation for HPLC. The thermostatted column compar-tment included a Peltier element for precise temperatures above, below and at ambient temperature.

For the separation we used a dedicated PNAs column that allowed the separation of PNA isomers. For economic reasons we used a column with an internal diameter of 2.1 nm that allowed a solvent flow of 0.4 ml/min. For evaluation of separation we used the 16-component EPA standard spiked with some other components that may be present in typical environmental samples and that may chromatographically interfere with the compounds of interest.

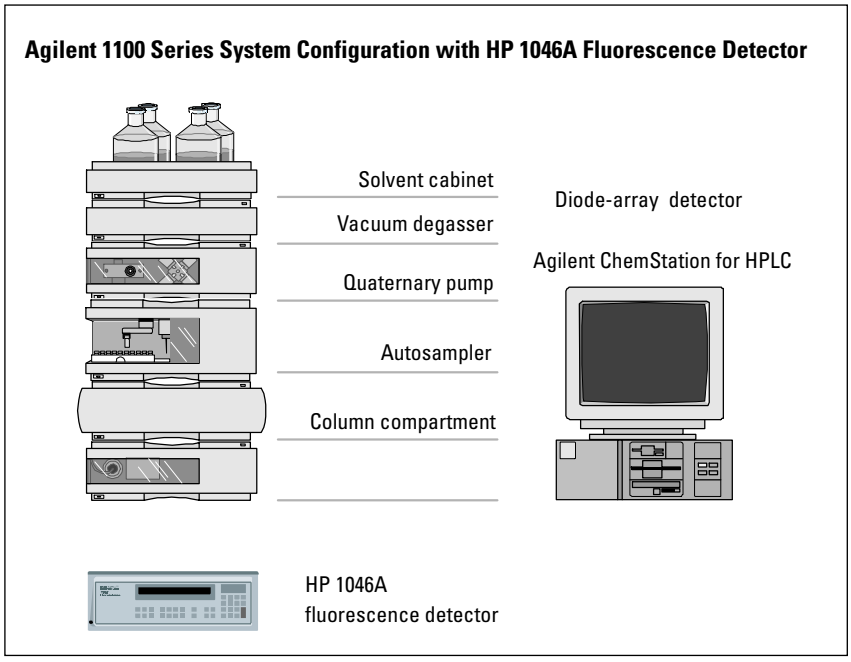


Figure 2
Schematic of instrumentation used

Column	250 _ 2.1 mm PAH column, 5 mm (Agilent part no. 79918PAH-582)
	Buffer A water
	Buffer B acetonitrile
Temperature	18 °C, 25 °C, 30 °C, see figure
Flow rate	0.4 ml/min
Gradient	50 % B to 60 % in 3 min
	to 90 % in 14.5 min
	to 95 % in 22.5 min
Detector	Sample wavelength 270 nm, bandwidth 40 nm
Samples	See table 1

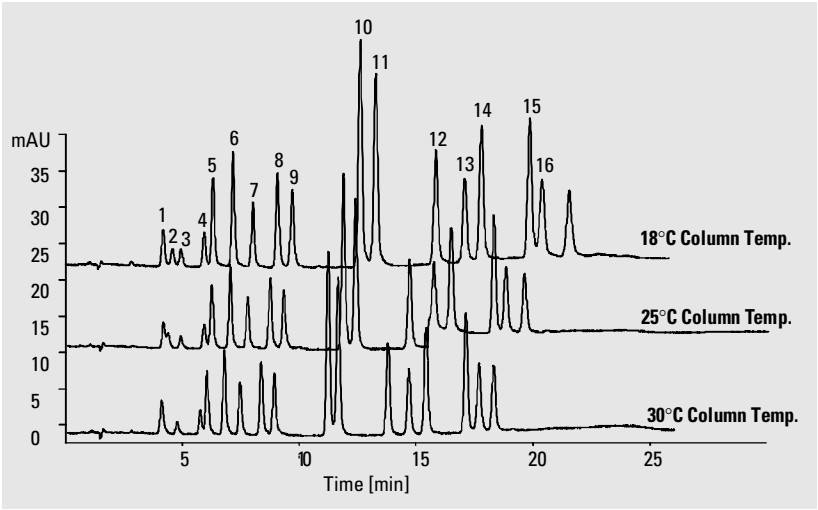


Figure 3
Separation of DIN/EPA standards at different column temperatures

Results

Impact of column temperature on separation

We investigated the impact of column temperature on the separation at three different temperatures: 30, 25 and 18 °C. The 16 EPA compounds could be separated at all temperatures (see figure 3), however a temperature of 18 °C had several advantages:

- The resolution between critical compound pairs such as benzo(ghi)perylene and indeno(1,2,3-cd)pyrene was better. This allowed trouble-free switching of excitation and emission wavelengths when using a programmable fluorescence detector.

- Additional interfering compounds such as anthrachinon could be separated from the PNAs. Figure 3 clearly shows the improved separation of PNAs far below 30 °C.

Peak #	Name of compound	RSD t_R (15 runs)	RSD area (15 runs)
1	Naphthalene	0.12%	1.41
2	Anthrachinon	0.05%	3.70
3	Acenaphthylene	0.10%	3.51
4	Acenaphthene	0.09%	3.71
5	Fluorene	0.08%	1.76
6	Phenanthrene	0.06%	1.72
7	Anthracene	0.05%	1.42
8	Fluoranthene	0.05%	1.40
9	Pyrene	0.05%	1.62
10	Benzo(a)anthracene	0.05%	1.59
11	Chrysene	0.06%	1.60
12	Benzo(b)fluoranthene	0.07%	1.90
13	Benzo(k)fluoranthene	0.08%	1.96
14	Benzo(a)pyrene	0,08%	1.76
15	Dibenzo(a,h)anthracene	0.09%	1.62
16	Benzo(ghi)perylene	0.09%	1.99
17	Indeno(123-cd)pyrene	0.11%	2.50

Table 1
Precision of retention times at 18 °C column temperature, with ambient temperature of 25 °C

Precision of retention times and peak areas

Stable retention times are important for correct identification of complex environmental matrices. When using a time-programmable fluorescence detector stable retention times are also important to avoid wavelength switching during an analyte's elution.

Precision of peak areas is important for obtaining reliable quantitative data.

Table 1 demonstrates typical RT precision of better than 0.2 %, obtained over 15 runs at 7 degrees below ambient. Peak-area precision for the low ng range is below 2 % RSD in general, if the peaks are well separated. It goes up to 4 % RSD if the peaks are not baseline separated.

Impact of vacuum degassing on fluorescence detection

PNAs can be quantified reproducibly down to the low picogram range with the correct fluorescence detection method. Careful selection of excitation and emission wavelengths and the use of mobile phase degassing, ensure high-sensitive PNA analysis. It is well known that the presence of oxygen in the mobile phase deteriorates detection limits because of quenching effects. Therefore thorough degassing is of utmost importance. We investigated the influence of no degas-sing, helium degassing and vacuum degassing on the response of critical compounds. As figure 4 demonstrates, the best results were achieved with vacuum degassing.

Spectral information with diode-array detection

For highly contamin-ated samples UV-Visible absorbance diode-array detection offers additional analytical tools using the spectral domain: peak-identity and peak-purity confirmation. Acenaphthylene (EPA compound) does not fluoresce, so for this compound UV-Visible absorption is the detection method of choice. An ideal detection method for PNAs is the serial detection with fluorescence and UV-Visible diode-array instrumentation. Here highest sensitivity is combined with optimum selectivity and additional identification tools for highly contaminated samples.

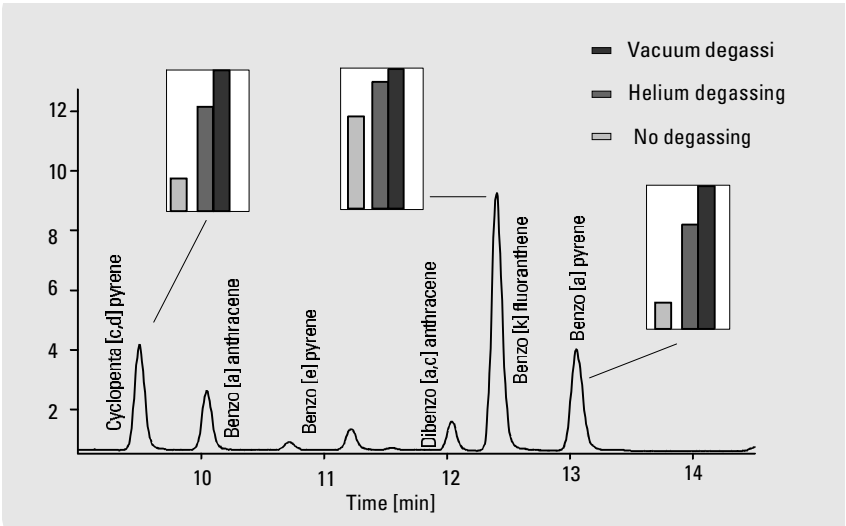


Figure 4
Quenching by dissolved oxygen can be avoided with a suitable mobile phase degassing technique

Automated positive identification of PNAs in soil with diode-array detection

Figure 5 shows the analysis of a soil extract using UV-Visible diode-array detection. The oven temperature was 22 °C. This still gave good resolution for early eluting peaks and allowed sensitive detection of late eluting peaks, for example, coronene.

The Agilent ChemStation's spectral search routine used here compares each spectrum with those stored in a spectral library compiled from analyses of standards run beforehand. The software recognizes those spectra that mach each other closely within the tolerance window specified. In those cases where a retention time has been tagged to the library records, the spectral match can be further qualified before being pronounced as specified. Figure 6 shows spectral overlay of sample spectra with library pyrene spectrum in the low mAU range. Normalization was done on the spectra library's spectra.

The complete method — HPLC separation with data acquisition, data evaluation, quantification, and identification — can be automated for multiple, unattended analyses. The reports that are generated include sample amounts, purity and library identity information.

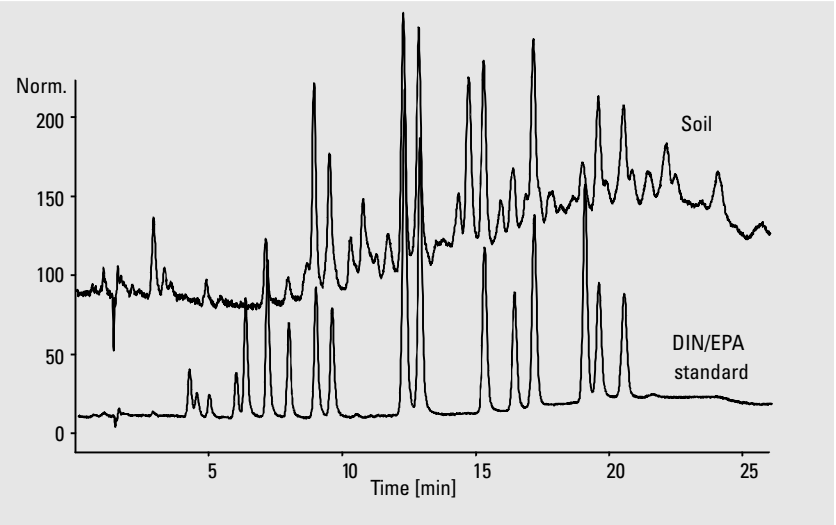
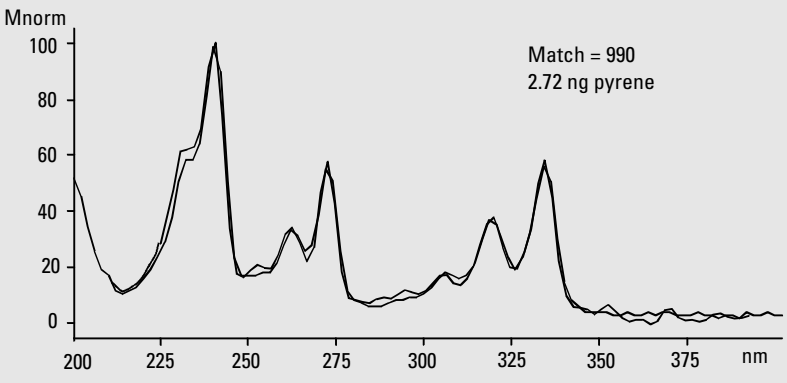


Figure 5
Analysis of PNAs in soil sample (extraction was made with super critical fluid extraction)1



Signal 1: DAD1 B, Sig=270,40 Ref=550,100

Calibrated compounds

Meas. RtTime [min]	Library RtTime [min]	CalTbl RtTime [min]	Sig	Amount [ng/ul]	Purity Factor	#	Library Match		Name
7.08	7.09	7.09	1	0.777	985	1	974		Phenanthrene
8.89	8.91	8.90	1	2.966	970	1	982		Fluoranthene
9.48	9.52	9.51	1	2.719	965	1	990		Pyrene
12.23	12.23	12.22	1	1.411	985	1	967		Benzo(a)anthracene
12.82	12.81	12.81	1	1.791	979	1	970		Chrysene
15.24	15.23	15.23	1	1.976	756	1	973		Benzo(b)fluoranthene
16.35	16.35	16.35	1	1.371	730	1	894	x	Benzo(k)fluoranthene
17.09	17.09	17.08	1	2.157	885	1	908	x	Benzo(a)pyrene
18.94	18.99	18.99	1	0.746	320	1	75	x	Dibenzo(ah)anthracene
19.52	19.50	19.50	1	1.907	920	1	969		Benzo(ghi)perylene
20.47	20.45	20.45	1	2.228	910	1	944	x	Indeno(123-cd)pyrene

Figure 6
Identification of pyrene using spectral library search with report containing quantitative and qualitative results

Conclusion

The new Agilent 1100 Series HPLC systems solved the problems usually associated with the analysis of PNAs.

The Peltier thermostatted column compartment allowed the analysis of PNAs at ambient and subambient column temperatures with high precision. Peltier cooling was preferred to water cooling systems, because no additional equipment was needed and control and setup was easier and convenient.

The vacuum degasser enabled quenching-free fluorescence detection of PNAs at lowest detection limits due to the highly-efficient removal of oxygen from the mobile phase.

The Agilent ChemStation for HPLC gave full automation capabilities starting with complete control of all modules, data acquisition, data evaluation and report presentation with spectrally confirmed *qualitative* and *quantitative* results.

The quaternary gradient pump, vacuum degasser and Peltier thermostatted column compartment provided excellent retention-time stability at a flow of 0.4 ml/min. This saved purchase and disposal costs for solvents and allowed to work with a factor of four lower sample volumes.

References

1
Helmut Schulenberg-Schell, Rainer Schuster (Agilent Technologies, Waldbronn, Germany), unpublished results presented as a poster at the *3rd International Capillary Chromatography Conference*, *Riva del Garda*, **1993**.

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Publication Number 5964-3540E

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Contract Laboratory Program (CLP) Pesticide Analysis with 0.18 mm ID High-Efficiency GC Columns Utilizing Helium Carrier Gas

Application

Environmental

Authors

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Abstract

Contract Laboratory Program (CLP) pesticide analysis is demonstrated on high-efficiency GC columns (20 m × 0.18 mm id × 0.18 µm film thickness) with helium carrier gas. DB-17ms stationary phase is used for primary analysis and DB-XLB stationary phase for confirmation. Primary analysis and confirmation of 22 CLP pesticides in the protocol is achieved in an 11-minute analysis, a 35% reduction in analysis time versus 0.32 mm id columns.

Method translation software is successfully employed to translate an original set of conditions with hydrogen carrier gas to a new set of conditions using helium carrier gas. Elution order and degree of separation are shown to translate precisely from the original method to the new method through use of this software (available for free download) [1].

Introduction

The determination of organochlorine pesticides (OCPs) in environmental remediation samples are important, high-volume analyses in the competitive contract laboratory marketplace. A standard Contract Laboratory Program (CLP) pesticide method is used for these analyses. In many cases a lab will analyze large numbers of samples over the course of a given project, adding costs to both the lab and its client. Here, 0.18 mm id high-efficiency GC columns are demonstrated as a means of enhancing laboratory productivity. These columns are fully compatible with standard gas chromatographs and helium carrier gas operation. The high efficiency these columns offer coupled with their full compatibility with existing GCs provide laboratories with a powerful tool for enhancing sample throughput. When analysis times for 30 m × 0.32 id and 20 m × 0.18 mm id columns were compared, a 17-minute analysis was reduced to only 11 minutes and with improved resolution [2].

Helium carrier gas was selected as a means to show the utility of 0.18 mm id columns in doing CLP pesticide analyses and to demonstrate the full compatibility of these columns with standard gas chromatographs. The operating gas pressures for these 20 m × 0.18 mm id columns range from 33 psi initially to 50 psi at the high point of the temperature program. The gas pressure range used with helium carrier gas with these 0.18 mm id columns was well within the operating range for standard chromatographs.

Experimental

This work was accomplished using an Agilent 6890N GC equipped with dual µECDs and a 7683B autosampler. A single split/splitless injection port was used for sample introduction at the head of a retention gap column connected through a Y-splitter with two analytical columns. Details of the chromatographic conditions are presented in Table 1.

Table 1. Chromatographic Conditions

GC	Agilent 6890N
Sampler:	Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Inlet	Split/splitless; 250 °C pulsed splitless (35 psi for 0.5 min)
Inlet liner	Deactivated single taper direct connect (Agilent p/n 1544-80730)
Carrier	Helium (constant flow, 49.5 cm/sec at 120 °C, purified through big universal trap Agilent p/n RMSH-2)
Retention gap	5 m × 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter	Quartz deactivated (Agilent p/n 5181-3398)
Columns:	
1	20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722)
2	20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222)
Oven	120 °C (0.49 min); 85 °C/min to 160 °C; 20 °C/min to 260 °C (0.20 min); 25 °C/min to 285 °C; 40 °C/min to 300 °C (3.5 min)
Detection	µECD 325 °C; nitrogen makeup; constant column + makeup flow 60 (mL/min)

The flow path supplies used in these experiments are listed in Table 2 below.

Table 2. Flow Path Supplies

		Agilent p/n
Vials	Amber screw cap	5182-0716
Vial caps	Blue screw cap	5282-0723
Vial inserts	100 µL glass/polymer feet	5181-1270
Syringe	5 µL	5181-1273
Septum	Advanced green	5183-4759
Inlet liner	Deactivated single taper direct connect	1544-80730
Ferrules	0.4 mm id short; 85/15 Vespel/graphite	5181-3323
Y-splitter	Quartz deactivated	5181-3398
Sealing resin	Polyimide sealing resin	500-1200
20x magnifier	20x magnifier loop	430-1020
Tubing cutter	Ceramic wafer column cutter	5181-8836

Sample Preparation

CLP pesticide standard solutions were purchased from AccuStandard, New Haven, CT 06513-USA. ULTRA RESI ANALYZED grade 2,2,4 trimethylpentane was purchased from J. T. Baker, Phillipsburg, NJ 08865-USA. CLP-023R-160X and CLP-024R-160X concentrates were diluted separately into two 100-mL volumetric flasks in 2,2,4 trimethylpentane and then combined in subsequent serial dilutions. Volumetric flasks and pipettes used were all class A. Standard concentration range for low-level target compounds in the protocol was from 1.6 to 40 ng/mL. On-column loading ranged from 0.4 to 10 pgs for low-level target compounds when a 0.5-µL injection over both columns is considered.

Column Installation Tip on Using Y Splitters

Installation of the Y splitter was accomplished by coating the outside of the fused silica tubing to be inserted into the Y splitter with a thin film of polyimide sealing resin prior to cutting the tubing. The cut was then made through the coated section of tubing. The cut end was then checked with a 20x magnification loop to make sure that the cut was clean and that excess sealant had not diffused inside the column. Once a clean cut was obtained, the fused silica with the polyimide sealant on the outside only was inserted into the desired branch of the Y and held for approximately 45 seconds to seal. Good sealing was indicated by a thin ring of sealant at the point of contact. The process was done first with the analytical columns and then repeated for the trunk of the Y into the retention gap. This approach gave tight, reliable connections that have lasted without any difficulty for more than 2 months (to date) and hundreds of oven temperature program cycles.

Results and Discussion

The starting point for this application was a set of conditions for CLP pesticide analyses using hydrogen carrier gas and 0.18-mm high-efficiency columns developed by Wool and Decker [3]. Using hydrogen carrier and flow programming, they were able to achieve primary separation and confirmation analysis of CLP pesticides in a 7-minute analysis. The chromatographic parameters for the hydrogen carrier separation were input as initial setpoints in method translation software to convert the method to use with helium carrier. Helium carrier was selected for use in laboratories reluctant to work with hydrogen carrier due to site safety policy or individual preference. High-efficiency GC columns give the chromatographer the option to work with either helium or hydrogen carrier gases and still achieve faster analyses.

Wool and Decker [3] indicated in their paper that frequent trimming of the front of the column was necessary for use with heavy matrix samples due primarily to the lower sample capacity of 0.18-mm columns. In this work a 5-m 0.25-mm id retention gap and Y connector were installed ahead of the

analytical columns to help offset the diminished sample capacity relative to wider bore capillary columns. Use of a retention gap will also shield the analytical columns from deleterious matrix affects and extend the useful lives of the columns.

Agilent's method translation software simplifies conversion from established laboratory GC methods to parallel sets of conditions suitable for high-efficiency GC columns. Chromatographic conditions from the original method, along with the new column dimensions, are entered into a menu-driven table within the software. The software then generates a translated method table with all the new chromatographic setpoints for the translated method. The new translated method setpoints produced by the software are often all that is required to successfully translate a method.

Three primary modes of method translation are available in the method translation software: translate only, best efficiency, and fast analysis. The "translate only" mode produces a set of conditions that most closely resembles the original method in terms of relative position on the Van Deemter curve, degree of separation, and elution order. The "best efficiency" mode generates a set of conditions where column efficiency is prioritized. The "fast analysis" mode generates a set of conditions where analysis speed is prioritized. By using the various modes available a translated method specific to a particular application can be developed quickly with a few keystrokes and iterative passes through the software.

The software is very useful in porting methods from the use of one carrier gas to another. Translation from the original method using one carrier to a method using another carrier is accomplished by entering the original method setpoints, the new column dimensions, and the desired carrier. The software then generates the translated method setpoints for the new column and carrier. For additional information on Agilent's method translation software, please visit this link: http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm.

Flow programming is not addressed in the method translation software, so minor adjustments to flow rate parameters may be required to achieve desired results. When translating flow-programmed methods, initial or intermediate flow rates can be entered into the original method parameters table to visualize the effect on the other parameters' output in the translated method table. The operator can then collect data at several different flow rates and select the best set of conditions for the application.

In this CLP pesticide example, the original method used a hydrogen carrier and flow programming. The initial flow parameters were entered into the method translation software, along with the new column dimensions, specifying helium as the carrier gas. Translate-only mode was selected in the software and produced the translated method setpoints that appear in Figure 1.

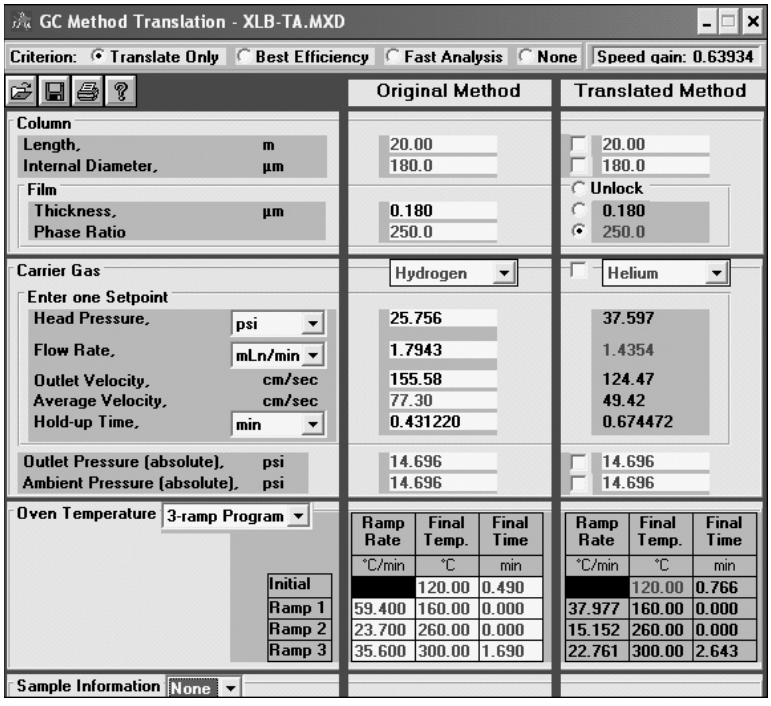

Figure 1. Method translation using translate-only mode.

Figure 2 shows the resulting CLP pesticide separations produced using translate-only mode in the method translation software on the DB-17ms column. Note that all 22 species are baseline resolved on the DB-17ms column where there is a partially separated triplet consisting of gamma chlordane, alpha chlordane, and endosulfan 1 on the DB-XLB column (Figure 3). This partially separated triplet was also observed in the original DB-XLB separation using hydrogen carrier.

Table 3 is a standard compound key for the numbered peaks in the chromatograms. Separation characteristics such as degree of separation and elution order were maintained exactly as they were in the original method using the new translated method with helium carrier. The original method was successfully translated with no additional method development.

Unfortunately, the unresolved triplet on DB-XLB observed in the original method remained unresolved in the translated method. Additional method development attempts focused on resolving the partially separated triplet on the DB-XLB confirmation column and reduction of analysis time. Some success was achieved; however, the trailing two peaks in the triplet remained partially resolved on the DB-XLB confirmation column while analysis time was reduced to 11 minutes. The DB-17ms column resolved all of the species in the protocol throughout these experiments (Figure 4). Triplet resolution on the DB-XLB (Figure 5), though not ideal, is adequate for the purpose of peak confirmation of well-resolved species on the DB-17ms.

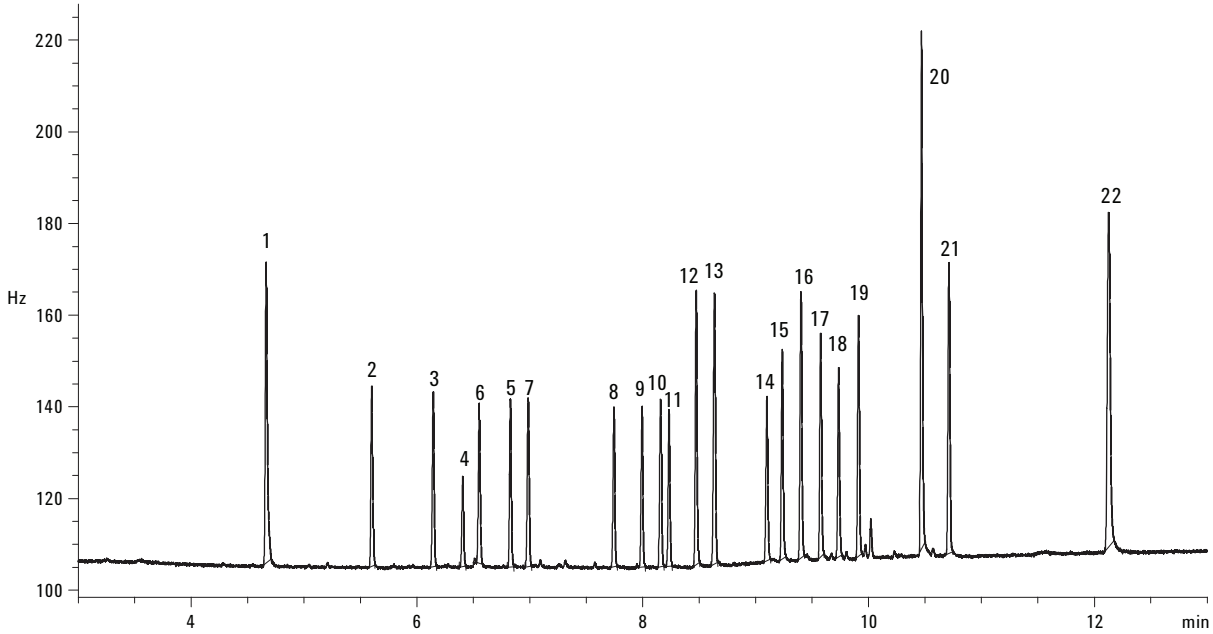

Figure 2. Translate-only separation (conditions as in Figure 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 0.4 pg/component loading for low-level target compounds.

Table 3. CLP Standard Compound List Key

1. Tetrachloro-m-xylene
2. Alpha BHC
3. Gamma BHC
4. Beta BHC
5. Delta BHC
6. Heptachlor
7. Aldrin
8. Heptachlor epoxide
9. Gamma chlordane
10. Alpha chlordane
11. Endosulfan I
12. 4,4′ DDE
13. Dieldrin
14. Endrin
15. 4,4′ DDD
16. Endosulfan II
17. 4,4′ DDT
18. Endrin aldehyde
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl

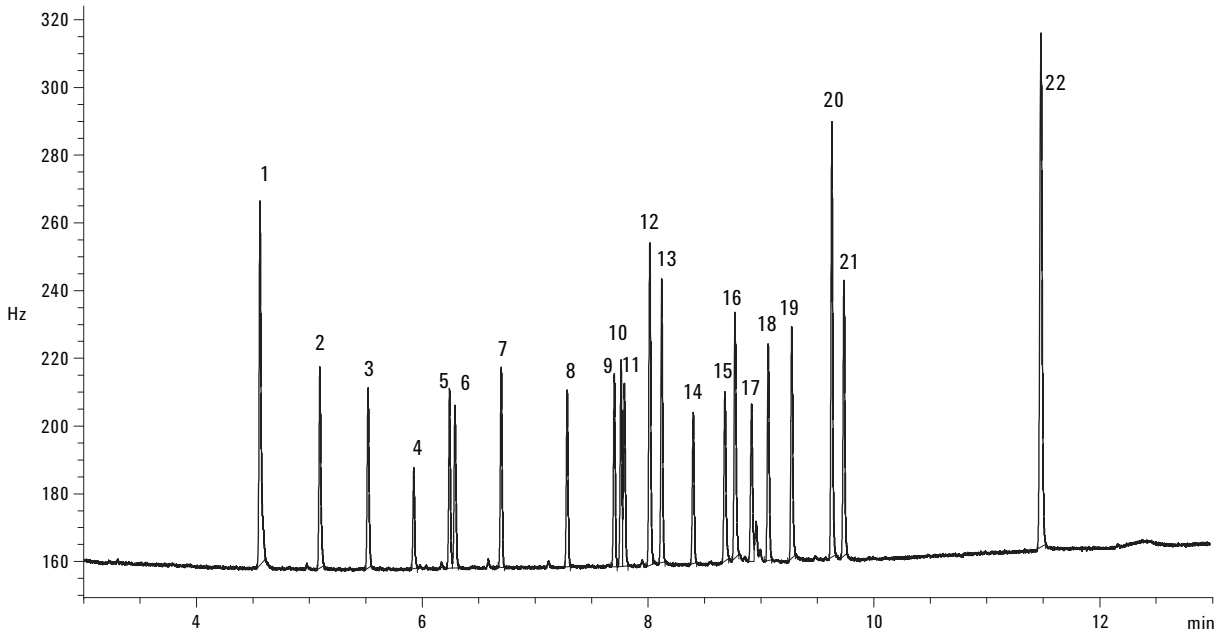

Figure 3. Translate-only separation (conditions as in Figure 1) on 20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222) with a 0.4 pg/component loading for low-level target compounds.

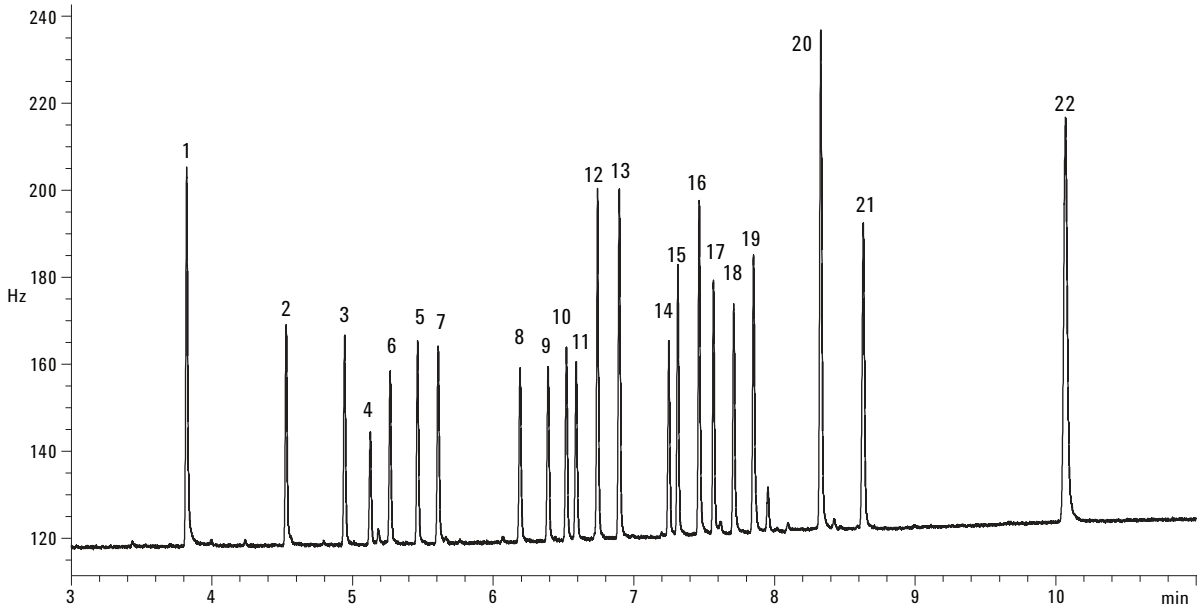

Figure 4. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 0.4 pg/component loading for low-level target compounds.

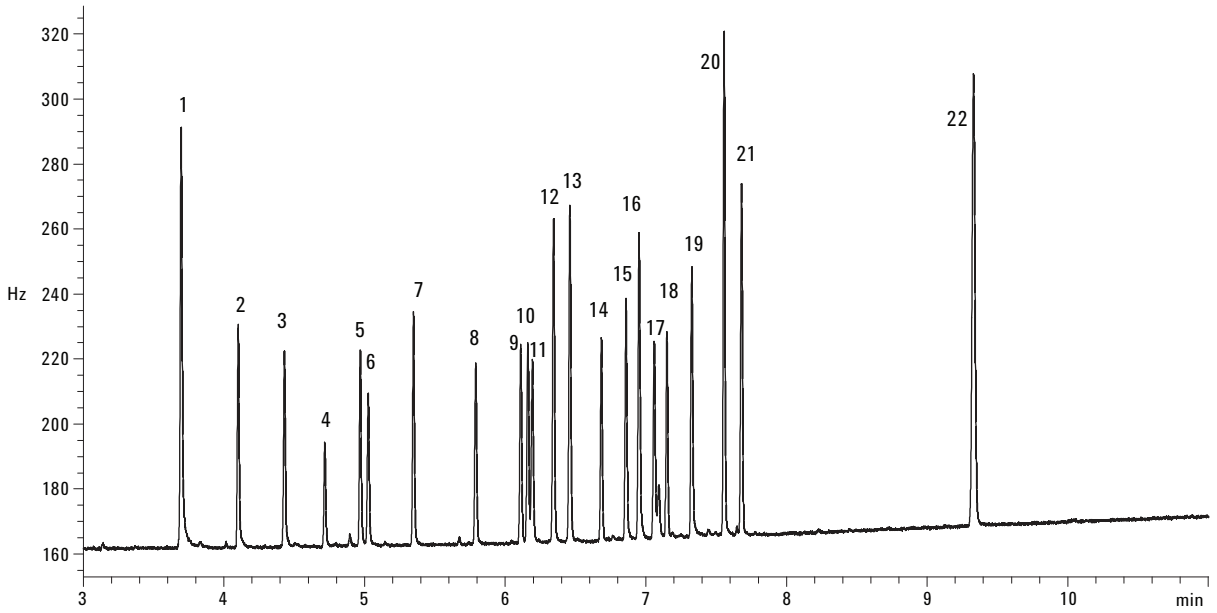

Figure 5. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222) with a 0.4 pg/component loading for low-level target compounds.

Detector Sensitivity and Linearity

The 0.5-µL injections were split between two columns for an on-column loading of 0.4 pg per component of the low-level target compounds. The data suggest that detection limits of at least an order of magnitude lower are possible. Sensitivity and linearity measurements conducted with these chemical species using µECD detection support this assertion [4]. Analyte concentration range investigated here was from 1.6 – 40 ng/mL. This range meets the 16-fold low- to high-check standard criteria for the protocol and appears to cover only the middle of the dynamic range the detector is capable of fielding. Figure 3 shows the DB-17ms separation where low-level component loading was 0.4 pg. Figure 6 shows the same separation with a 10-pg loading for the same components.

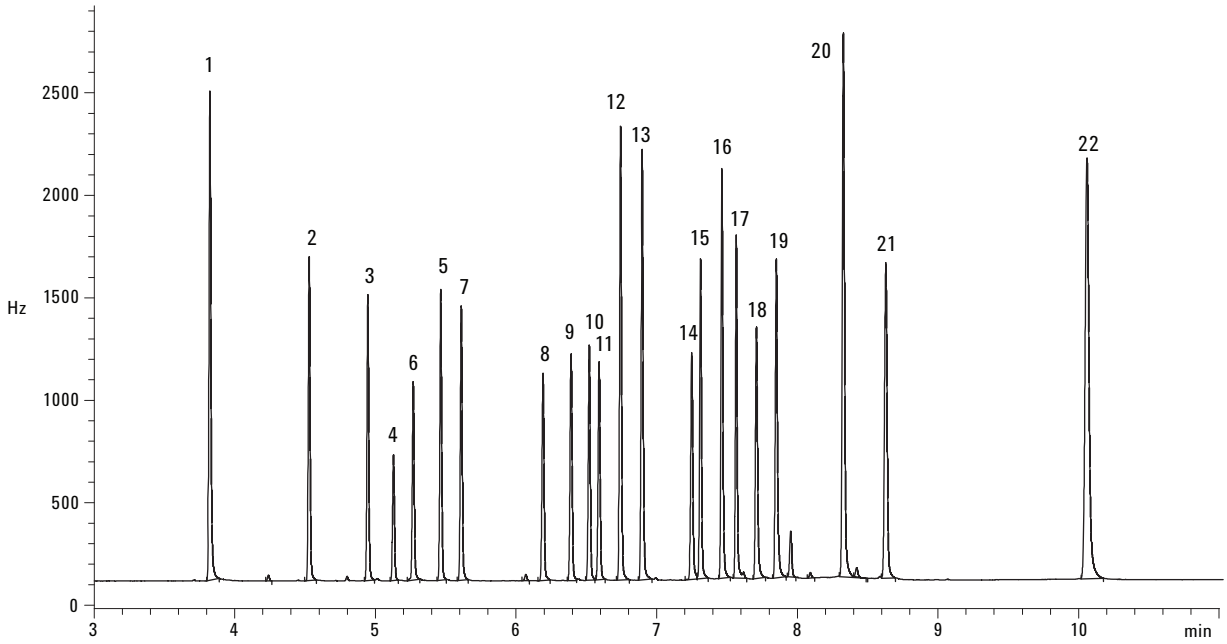

Figure 6. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 10-pg/component loading for low-level target compounds.

Conclusions

Complete separation and confirmation of all 22 species in the CLP pesticide protocol were accomplished in an 11-minute analysis with helium carrier gas. These results demonstrate the utility of these 0.18-mm id high-efficiency GC columns for CLP pesticide analysis. Using a 0.5-µL injection of pesticide standard solutions over a concentration range of 1.6 – 40 ng/mL gave excellent results. These results easily meet the 16x high/low dynamic range requirement for the protocol and suggest that expanding the range to both lower and higher concentrations is certainly possible with these 0.18-mm columns.

Full compatibility for use of these columns with standard GC equipment and helium carrier was also established by this successful separation. Operating pressure for use of these columns at the high point of the temperature program (300 °C) was 50 psi, well within the operation pressure range for standard GC equipment.

Method translation software successfully translated the original method using hydrogen carrier to the new method using helium carrier. Separation characteristics from the original method, such as elution order and degree of separation, were matched exactly in the translated method. This exercise served once again to validate the simplicity of method translation using the software. Method development beyond the translated method setpoint only became necessary when improvements to the original separation were attempted.

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2. C. George, “Rapid Analysis of CLP Pesticides Using High-Temperature DB-35ms and DB-XLB Columns,” Application Note 5988-4973EN, December 18, 2001
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Printed in the USA
January 16, 2008
5989-7818EN

Agilent Technologies

A Direct Column-Performance Comparison for Rapid Contract Laboratory Program (CLP) Pesticide Analysis

Application

Environmental

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Abstract

Agilent J&W High Efficiency GC columns with internal diameter of 0.18 mm for Contract Laboratory Program (CPL) pesticide analyses gave superior results for CPL pesticide primary analysis and confirmation. Chromatograms depicting peak shape characteristics, peak resolution, and baseline stability for two sets of 0.18-mm id columns are presented in a head-to-head comparison. Complete primary and confirmatory analysis of the 20 pesticides in the protocol is accomplished in less than 6 minutes using hydrogen carrier gas and flow programming. Successful primary and confirmatory analyses were achievable only on Agilent J&W High Efficiency GC columns.

Introduction

The analyses of organochlorine pesticides (OCPs) in environmental remediation samples are important, high volume, analyses in the competitive contract laboratory marketplace. A standard Contract Laboratory Program (CLP) pesticide method is used for these analyses. In many cases a lab will analyze large numbers of samples over the course of a given project, accumulating costs to both the lab and its client. Use of Agilent J&W 0.18-mm id High Efficiency GC columns is a means of enhancing laboratory productivity [1-3].

Wool and Decker [3] reported their findings at the U.S. Environmental Protection Agency (Region VI, Houston, TX) laboratory and described the value of columns in the 20 m × 0.18 mm format for CLP pesticide analysis. Their suggestion to use a retention gap to protect the analytical columns from deleterious matrix effects and to help offset the lower sample capacity of these columns relative to wider bore columns was incorporated into this column comparison. Deactivated 5 m × 0.25 mm id retention gaps were used in this series of experiments on each column set used.

Columns with 0.18 mm id capable of doing CPL pesticide analysis are available from several leading column manufacturers. Agilent's suggested pair for CLP pesticide analysis in the 0.18 mm id format is a DB-17ms column for primary analysis and a DB-XLB column for confirmation. Vendor R's offering is a set of proprietary phase 0.18 mm id columns for both primary and confirmation analysis of CLP pesticides.

Experimental

The chromatograph used was an Agilent 6890N GC equipped with dual electron capture detectors (μECDs) and a 7683B autosampler. Sample introduction was done by a single split/splitless injection port at the head of a retention gap column connected through a Y-splitter with two analytical columns. Details of the initial chromatographic conditions appear in Table 1.

Table 1. Chromatographic Conditions

GC :		Agilent 6890N
Sampler:		Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Carrier:		Hydrogen (flow programmed, 69 cm/s at 120 °C, ramped at 99 mL/min to 106 cm/s at 4.4 minutes, purified through a Big Universal Trap (Agilent p/n RMSH-2)
Inlet:		Split/splitless; 220 °C, pulsed splitless (35 psi for 0.5 min, purge flow of 40 mL/min on at 1 minute, gas saver flow 20 mL/min on 3 minutes
Inlet liner:		Deactivated single taper direct connect (Agilent p/n 1544-80730)
Retention gap:		5 m × 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter:		Quartz deactivated (Agilent p/n 5181-3398)
Columns:	1	20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722)
	2	20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222)
Oven:		120 °C (0.32 min); 120 °C/min to 160 °C; 30 °C/min to 258 °C (0.18 min); 38.81 °C/min to 300 °C (1.5 min)
Detection:		µECD 320 °C; nitrogen makeup; constant column + makeup flow (60 mL/min)

The flow path supplies used in these experiments are listed in Table 2.

Table 2. Flow Path Supplies

	Description	Agilent p/n
Vials:	Amber screw cap	5182-0716
Vial caps:	Blue screw cap	5282-0723
Vial inserts:	100 µL glass/polymer feet	5181-1270
Syringe:	5 µL	5181-1273
Septum:	Advanced green	5183-4759
Inlet liner:	Deactivated single taper direct connect	1544-80730
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite	5181-3323
Y-splitter:	Quartz deactivated	5181-3398
Sealing resin:	Polyimide sealing resin	500-1200
20x magnifier:	20x magnifier loupe	430-1020
Tubing cutter:	Ceramic wafer column cutter	5181-8836

Both sets of columns used in this comparison were installed into the GC in the same manner. The same retention gap and inlet liner were used for both sets of columns. The chromatographic conditions (except for the columns) in Tables 1 and 2 were used to evaluate both the proprietary columns recommended by Vendor R and Agilent's columns. The primary analysis column from Agilent was a 20 m × 0.18 mm x 0.18 µm DB-17ms. The column from Vendor R was a 20 m x 0.18 mm × 0.18 µm with a proprietary stationary phase. The confirmatory analysis column from Agilent was a 20 m × 0.18 mm × 0.18 µm DB-XLB. The column from Vendor R was a 20 m × 0.18 mm x 0.14 µm with a proprietary stationary phase.

Sample Preparation

CLP pesticide standard solutions were purchased from AccuStandard (New Haven, CT 06513 USA). ULTRA RESI ANALYZED grade 2,2,4 trimethylpentane was purchases from J.T. Baker (Phillipsburg, NJ 08865 USA).

CLP-023R-160X and CLP-024R-160X concentrates were diluted first into 50-mL volumetric flasks in 2,2,4 trimethylpentane and then serially diluted. Volumetric flasks and pipettes used were all class A. The standard concentration range for low-level target compounds in the protocol was from 3.2 to 80 ng/mL. On-column loading ranged from 0.8 to 20 pg for low-level target compounds when a 0.5-µL injection over both columns is considered.

Column Installation Using Y Splitters

Installation of the Y splitter was accomplished by coating the outside of the fused silica tubing to be inserted into the Y splitter with a thin film of polyimide sealing resin prior to cutting the tubing. The cut was then made through the coated section of tubing. The cut end was then checked with a 20x magnification loupe to make sure that the cut was clean and that excess sealant had not diffused inside the column. Once a clean cut was obtained, the fused silica with the polyimide sealant on the outside only was inserted into the desired branch of the Y and held for approximately 45 seconds to seal. Good sealing was indicated by a thin ring of sealant at the point of contact. The process was done first with the analytical columns and then repeated for the trunk of the Y into the retention gap. This approach has given tight, reliable connections that have lasted without difficulty for over 2 months and through hundreds of oven temperature program cycles.

Method Translation Software/Path to Successful Conditions

The starting point for this comparison was the conversion of a successful set of separation conditions using helium carrier on Agilent's DB-17ms and DB-XLB 0.18 mm id columns [4] to a set of conditions using hydrogen carrier. The chromatographic parameters for the helium carrier separation were keyed into the translation table in the Agilent GC Method Translation software [5–6] to convert the method to use with hydrogen carrier. In the software, the "Translate Only" mode was used to convert the 11-minute helium carrier method to a 7.3-minute hydrogen carrier method using the same columns.

Method development effort beyond conversion from helium carrier to hydrogen carrier gas became necessary only when the goal of the analysis shifted to emphasize speed of analysis using flow programming. Flow programming is outside the scope of the Method Translation software. In this series of experiments, flow programming helped to elute highly retained peaks faster. Further temperature program modifications also increased the speed of analysis with minimal loss of resolution on the Agilent columns.

Results and Discussion

Successful separation of CLP pesticides using hydrogen carrier was demonstrated on Agilent's DB-17ms and DB-XLB 0.18-mm id columns using the conditions shown in Table 1. Vendor R's 0.18-mm ID columns were evaluated using the following conditions: the conditions shown in Table 1, the conditions obtained on Vendor R's Web site (to the extent practical), and with a set of conditions optimized specifically on Vendor R's columns for this analysis. The goal throughout these experiments was to show as fair and objective a comparison as possible.

To compare chromatograms, injections at a standard concentration of 3.2 ng/mL for low-level target species in the CLP protocol were selected. Using this concentration consistently provides a fixed point of reference and at the same time alleviates the potential for masking deleterious chromatographic effects often seen at higher concentrations. Inclusion of a Y scale in each chromatogram provides another fixed reference within each figure to facilitate comparison. Key aspects to look for in the example chromatograms are peak resolution, indications of peak tailing, and temperature-dependent drift on the μECD.

An Agilent DB-17ms column was used as the primary analysis column in these experiments. An example chromatogram from an injection at a nominal concentration of 3.2 ng/mL for low target level pesticides is shown in the upper portion of Figure 1. This column resolved all the peaks of interest in less than 6 minutes, gave sharp symmetrical peaks, and had minimal background drift on the μECD. A compound label key for the numbered peaks in the chromatogram is located in Table 3.

An Agilent DB-XLB 20 m x 0.18 mm x 0.18 μm column was used for confirmatory analysis on these experiments. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in the lower portion of Figure 1. This column resolved 20 of the peaks of interest in less than 6 minutes and gave near baseline resolution of peaks 10 and 11. Again, sharp symmetrical peaks and minimal temperature-dependent baseline drift were observed on the μECD. Although complete resolution of 20 of the 22 peaks of interest on the confirmatory columns is not ideal, the observed resolution is satisfactory for peak confirmation.

The peak identification table applies to Figure 1, depicting CPL pesticide separation on Agilent's column only. Elution order for these columns with their particular selectivity was established in previous work. To establish elution order on Vendor R's columns with different selectivity, injection of individual standards or mass spectral confirmation is required.

Table 3. CLP Standard Compound List Key

1. Tetrachloro-m-xylene	12. 4,4' DDE
2. Alpha BHC	13. Dieldrin
3. Gamma BHC	14. Endrin
4. Beta BHC	15. 4,4' DDD
5. Delta BHC	16. Endosulfan II
6. Heptachlor	17. 4,4' DDT
7. Aldrin	18. Endrin aldehyde
8. Heptachlor epoxide	19. Endosulfan sulfate
9. Gamma chlordane	20. Methoxychlor
10. Alpha chlordane	21. Endrin ketone
11. Endosulfan I	22. Decachlorobiphenyl

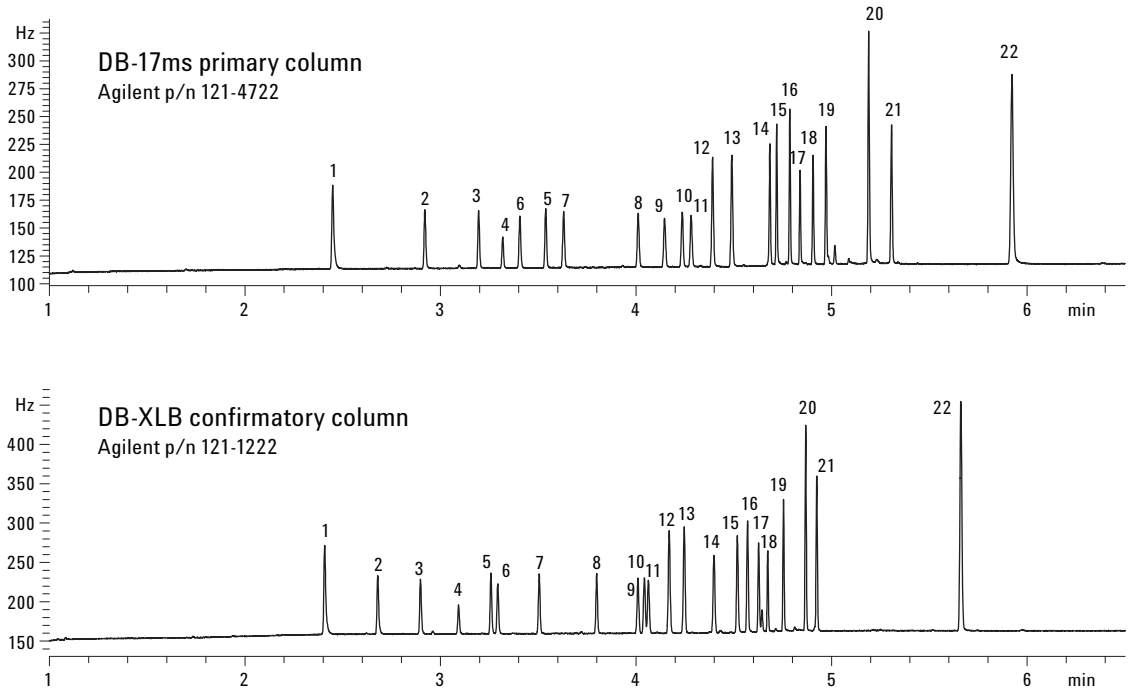

Figure 1. Chromatogram of 0.5-µl injection of 3.2 ng/ml low-level target pesticide standard solution injected through a Y-splitter onto DB-17ms (Agilent p/n 121-4722) primary analysis column and a DB-XLB (Agilent p/n 121-1222) confirmatory analysis column, conditions as in Table 1.

The primary analysis column from Vendor R was a 20 m × 0.18 mm × 0.18 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 2. This column gave resolution of 20 of the 22 peaks of interest, peak tailing for some species, and minimal temperature dependent baseline drift on the µECD. The arrows within the figure point to co-eluting and tailing peaks.

The confirmatory analysis column from Vendor R was a 20 m × 0.18 mm × 0.14 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 2. This column yielded resolution of all 22 peaks of interest, indication of peak tailing for some species, and significant temperature-dependent baseline drift on the µECD. The arrows within the figure point to tailing peaks and highlight baseline drift, in this case over 100 Hz.

Vendor R's suggested separation conditions for their column pair were unsuccessful at producing results equivalent to those shown on their Web site. This appears to stem from an oversight on their part. Suggested conditions found in a figure caption on the Web site called for a 2-min hold 10° C above the maximum recommended temperature. A temperature of 330 °C was called for; however, the label on the column box listed the upper temperature program limit as 320 °C for the confirmation column. Vendor R's confirmation column demonstrated significant bleed even with a temperature program that reached only 300 °C, a full 20 °C below the upper limit.

A series of attempts to resolve the co-eluting pair of pesticides on Vendor R's primary analysis column gave improved but still incomplete resolution. It was necessary to substantially reduce flow rate and modify both temperature and flow programming parameters to achieve the results shown in Figure 3. The chromatographic conditions used for these injections appear in Table 4; the flow path supplies were the same as those listed in Table 2.

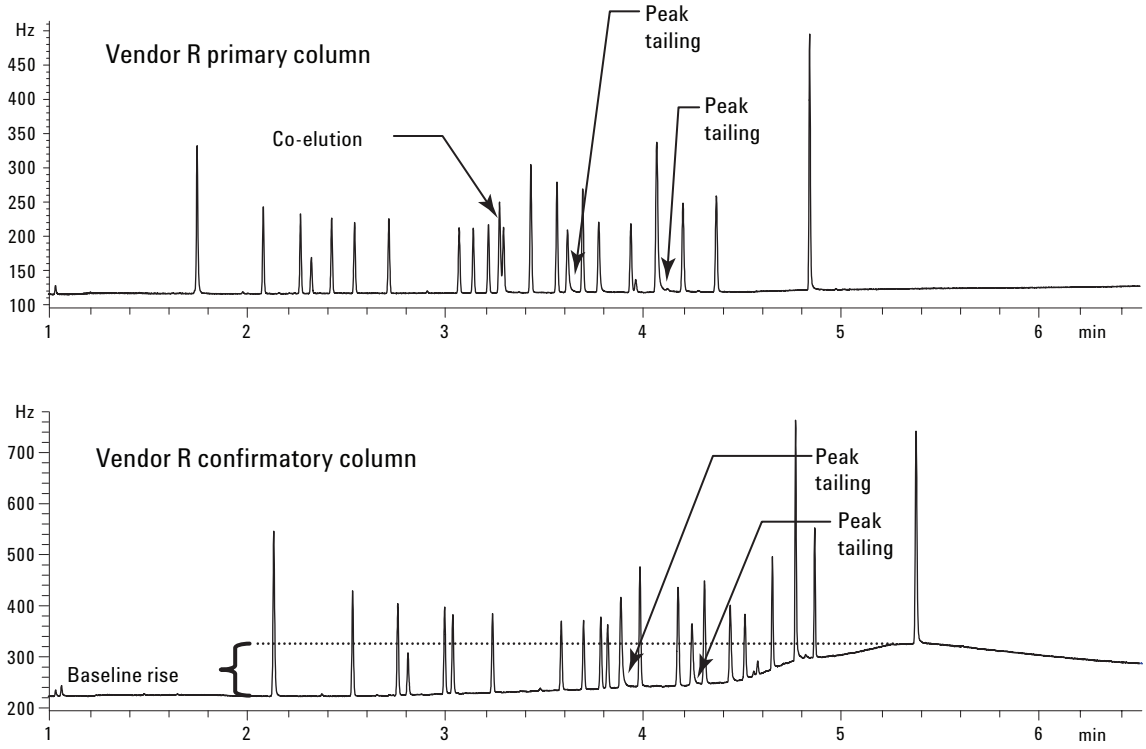

Figure 2. Chromatogram of 0.5 µL injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary analysis column and confirmatory columns, conditions as in Table 1. Figure 2. Chromatogram of 0.5 µL injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary analysis column and confirmatory columns, conditions as in Table 1.

The primary analysis column from Vendor R was a 20 m × 0.18 mm × 0.18 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 3. This column still gave resolution of 20 of the 22 peaks of interest, indication of peak tailing for some species, and minimal temperature-dependent baseline drift on the µECD. The arrows within the figure point to the unresolved peaks and tailing peaks.

The confirmatory analysis column from Vendor R was a 20 m × 0.18 mm × 0.14 µm with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 3. This column yielded resolution of all 22 peaks of interest, indication of peak tailing for some species, and significant temperature-dependent baseline drift on the µECD. The arrows within the figure point to tailing peaks and highlight baseline drift.

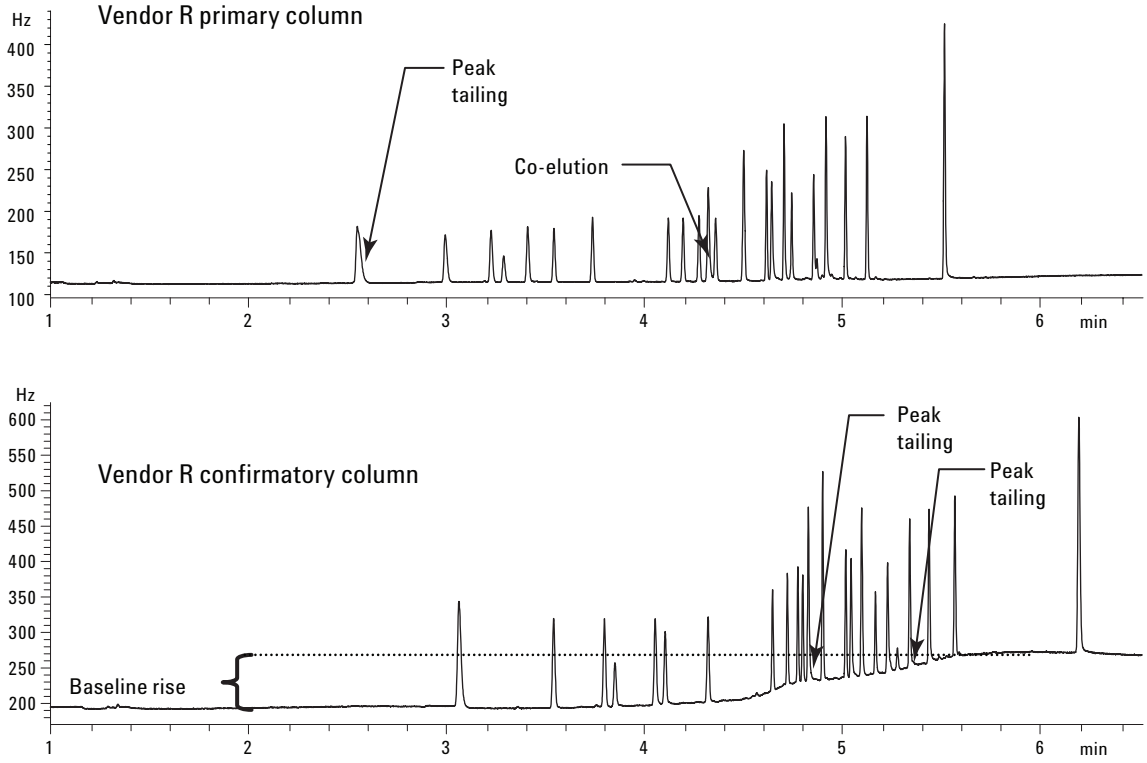

Figure 3. Chromatogram of 0.5-µl-injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary and confirmatory analysis columns, conditions as in Table 4.

Table 4. Chromatographic Conditions

GC:		Agilent 6890N
Sampler:		Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Carrier:		Hydrogen (flow programmed , 45 cm/s at 120 °C, ramped at 99 mL/min to 72 cm/s at 4.4 minutes, purified through a Big Universal Trap (Agilent p/n RMSH-2)
Inlet:		Split/splitless; 220 °C pulsed splitless (35 psi for 0.5 min, purge flow 40 mL/min on 1 minute, gas saver 20 mL/min at 3 minutes)
Inlet liner:		Deactivated single taper direct connect (Agilent p/n 1544-80730)
Retention gap:		5 m x 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter:		Quartz deactivated (Agilent p/n 5181-3398)
Columns:	1	20 m × 0.18 mm × 0.18 µm primary analysis column
	2	20 m x 0.18 mm × 0.14 µm confirmatory analysis column
Oven:		120 °C (0.50 min); 60 °C/min to 160 °C; 30 °C/min to 260 °C; 40 °C/min to 300 °C (2.0 min)
Detection:		µECD 320 °C; nitrogen makeup; constant column + makeup flow 60 (mL/min)

Conclusions

Agilent's 0.18-mm id primary analysis column is superior to Vendor R's offering. All 22 peaks of interest were resolved on the DB-17ms primary analysis column in less than 6 minutes with sharp, symmetrical peaks and minimal baseline drift. Vendor R's primary analysis column resolved 20 of 22 peaks of interest and displayed evidence of peak tailing for some of the peaks of interest.

Agilent's 0.18-mm id confirmatory analysis column offering is superior to Vendor R's offering. Twenty of 22 peaks of interest were resolved on the DB-XLB, with the other two peaks being almost baseline resolved in less than 6 minutes, with sharp, symmetrical peaks and minimal temperature-dependent baseline drift. Resolution of 20 of 22 peaks is less than ideal but should serve well for peak confirmation. Vendor R's confirmatory column resolved all 22 peaks of interest but showed evidence of peak tailing and an unacceptable level of temperature-dependent baseline drift.

The DB-17ms and the DB-XLB columns used in these experiments gave very low bleed profiles on the µECDs. The stable baselines produced by both of these columns can lead to lower detection limits,

simpler integration and more reliable results over time. These columns also have the versatility of use with other analyses beyond CLP pesticides.

Reliable CLP pesticide primary and confirmation analyses are achievable using Agilent J&W high-efficiency GC columns in less than 6 minutes with standard gas chromatographic equipment.

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Printed in the USA
February 26, 2008
5989-8031EN

Agilent Technologies

Analysis of Atrazine in Drinking Water at the ppb Level Using New Agilent Reversed Phase LC Columns

Application

Environmental

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Abstract

The herbicide atrazine was analyzed in drinking water samples using the new Agilent reversed phase columns, Agilent TC-C18(2) and HC-C18(2). These columns provided good resolution from interfering or coeluting compounds as well as highly symmetrical peaks and a high performance result. The limit of detection (LOD) is 0.5 ng, which meets China's drinking water standards. The method on these new columns is very suitable for atrazine analysis in drinking water.

Introduction

Atrazine, which is one of the triazine herbicides (structure shown in Figure 1), is a widely used herbicide for control of broadleaf and grassy weeds in the U.S. and some other countries. Atrazine is highly persistent in soil and is leached directly from the soil into groundwater, surface water, and drinking water. Atrazine has potential short-term and long-term health effects. Short-term potential health effects include heart, lung, and kidney congestion, as well as low blood pressure and muscle spasms. Potential health effects from longterm exposure at low levels include weight loss, retinal degradation, cardiovascular damage, and, potentially, even cancer. Therefore, actions have been taken to control this compound and require measuring amounts in drinking water. The maximum contamination level (MCL) regulated by the EPA (U.S. Environmental Protection Agency) is 3 ppb [1]. In China's new drinking water standards, the MCL is set at 2 ppb [2].

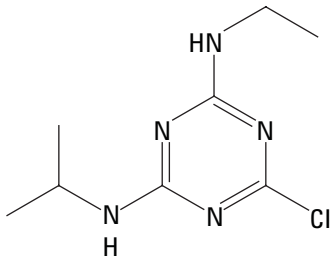

Figure 1. Structure of atrazine.

The HPLC method shown here was developed to meet the requirements for atrazine analysis in drinking water in China's new drinking water standards. We chose AccuBond C18 SPE sample cartridges for sample preparation instead of traditional liquid-liquid extraction because of the concentration effect on the atrazine. After water enrichment, the sample was then analyzed with Agilent TC-C18(2) and HC-C18(2) columns, which provide symmetrical peak shape and high sensitivity. It's a simple, fast, and high recovery method that is fit for quality control of drinking water.

Experimental

Standards for Calibration Curve

Accurately weigh 0.01 g atrazine standard and dissolve in methanol to a volume of 100 mL. This is a stock standard solution of 100 µg/mL. Dilute aliquots of the stock standard solution with

methanol into a series of standard solutions of 0, 0.05, 0.1, 0.5, 1.0, and 5 µg/mL.

Sample Preparation

We used the sample preparation method described by Yang et al. [3]. An AccuBond C18 SPE cartridge (Agilent p/n 188-1356) was used to extract the water sample and concentrate the atrazine in the water. Each cartridge was washed with 5-mL aliquots of methanol and reagent water successively, and the cartridge was kept wet after the reagent water wash. The entire sample was vacuum filtered through the cartridge at a flow rate 5 mL/min. The cartridge was washed with 5 mL reagent water and allowed to drain after washing. The sample was eluted from the cartridge with 5-mL portions of methanol and evaporated with a stream of N_2 to a volume of 1 mL. Following the same procedure, 50 mL of reagent water and tap water spiked with 5 ppb standard were treated to get the recovery sample.

HPLC Conditions

Instrument	Agilent 1200SL with DAD
Columns	Agilent TC-C18(2) (p/n: 588935-902) and HC-C18(2) (p/n: 588915-902), 4.6 mm × 150 mm, 5 µm
Mobile phase	55% Methanol:45%Water
Flow rate	1 mL/min
Wavelength	254 nm
Temperature	40 °C
Injection volume	10 µL

Results and Discussion

The standard solutions were analyzed by injecting 10 µL of each of the standard solutions in methanol onto the Agilent TC-C18(2) and HC-C18(2) columns. The calibration curve resulting from these standard injections on the TC-C18(2) column is shown in Figure 2. The method shows excellent linearity, being very close to 1.0 (0.9998). The chromatograms from the standard atrazine injections (Figure 3) show high performance and symmetrical peaks. Some differences in retention were seen on the two columns, which have different carbon loads (the HC-C18(2) has a load of 17% and the TC-C18(2) has a lower load of 12%). These differences impact retention, and nonpolar and moderately polar compounds are typically more retained on the HC-C18(2) column compared with the TC-C18(2) column. The mobile phase for this method used 55% methanol, which is suitable for both columns, but the TC-C18(2) provided a slightly shorter analysis time at just over 7 minutes. We therefore chose the TC-C18(2) for this method.

To evaluate the reproducibility of this method on the TC-C18(2) column, 5 ng of atrazine was injected 10 times. The reproducibility of the peak area is 2.7% and of the retention times is 0.03%; therefore, the TC-C18(2) column provided excellent reproducibility.

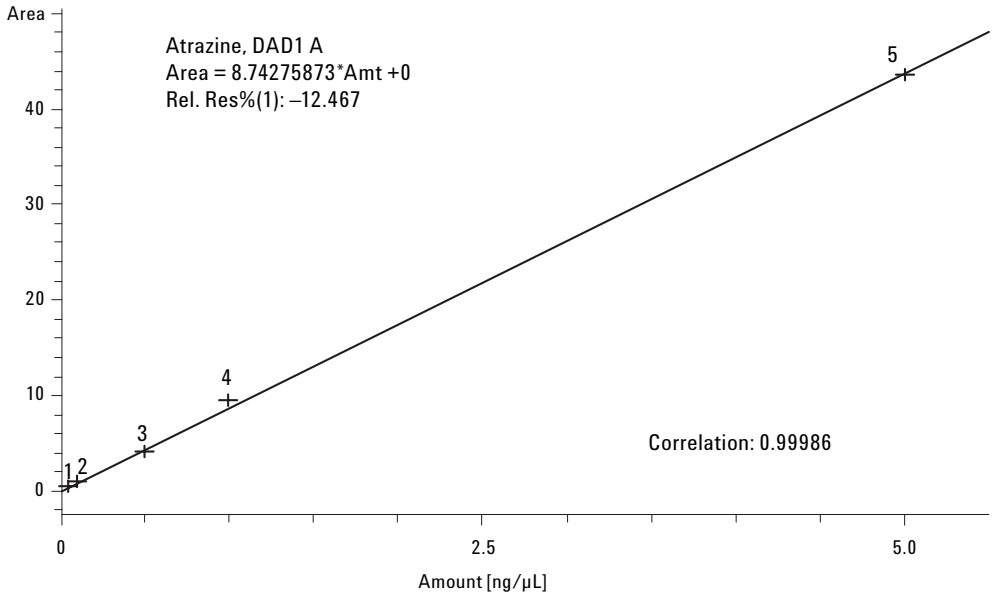

Figure 2. Calibration curve of atrazineon on TC-C18(2) and HC-C18(2), 4.6 mm × 150 mm, 5 µm columns.

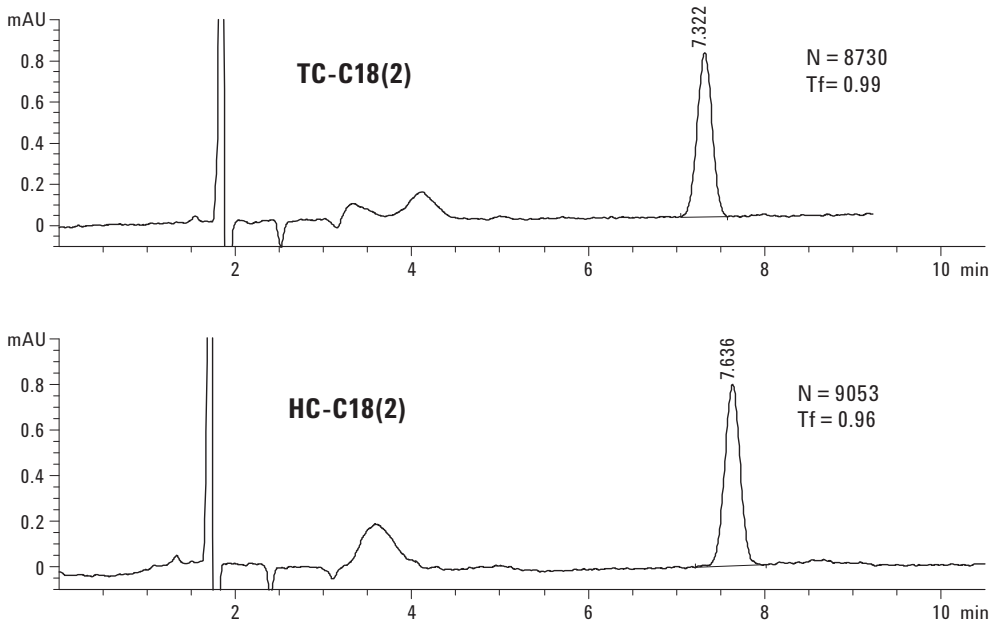

Figure 3. Chromatogram of atrazine standard on TC-C18(2) and HC-C18(2), 4.6 mm × 150 mm, 5 µm columns.

Figure 4 shows a chromatogram for a 0.5-ng atrazine injection. The signal-to-noise ratio is 3:1, so the LOD of this method is about 0.5ng which meets China's new drinking water standards.

An AccuBond C18 SPE cartridge was used in this method to extract trace atrazine in the water sample. The average recovery achieved is 88.2% (n = 3, RSD = 4.1%). This sample preparation method is simple and fast, and a low volume of organic solvents was consumed, making it an economical sample preparation method as well.

The chromatograms of reagent water and tap water and their spiked samples are shown in Figures 5 and 6. All the potential interfering compounds in reagent and tap water are well separated from the target compound atrazine; therefore, the method selectivity was also very good.

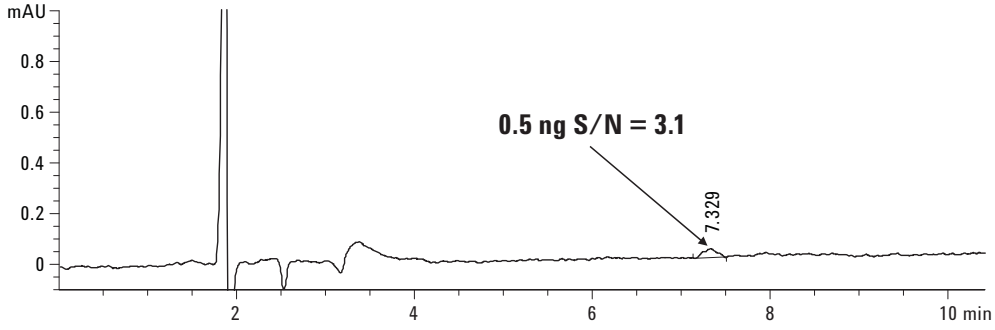

Figure 4. Chromatogram of standard with 0.5-ng injection on TC-C18(2), 4.6 mm × 150 mm, 5 µm columns.

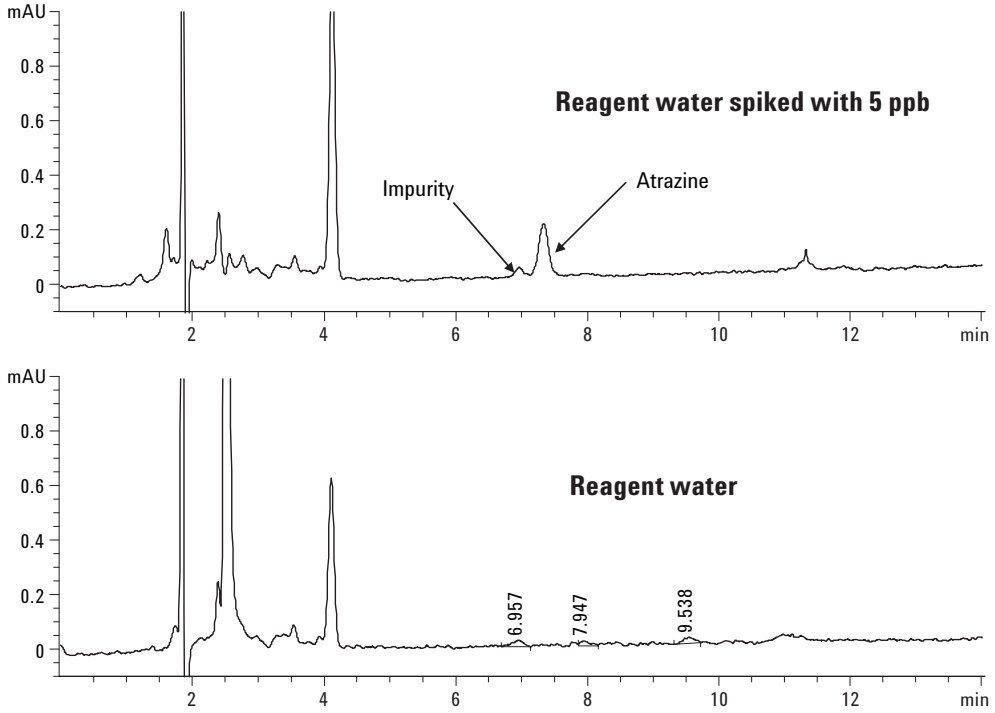

Figure 5. Chromatogram of reagent water and its spiked sample on TC-C18(2), 4.6 mm × 150 mm, 5 µm) columns.

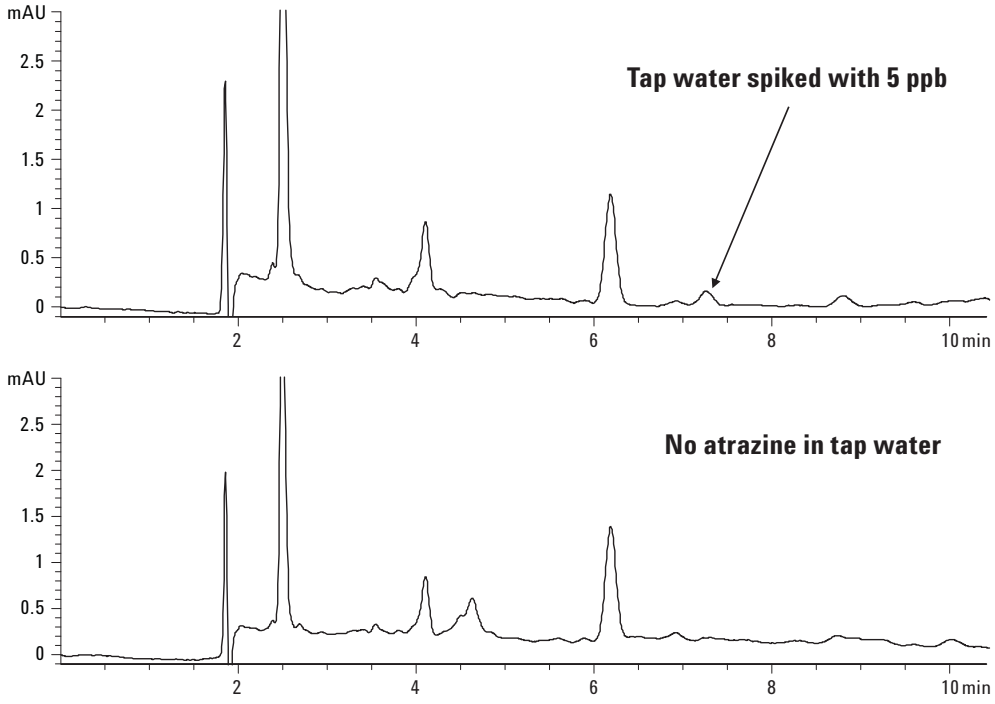

Figure 6. Chromatograms of tap water and its spiked sample on TC-C18(2), 4.6 mm × 150 mm, 5 µm columns.

Conclusions

The herbicide atrazine in drinking water can be easily analyzed at low levels and is well separated from potential interfering compounds in about 7 minutes using a new Agilent TC-C18(2) column. The AccuBond C18 SPE cartridge was used for sample preparation to concentrate the sample and meet the detection limits required of 0.5 ng on column. This total method can be used effectively to measure atrazine in drinking water quickly.

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Printed in the USA
April 1, 2008
5989-8328EN

Agilent Technologies

Determination of Estrone-3-Sulfate in Water at Sub-ppt Level by LC-(ESI-)-MS/MS

Application

Environmental

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Abstract

Using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC-MS/MS), in negative electrospray mode, estrone-3-sulfate was analyzed down to sub-ppt level (< ng L^{-1}).

Introduction

The occurrence of steroids in the aquatic environment and their effects on normal endocrine function in aquatic organisms are subjects of current concern. Several studies have also shown that birds, reptiles, and mammals in polluted areas undergo alterations of the endocrine-reproductive system.

At present, a multitude of chemicals have been demonstrated to be endocrine disrupters. Among these, natural and synthetic estrogens already show adverse effects at the lower ng/L. Their efficient control in environmental waters is made possible nowadays thanks to numerous analytical approaches available in the literature. This is the case for estradiol (E2), estrone (E1), estriol (E3), and ethinylestradiol (EE2) measurement. Estrone-3-sulfate (E1S) should also be highlighted, because of its stability in the environment, but until now fewer papers address this xenobiotic.

In this context, this application deals with the development of an analytical method dedicated to the quantification of estrone-3-sulfate in water. Identification relies upon the 2002/657/EC decision to confirm unambiguously the presence of steroids, even at ultra-trace levels (< 1 ng.L^{-1}).

Experimental

Sample Preparation Procedure

1. Add 10 ng internal standard (boldenone-sulfate-d_3).
2. If needed, filter water samples using 0.45-µm glass fiber filters.
3. Load the water sample onto an MM4 SPE cartridge (1 g, 6 mL) (Interchim, France).
4. Elute the compounds with 12 mL MeOH/NH_4OH (98:2, v/v).
5. Evaporate the extract to dryness.
6. Reconstitute the extract in 50 µL MeOH/H_2O (80:20, v/v).

Calibration Curve

Six calibration samples were fortified at 0, 1, 2, 3, 4, and 5 ng/L (ppt) and extracted using the described sample preparation procedure.

LC-MS/MS Measurement (Negative Electrospray Mode)

LC:	Agilent 1200
Column:	Gemini (50 mm x 2 mm, 3 µm) C18 110 A (Phenomenex)/Agilent equivalent: Extend-C18 3.5 µm, 2.1 x 50 mm (p/n 735700-902)
Column temperature:	40 °C
Mobile phases:	A: Ammonium acetate buffer 25 mM, pH 9.2 B: Acetonitrile (ACN)
Flow rate:	0.3 mL/min

Table 1. Elution Gradient

	Time (min)	%B
	0	5
	0.5	5
	15.5	100
	18.5	100
	21	5
	26	5
Injected volume:	10 µL	

MS:	Agilent 6410 LC/MS Triple Quadrupole
Ionization:	ESI (–)
Capillary:	3500 V
Nebulizer pressure:	40 psi
Drying gas:	13 L/min
Gas temperature:	275 °C

MS/MS Parameters

Table 2. MS/MS Parameters

Molecule	Precursor ion	MS1 resolution	Product ion	MS2 resolution	Dwell time (ms)	Fragmentor	Collision energy
Boldenone sulphate-d_3	368.3	Wide	353.3	Widest	200	120	15
Estrone-3-sulfate T1	349.2	Wide	269.2	Widest	300	200	35
Estrone-3-sulfate T2	349.2	Wide	145.2	Widest	300	200	60

Results and Discussion

Analytical Performance

Validation relies upon the 2002/657/EC decision to assess the methodology performances at sub-ppt levels. Various water samples coming from different origins (surface water or groundwater) were analyzed as blank samples. Five calibration curves were realized from spiked samples (1, 2, 3, 4, and 5 ppt of estrone-3-sulfate). All these samples were extracted according to the sample preparation procedure described previously. Two diagnostic signals (MRM transitions) were monitored for estrone-3-sulfate and one for boldenone-sulfate-d_3. Figure 1 illustrates the high specificity of the signals monitored and the good performance in terms of linearity.

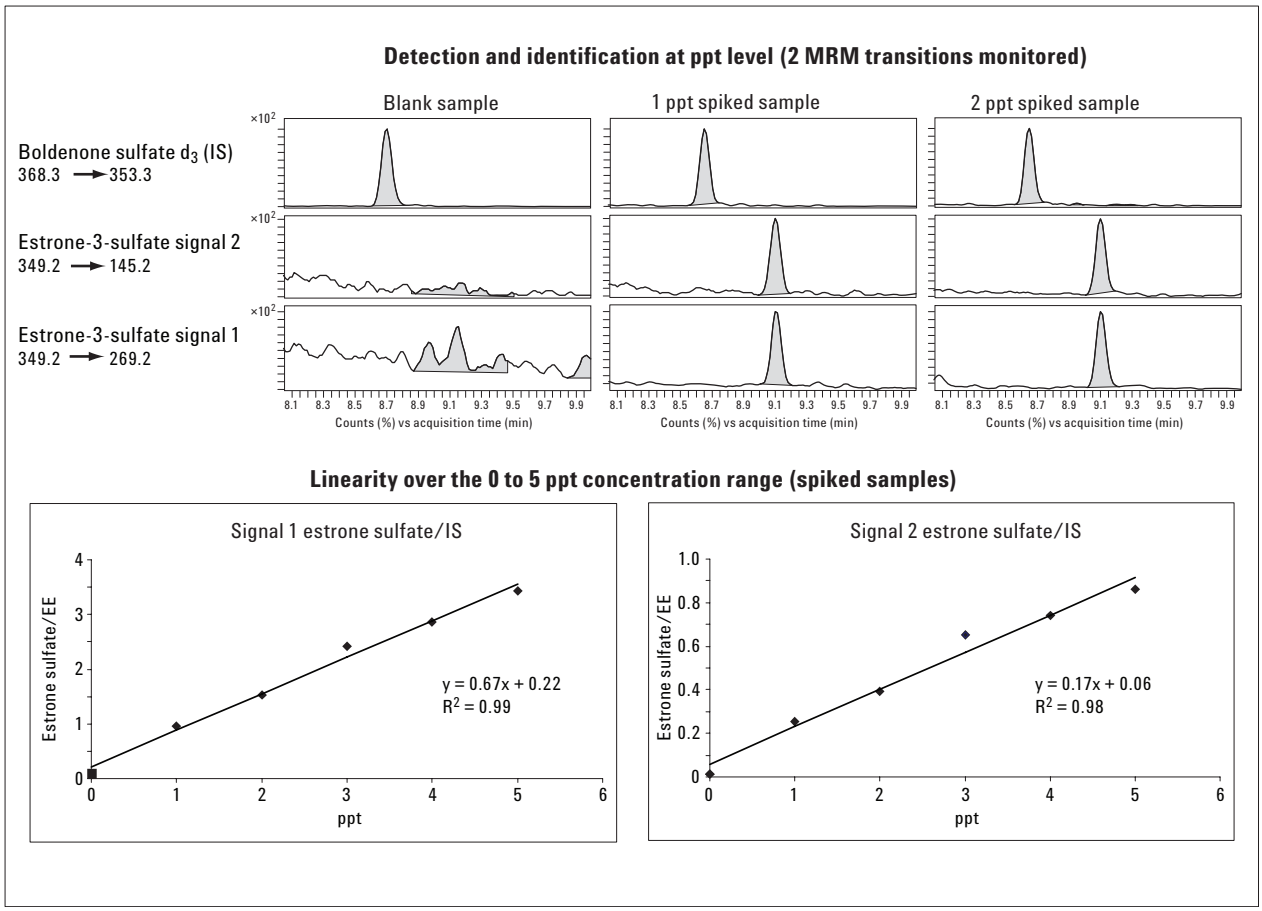

Figure 1. Illustration of the main performance parameters for the developed method (specificity, sensitivity and linearity).

Validation parameters

Table 3 presents the performances measured for each transition in term of linearity (for five different calibration curves), repeatability (at 1, 2, 3, 4, and 5 ppt fortified levels, on five different water), detection limit (CCα), and detection capability (CCβ).

Table 3. Validation Parameters

Estrone-3-sulfate	Linearity (range of R^2 on five calibration curves)	Repeatability (n=25) (5 replicates for each of the 5 tested concentration levels	CCα ($ng.L^{-1}$)	CCβ ($ng.L^{-1}$)
349.2 → 145.2	0.954 – 0.977	6.8 – 22.6 %	0.08	0.15
349.2 → 269.2	0.976 – 0.991	3.9 – 17.2 %	0.10	0.53

Analysis of Unknown Samples

Figure 2 illustrates the results obtained after application of the developed method to a batch of surface and groundwater samples. These results confirmed the efficiency of the method, demonstrating sensitivity and specificity.

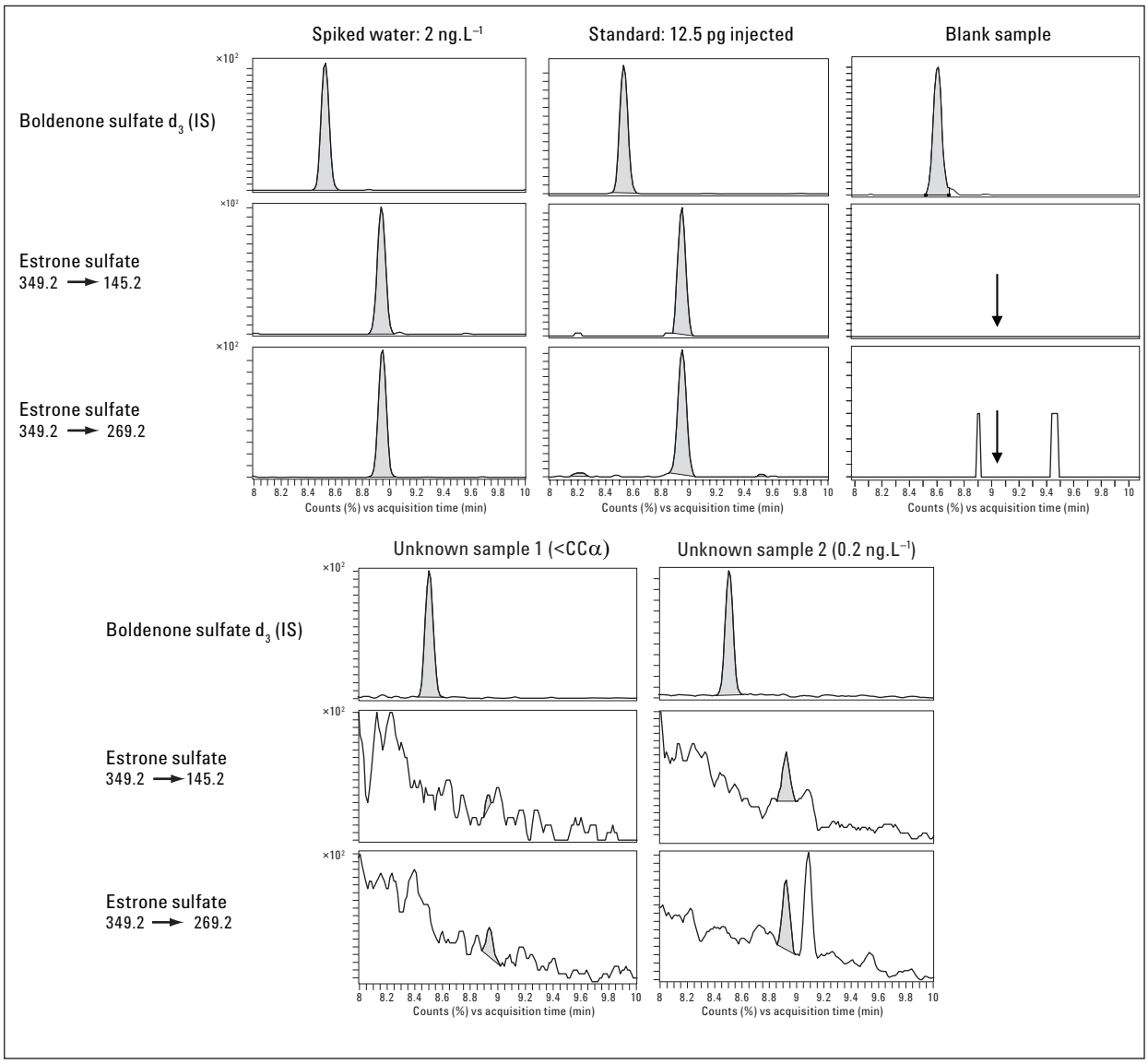

Figure 2. Typical MRM chromatograms obtained for routine analysis series (including spiked, standard, blank, and two unknown water samples).

Conclusions

The developed method, focused on estrone-3-sulfate, associates a selective SPE preparation and a specific LC-MS/MS detection (Gemini column, Phenomenex, Agilent 6410 LC-MS/MS system). The performed validation according to 2002/657/EC criteria allowed unambiguous confirmation of steroid presence, even at ultra-trace levels (< 1 ng.L^{-1}).

For More Information

For more information on this application, you may also contact Agilent Technologies, Inc., Attention: Lea Bonnington, European Field Support Centre, Hewlett-Packard Strasse 8, 76337 Waldbronn, Germany.

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Printed in the USA
June 24, 2008
5989-8480EN

Agilent Technologies

Analysis of Carbaryl and Carbofuran in Drinking Water with Post-Column Derivatization Using Agilent's New LC Column and SampliQ SPE Cartridges

Application Note

Environmental

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Abstract

The herbicides carbaryl and carbofuran were analyzed in drinking water samples using Agilent's new reversed-phase columns, Agilent TC-C18(2), and Agilent's new line of SampliQ C18 SPE cartridges. To meet the requirements of EPA Method 531.1 [1], we tried a 400-µL direct injection onto the new column. Even with such a large injection volume, the new column provided good resolution from interfering or coeluting compounds, as well as highly symmetrical peaks and a high performance result, which gives high sensitivity. In addition, post-column derivatization and a fluorescence detector were used, making the limit of detection (LOD) less than 0.01 ng on column, a lower LOD than that specified in China's drinking water standards [2]. This method used new Agilent SampliQ C18 cartridges to concentrate the water samples and made it possible to determine carbaryl and carbofuran in drinking water at the ppt level. This total solution is very suitable for trace-level analysis of carbaryl and carbofuran in drinking water.

Agilent Technologies

Introduction

Carbaryl and carbofuran are N-methyl carbamate pesticides (structure shown in Figure 1), which are classified as broad-spectrum insecticides and are mostly used on rice and corn crops. Excess pesticide can contaminate ground water, surface water, and drinking water. Because this kind of pesticide has been shown to create health problems with blood and with the nervous and reproductive systems, actions have been taken to control these compounds and require that their amounts be measured in drinking water.

Regulatory methods are set in many countries. The China drinking water standards published in 2006 restrict the maximum limit of carbofuran in drinking water to 7 ppb and use liquid-liquid extraction to test the water samples. The U.S. Environmental Protection Agency (EPA) Method 531.1 sets the maximum contamination level (MCL) at 40 ppb and uses direct aqueous injection [1]. Each method has its own disadvantages. Liquid-liquid extraction is laborious, time-consuming work that requires a large amount of organic solvents. Direct aqueous injection is a simple method with less interferential peaks; however, it is not suitable for measuring lower levels of compounds in drinking water.

Solid-phase extraction (SPE) is often used for the enrichment of water samples. This process is typically done using a standard SPE vacuum manifold but can be easily automated for the enhanced sensitivity required to measure low-level organic compounds in drinking water.

This application note describes an improved method for determining low levels of carbaryl and carbofuran in drinking water. The method applies an automated procedure of sample concentration and post-column derivatization to successfully measure carbaryl and carbofuran in drinking water at the ppt level. New Agilent SampliQ C18 SPE cartridges were used for sample enrichment from water. The sample was then analyzed with Agilent TC-C18(2) columns, which provide symmetrical peak shape and high sensitivity. Last, the separated compounds were derivatizated before detection with a fluorescence detector. The derivatization reaction is shown in Figure 2. Carbaryl and carbofuran are first hydrolyzed under basic conditions at high temperature into methylamine and then react with o-phthalaldehyde (OPA) and 2-mercaptoethanol into isoindole, which has strong fluorescence. This is a simple, automated, fast, and high recovery method that is fit for quality control of drinking water.

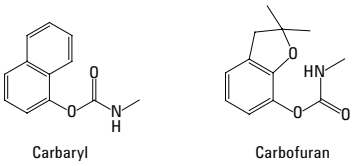

Figure 1. Structures of carbaryl and carbofuran.

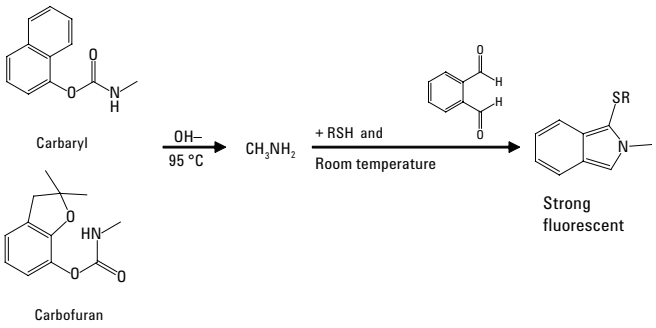

Figure 2. Derivatization reaction of carbaryl and carbofuran.

Experimental

Instruments

HPLC Conditions

Instrument	Agilent 1100 or 1200 with fluorescence detector (FLD): A multidraw upgrade kit (p/n G1313-68711) can be installed when 400-µL injections are needed.
Column	Agilent TC-C18(2) 4.6 × 150 mm, 5 µm (p/n 588935-902)
Mobile phase	A: Water, B: Methanol
	0 min 42% B, 5 min 55% B, 12 min 60% B, 13 min 42% B, stop time 15 min, post-run 2 min
Flow rate	1.0 mL/min
Column temperature	30 °C
FLD detector	Ex 339 nm; Em 445 nm
Injection volume	10 µL
Pinnacle PCX derivatization instrument	Pickering Laboratories, Inc.

Post-Column Reaction Conditions

Flow rate of reagents	0.1 mL/min
Reactor temperature	95 °C
Derivatization temperature	Room temperature
Caliper LS Autotrace SPE Workstation	Caliper Life Sciences, Inc.
SPE Cartridges Agilent Technologies, Inc.	SampliQ C18, 6ml/500mg (p/n 5982-1165)
TurboVap II Evaporation System	Caliper Life Sciences, Inc.

Standards for Calibration Curves

Accurately weigh 10 mg of carbofuran and 12.2 mg of carbaryl standards and separately dissolve in methanol to a volume of 10 mL. Make the stock solution by mixing 60 µL of the carbofuran and 40 µL of the carbaryl solutions into a 10 mL volumetric flask. Dilute to volume with methanol to obtain a concentration of 4.88 µg/mL of carbaryl and 6.0 µg/mL of carbofuran. Dilute aliquots of the stock standard solution with methanol into a series of standard solutions shown in Table 1.

Table 1. Standard Solutions for Calibration Curves

Level	**1**	**2**	**3**	**4**	**5**
Carbaryl (ppb)	48.8	97.6	244.0	488.0	976.0
Carbofuran (ppb)	60.0	120	300.0	600.0	1200

Derivatization Reagents

Sodium hydroxide	0.05 N – Dissolve 2.0 g of sodium hydroxide (NaOH) in reagent water. Dilute to 1.0 L with reagent water. Filter and degas just before use.
Mercaptoethanol (1+1)	Mix 10.0 mL of 2-mercaptoethanol and 10.0 mL of acetonitrile. Cap. Store in hood.
Sodium borate	0.05 N – Dissolve 19.1 g of sodium borate (Na2B407·10H2O) in reagent water. Dilute to 1.0 L with reagent water. The sodium borate will completely dissolve at room temperature if prepared a day before use.
OPA reaction solution	Dissolve 100 ± 10 mg of o-phthalaldehyde (melting point = 55 to 58 °C) in 10 mL of methanol. Add to 1.0 L of 0.05 N sodium borate. Mix, filter, and degas. Add 100 µL of 2-mercaptoethanol (1 + 1) and mix. Make up fresh solution daily.

Other reagents

Methanol	HPLC grade
Water	Milli-Q water as mobile phase and reagent water; tap water as sample
Acetic acid	AR grade
Sodium thiosulfate	AR grade
Dichloromethane	HPLC grade

Sample Preparation Method

We used an automated SPE workstation for sample preparation instead of traditional liquid-liquid extraction in China drinking water standard. Before extraction with SPE, acetic acid was added to the water sample to a concentration of 0.1%. If tap water is used, sodium thiosulfate should be also added to a concentration of 80 mg/L.

The SPE extraction procedure is as follows:

1. Wash syringe with 5.0 mL of CH_2CL_2.
2. Condition cartridge with 5.0 mL of CH_2CL_2 into solvent waste.
3. Wash syringe with 5.0 mL of methanol.
4. Condition cartridge with 5.0 mL of methanol into solvent waste.
5. Condition cartridge with 5.0 mL of reagent water into aqueous waste.
6. Load 1,000.0 mL (1 L) of sample onto column.
7. Rinse cartridge with 5.0 mL of reagent water into aqueous waste.
8. Dry cartridge with N_2 for 10 minutes.
9. Wash syringe with 5.0 mL of CH_2CL_2.
10. Soak for two minutes with 5.0 mL CH_2CL_2 and collect the eluent.
11. Repeat step 10.
12. Collect 5.0-mL fractions into sample tube using air.
13. Evaporate the eluted sample with a stream of N_2 to a volume of 0.5 mL.
14. Inject 10 µL into HPLC for analysis.

Flow Rates Set

Condition flow	1.0 mL/min
Load flow	5.0 mL/min
Rinse flow	1.0 mL/min
Elute flow	1.0 mL/min
Condition air push	15.0 mL/min
Rinse air push	10.0 mL/min
Elute air push	5.0 mL/min

If you do not have an automated SPE workstation, an Agilent vacuum manifold (p/n 5182-9110 or 5182-9120) is recommended. To automatically pull the water sample through, attach an adapter (p/n 5182-9109) with a pipe onto the SPE cartridge. The water sample should be pulled through the SPE by vacuum at the flow rate of no more than 5 mL/min for good recovery.

Results and Discussion

Separation

The standard solutions were analyzed by injecting 10 µL of each of the standard solutions onto the Agilent TC-C18(2) column. The chromatograms from the standard injections (Figure 3) show high performance, high resolution, and symmetrical peaks. We have also run this on an HC-C18(2) column. A little longer retention was achieved with almost the same performance as with the TC-C18(2) column. These columns have a different carbon load: 17 percent for the HC-C18(2) column and 12 percent for the TC-C18(2) column. These differences impact retention, with the HC-C18(2) column typically retaining nonpolar and moderately polar compounds more when compared with the TC-C18(2) column. We prefer the column providing a shorter analysis time but resolving all the peaks. Although both columns are suitable for this method, we chose the TC-C18(2) because it provided a slightly shorter analysis time.

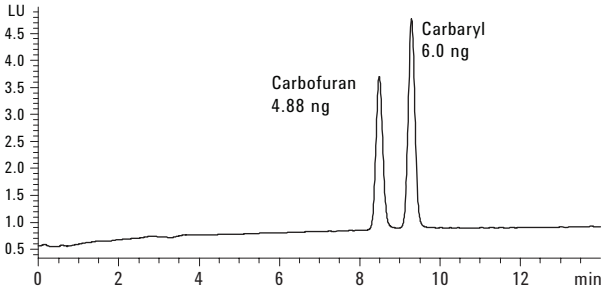

Figure 3. Chromatogram of carbofuran and carbaryl standards on Agilent TC-C18(2), 4.6 × 150 mm, 5 µm columns.

Linearity, Reproducibility, and Limit of Detection

The calibration curves resulting from these standard injections on the TC-C18(2) column are shown in Figure 4. The method shows excellent linearity, being very close to 1.0 (0.9997). To evaluate the reproducibility of this method on the TC-C18(2) column, two concentrations of carbofuran and carbaryl were each injected 10 times. The reproducibility of the peak areas is shown in Table 2; the absolute peak area reproducibility is superior. The average relative standard deviation (RSD) is below 3 percent. We calculate the LOD from the level 1 standard with a signal-to-noise ratio of 3. The LOD is 0.066 ng for carbofuran and 0.080 ng for carbaryl, which is three to four times better than the 0.25 ng that is regulated by China's drinking water standard.

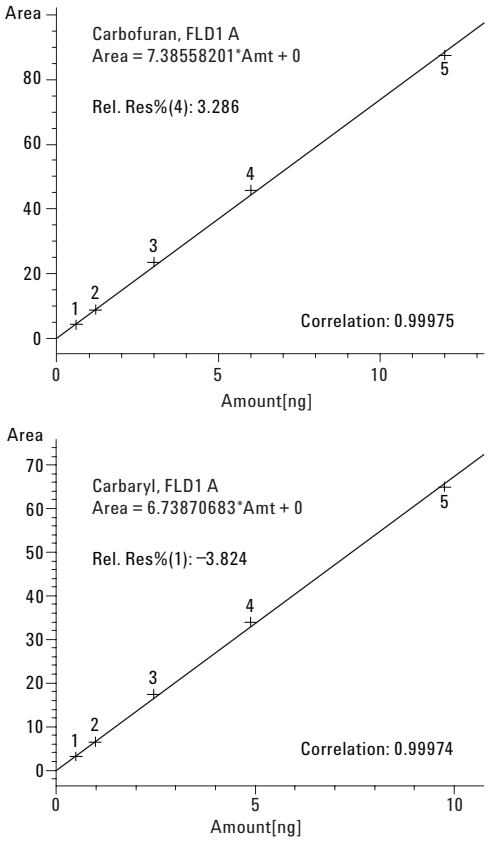

Figure 4. Calibration curves of carbofuran and carbaryl [Agilent TC-C18(2), 4.6 × 150 mm, 5 µm columns].

Table 2. Reproducibility of Standards Injections

Analyte	RSD (%) n = 10 Carbofuran 48.8 ppb Carbaryl 60.0 ppb	RSD (%) n = 10 Carbofuran 488 ppb Carbaryl 600 ppb
Carbofuran	2.3	1.6
Carbaryl	1.8	1.5

Recovery

Two different levels were spiked in reagent water and tap water, respectively, and then followed the sample preparation procedure. The recovery data are good, with a typical recovery in the range of 80 to 110 percent (Table 3).

To better match the China drinking water method, in which dichloromethane is used for liquid-liquid extraction, we chose dichloromethane as the SPE cartridge eluent. That provided us clean chromatograms.

It has been reported that the two compounds are not stable under neutral and basic conditions. [1,3] So the water samples were stabilized by the addition of 0.1 percent acetic acid. According to the China drinking water regulatory method, the residual chlorine present in tap water may result in low recovery, thus sodium thiosulfate should be added to the tap water to eliminate the residual chlorine. We therefore compared the recovery of the tap water with and without sodium thiosulfate. The recovery values obtained without sodium thiosulfate were significantly lower than those with sodium thiosulfate. Therefore, to get good recovery values, we added 80 mg/L of sodium thiosulfate to the tap water sample.

Table 3. Recovery for Carbofuran and Carbaryl from Tap Water and Reagent Water

Analyte	Spiked level (ppb)	Tap water R (%) n = 6	Tap water RSD (%) n = 6	Pure water R (%) n = 3	Pure water RSD (%) n = 3
Carbafuran	0.1	107.8	13.5	84.4	9.5
	0.5	98.8	9.2	98.1	6.8
Carbaryl	0.084	87.0	10.1	83.7	6.2
	0.41	91.8	9.6	94.3	3.3

The chromatograms of reagent water and tap water and their spiked samples are shown in Figures 5 and 6. All the potential interfering compounds in reagent and tap water are well separated from the target compounds, demonstrating good method selectivity.

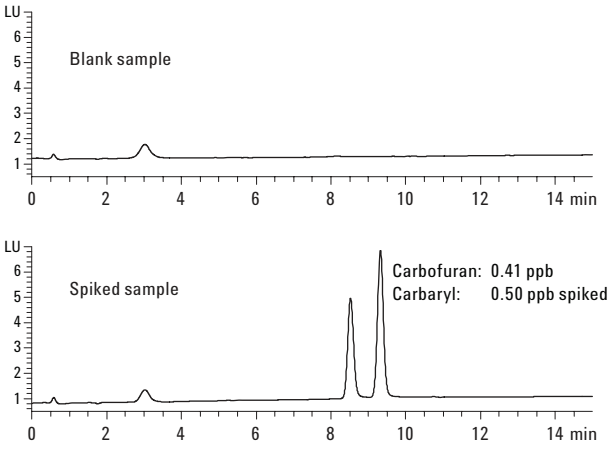

Figure 5. Chromatograms of reagent water and its spiked sample [Agilent TC-C18(2), 4.6 × 150 mm, 5 µm columns].

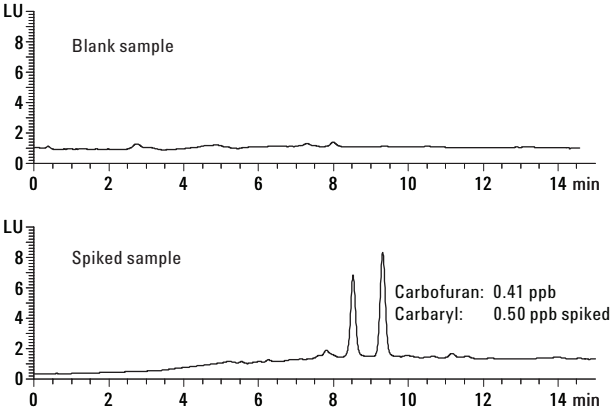

Figure 6. Chromatograms of tap water and its spiked sample [TC C18(2), 4.6 × 150 mm, 5 µm columns].

Direct Injection

A large-volume 400-µL direct injection was applied as described in EPA Method 531.1. We tried a 400-µL injection by using the multidraw attachment on the LC and the TC-C18(2) column. The column has a large surface area (290 m2/g), which allows large-volume injections. The chromatograms in Figure 7 show superior peak shape and high performance. Retention of the two target peaks was excellent and greater-than-baseline resolution was achieved. In addition, the peaks were eluted in a region without any interferences. The column, therefore, can be used for EPA Method 531.1.

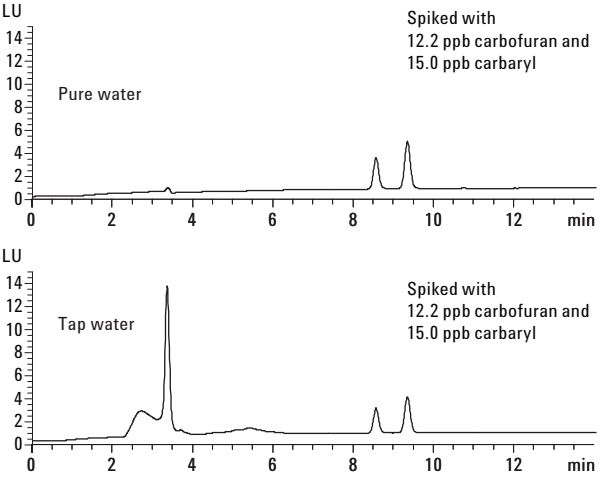

Figure 7. Chromatograms of pure water and tap water spiked with carbofuran and carbaryl.

Conclusions

This total solution enables you to easily analyze drinking water for low levels of the herbicides carbaryl and carbofuran. The Agilent TC-C18(2) column and the new SampliQ C18 SPE cartridge were used for their high sensitivity and low LOD of the two compounds. This method can be used to measure carbaryl and carbofuran in drinking water effectively and quickly.

References

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2. Standards of People's Republic of China, "Standard Examination Methods for Drinking Water – Pesticides, GB/T 5750.9-2006:15.
3. Yuliang Su, et al., "Determination of Carbaryl in Water by HPLC with Direct Injection," *Modern Scientific Instruments*, 2007, 5:125.

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Published in the USA
October 20, 2008
5989-9802EN

Agilent Technologies

Organophosphorus Pesticides Analysis Using an Agilent J&W DB-5ms Ultra Inert Capillary GC Column

Application Note

Environmental

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Abstract

Agilent Technologies Inc. has implemented new testing procedures to more effectively evaluate GC column inertness performance. This new testing procedure employs deliberately aggressive probes to thoroughly investigate and verify column inertness and quality. In challenging separations, knowing that the GC column has been thoroughly investigated for column inertness gives analysts higher confidence in the accuracy of their results.

Trace- and ultra trace-level pesticide analyses are important tools for accessing food supply and environmental quality worldwide. In this application note, trace-level organophosphorus pesticide analysis is demonstrated using electron impact single quadrupole scanning mass spectrometry. Agilent's J&W DB-5ms Ultra Inert capillary GC column provides excellent peak shape for even the most problematic pesticides.

Agilent Technologies

Introduction

Pesticides are commonly used in agricultural and residential applications throughout the world. Organophosphorus pesticides make up approximately 70 percent of the insecticides currently in use. Unfortunately, these highly toxic materials have three main routes of human exposure: inhalation, ingestion, and skin penetration. Sources of these exposures include consumption of foodstuff containing pesticide residues, aerosol inhalation, and dermal contact during pesticide application. [1]

Organophosphorus pesticides use the same mechanism of action as deadly nerve agents such as sarin, soman, and VX. These pesticides affect the nervous system of insects, mammals, and wildlife by inhibiting the enzyme cholinesterase, important in helping regulate nerve impulses. Inactivation of cholinesterase leads to the accumulation of the neurotransmitter acetylcholine in the central and peripheral nervous system, which leads to depressed motor function and respiratory depression. Human toxicities for this class of molecules have shown acute as well as chronic effects from pesticide poisoning. [2,3]

Organophosphorus pesticides tend to be difficult to quantify due to poor peak shape, as evidenced by broad, asymmetrical peaks. An EPA Method 525.2 standard containing organophosphorus pesticides along with a custom pesticide mix acquired from Ultra Scientific (North Kingstown, RI) were analyzed to highlight the value of using a 30-m Agilent J&W DB-5ms Ultra Inert capillary GC column for difficult pesticide analysis. Many pesticides are sensitive to chromatographic system activity and will readily breakdown. The Ultra Scientific custom mix contains several types of these pesticides, which are useful in quickly evaluating system performance with particularly challenging pesticide analytes. Capillary GC column activity as a potential source of result uncertainty has been virtually eliminated with the Ultra Inert series of columns. [4]

Experimental

An Agilent 6890N GC/5975B MSD equipped with a 7683B autosampler was used for this series of experiments. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1A. Chromatographic Conditions for EPA Method 525.2 Calibration Standards

GC	Agilent 6890N/5975B MSD
Sampler	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5181-1273) 1.0-µL splitless injection
Carrier	Helium 44 cm/s, constant flow
Inlet	Pulsed splitless; 250 °C, 40 psi until 0.75 min, purge flow 50 mL/min at 1.0 min
Inlet liner	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column	Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven	40 °C (1 min) to 110 °C (50 °C/min), 7 °C/min to 190 °C, 12 °C/min to 285 °C, hold 2 min.
Detection	MSD source at 250 °C, quadrupole at 150 °C, transfer line at 280 °C, EI mode, scan range 45–450 amu

Table 1B. Chromatographic Conditions for Ultra Scientific Calibration Standards

GC	Agilent 6890N/5975B MSD
Sampler	Agilent 7683B, 5.0-µL syringe (Agilent p/n 5181-1273) 1.0-µL splitless injection
Carrier	Helium 52 cm/s, constant flow
Inlet	Pulsed splitless; 250 °C, 40 psi until 0.75 min, purge flow 50 mL/min at 1.0 min
Inlet liner	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column	Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven	75 °C to 175 °C (15 °C/min), 10 °C/min to 275 °C (1 min)
Detection	MSD source at 250 °C, quadrupole at 150 °C, transfer line at 280 °C, EI mode, scan range 45–450 amu

Table 2. Flow Path Supplies

Vials	Amber crimp-top glass vials (Agilent p/n 5183-4496)
Vial caps	Crimp caps with 11-mm septa (Agilent p/n 5181-1210)
Vial inserts	100-µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe	5 µL (Agilent p/n 5181-1273)
Septum	Advanced Green (Agilent p/n 5183-4759)
Inlet liners	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Ferrules	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
20x magnifier	20x magnifier loupe (Agilent p/n 430-1020)

Sample Preparation

A six-component EPA Method 525.2 pesticide standard mix and internal/surrogate standard mix were purchased from Accu-Standard (New Haven, CT) and used to prepare a six-level calibration standard set. The stock pesticide solution as delivered had a nominal concentration of 1,000 µg/mL. The internal/surrogate solution as delivered had a nominal concentration of 500 µg/mL. The calibration standards were prepared with component concentrations of 10, 5, 2, 1, 0.5, and 0.1 µg/mL and a constant level of 5 µg/mL of internal/surrogate standard as per EPA Method 525.2. All solutions were prepared in acetone using class A volumetric pipettes and flasks. Acetone used was JT Baker Ultra Resi Grade purchased thorough VWR International (West Chester, PA). Acetone was used as a reagent blank and syringe wash solvent.

An 11-component pesticide standard mix was purchased from Ultra Scientific and used to prepare a seven-level calibration standard set. The stock pesticide solution as delivered had a nominal concentration of 1,000 µg/mL. The calibration standards were prepared with component concentrations of 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 µg/mL. All solutions were prepared in 2,2,4-trimethylpentane using class A volumetric pipettes and flasks. The 2,2,4-trimethylpentane used was JT Baker Ultra Resi Grade purchased thorough VWR International (West Chester, PA). 2,2,4-Trimethylpentane was used as a reagent blank and syringe wash solvent.

Results and Discussion

Baseline Inertness Profile for Ultra Inert Columns

The basic approach for inertness verification for the Agilent J&W Ultra Inert series of capillary GC columns is testing with aggressive active probes at low concentration and low temperature. [5] This is a rigorous approach that establishes consistent baseline inertness profiles for each column in the Agilent J&W Ultra Inert GC column series. The baseline inertness profile then serves as a predictor for successful analysis of chemically active species that tend to adsorb onto active sites, particularly at trace level, like the organophosphorus pesticides in this application example. A more detailed description of the test mix and additional application examples can be found in references 6 through 8.

Organophosphorus Pesticide Analysis

In this application note, a multilevel pesticide calibration curve set was evaluated over the concentration range of 0.1 to 10 µg/mL on an Agilent J&W Ultra Inert DB-5 ms 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI). Separate calibration curves were developed for both the EPA 525.2 organophosphorus and Ultra Scientific standards. The standard levels used for the 525.2 calibration were 0.1, 0.5, 1, 2, 5, and 10 µg/mL, while the Ultra Scientific calibration levels were 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 µg/mL. The custom pesticide standard from Ultra Scientific was used to determine system performance by analyzing difficult pesticides, such as endrin and p,p'-DDT, which are prone to analyte breakdown.

No tailing was observed for any of the organophosphorus pesticide peaks across the range studied in either standard set. Sharp, symmetrical peak shapes were noted for all the organophosphorus pesticides analyzed. Good resolution was obtained for each of the pesticides investigated.

Linearity for the 525.2 standard components was excellent across the range studied, giving R^2 values of 0.997 or greater in all cases but fenamiphos, which had an R^2 value of 0.978. This value increases to 0.991 at the midlevel concentrations as suggested by EPA Method 525.2 Sec. 13.2.3.3. Figure 5 indicates the correlation coefficients for each of the individual pesticides and shows an example linear regression plot for disulfoton.

Linearity for the Ultra Scientific standard components was also quite good across the range studied. R^2 values of 0.990 or greater were obtained for the organophosphorus pesticides. Figure 6 indicates the correlation coefficients for each of the individual pesticides and shows an example linear regression plot for mevinphos.

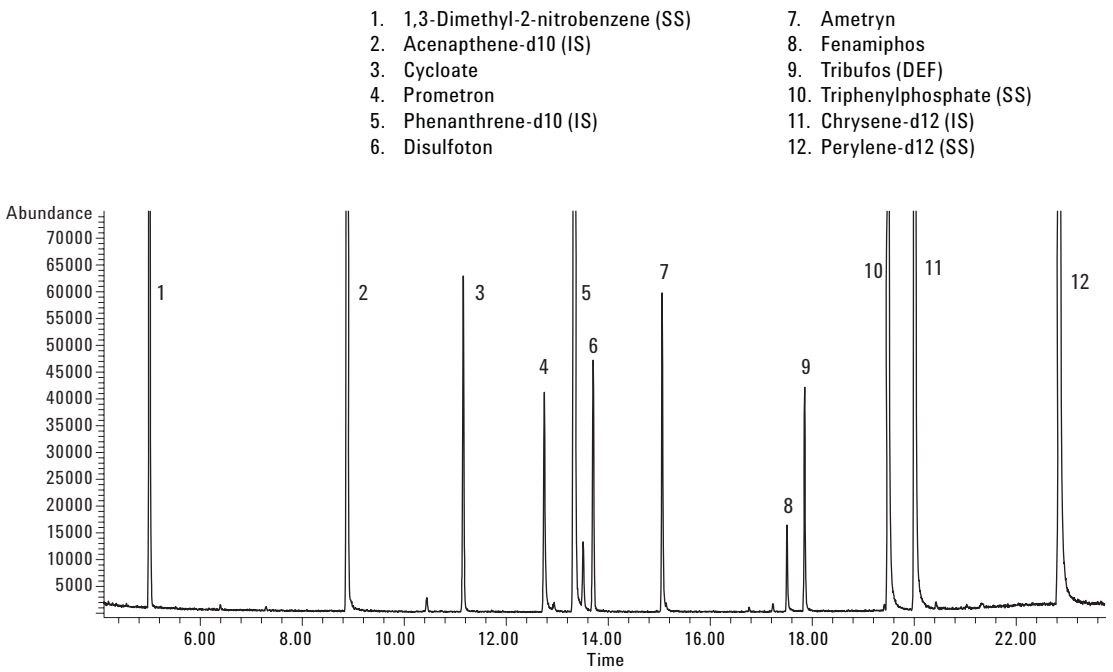

Figure 1. Total ion chromatogram (scan mode) of the 1-ng on-column EPA Method 525.2 standard solution loading on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). Chromatographic conditions are listed in Table 1A.

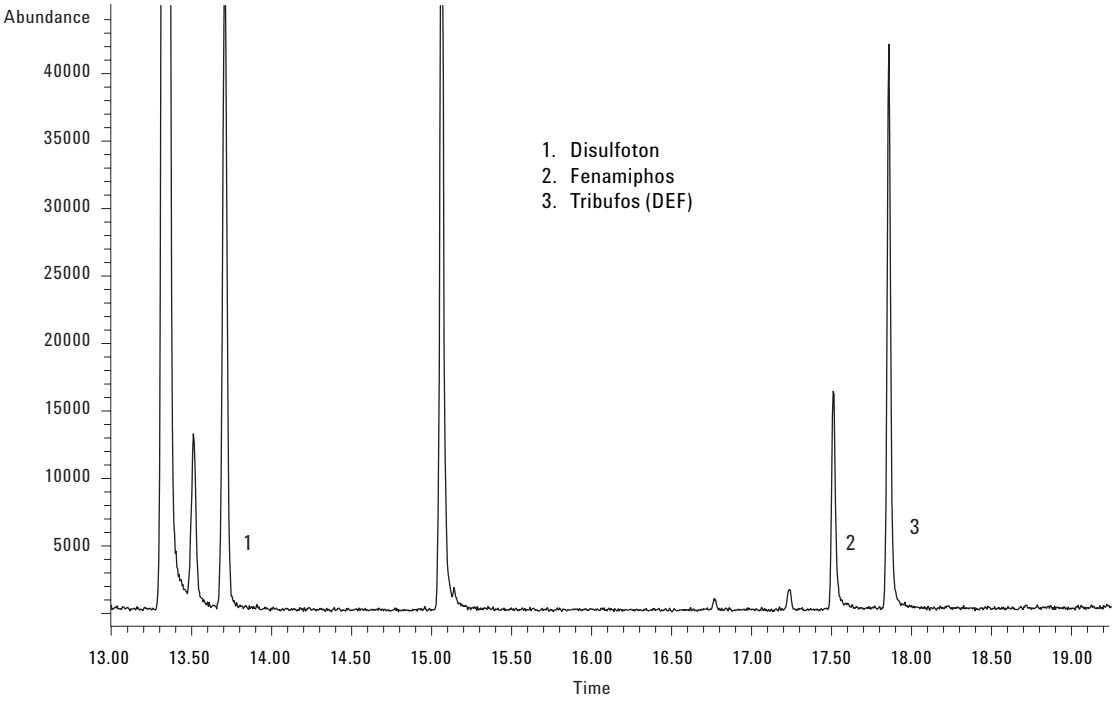

Figure 2. Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 1.0 µg/mL EPA Method 525.2 standard pesticide mix. The peaks noted in the figure are the three organophosphorus pesticides of interest. Chromatographic conditions are listed in Table 1A.

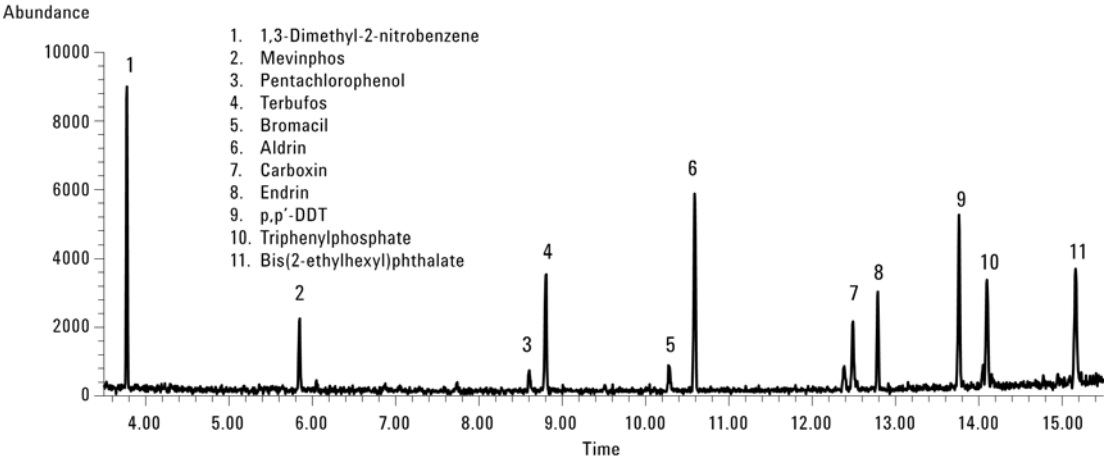

Figure 3. Total ion chromatogram (scan mode) of the 0.1-ng on-column Ultra Scientific standard solution loading on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). Chromatographic conditions are listed in Table 1B.

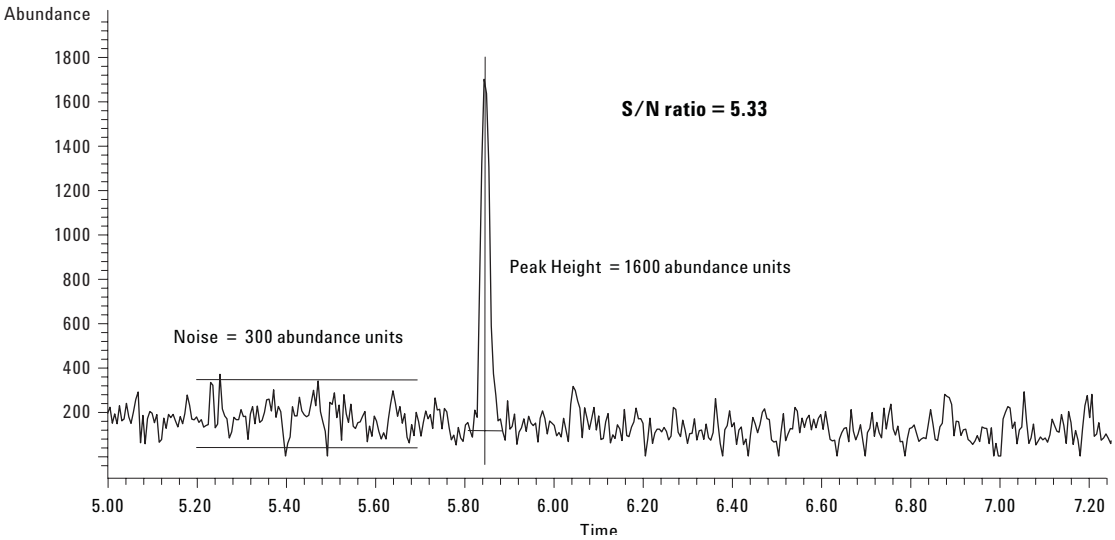

Figure 4. Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 0.1 µg/mL Ultra Scientific standard pesticide mix on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). The peak in the figure is mevinphos, an organophosphorus pesticide of interest. This injection represents an on-column loading of 0.1 ng per component. Chromatographic conditions are listed in Table 1B.

Component	R^2
Cycloate	1.000
Prometon	0.999
Disulfoton	0.999
Ametryn	0.999
Fenamiphos	0.978
Tribufos (DEF)	0.997

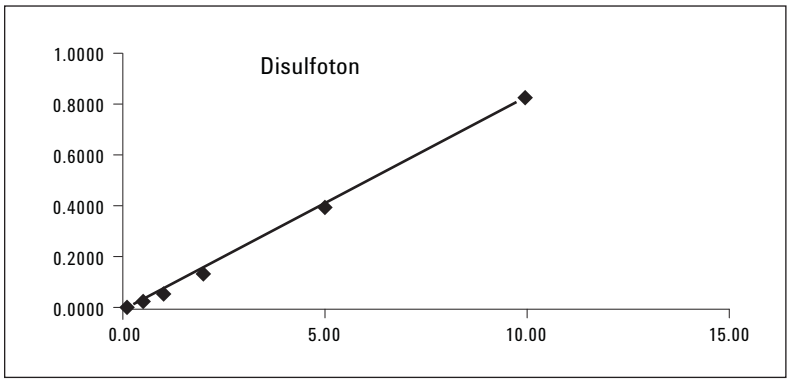

Figure 5. Correlation coefficients for the EPA Method 525.2 pesticide components over the 0.1 to 10 µg/mL range of this study and an example linear regression plot for disulfoton.

Component	R^2
1,3-Dimethyl-2-nitrobenzene	0.999
Mevinphos	0.990
Pentachlorophenol	0.989
Terbufos	0.996
Bromacil	0.988
Aldrin	0.999
Carboxin	0.996
Endrin	0.998
p,p'-DDT	0.996
Triphenylphosphate	0.997
DEHP	0.996

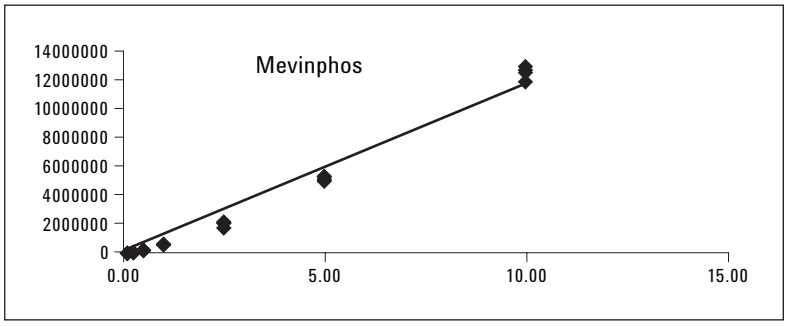

Figure 6. Correlation coefficients for the Ultra Scientific pesticide components over the 0.1 to 10 µg/mL range of this study and an example linear regression plot for mevinphos.

Conclusions

This application successfully demonstrates the use of an Agilent J&W DB-5ms Ultra Inert capillary GC column for trace-level organophosphorus pesticides. Linearity was excellent for all organophosphorus pesticides studied, yielding 0.99 or greater R^2 values down to a 0.1-ng on-column loading of each component. One of the reasons for excellent linearity and high R^2 values is the highly inert surface of the column. The lack of chemically active sites makes these columns an excellent choice for trace-level applications.

This study was done using scan mode on an Agilent 6890/5975B GC/MSD equipped with an inert electron impact source. The signal-to-noise ratio for a 0.1-ng on-column loading of mevinphos was greater than 5 to 1 with this system. This result shows clearly the power of using an Agilent J&W DB-5ms Ultra Inert column for trace-level organophosphorus pesticides analysis. Lower limits of quantification are expected when using one of Agilent's latest GC/MS offerings, such as the 7890/5975C GC/MSD Triple-Axis Detector coupled with an Agilent J&W DB-5ms Ultra Inert GC capillary column.

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Published in the USA
November 12, 2008
5989-9879EN

Agilent Technologies

A Rapid Method for Trace Analysis of Organophosphorus Pesticides in Drinking Water

Application Note

Environmental

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Abstract

A simple and quick method for the determination of organophosphorus pesticides (OPs) in drinking water has been developed. After sample extraction with methylene chloride, analysis was directly carried out without further treatment using GC with a specific detector FPD on a DB-1701P column. A linear relationship between concentration and peak area was obtained within the range of 0.005 to 0.500 ng with correlation coefficients greater than 0.999 and detection limits less than 0.03 µg/L. Recoveries of six OPs at spiked levels of 0.50, 2.50, and 4.50 µg/L ranged from 88 to 104% These OPs were reproducibly detected well below the maximum residue limits (MRLs) of EPA Method 525 and European Union regulations for pesticide residues in drinking water.

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Introduction

Organophosphorus pesticides (OPs) are among the most common pesticides used in industrialized countries. These compounds are very toxic when absorbed by human organisms because of acetylcholinesterase deactivation. Due to their universal application in agriculture, OPs represent an important source of environmental contamination. Maximum residue limits (MRLs) have been established for pesticides in foodstuff and drinking water in most countries to avoid any adverse impact on public health. U.S. Environmental Protection Agency (EPA) Method 525 has a maximum allowable risk level for OPs in drinking water ranging from 0.001 to 0.25 mg/L [1]. In the European Union (EU), a maximum allowable concentration of 0.0001 mg/L for each individual pesticide in drinking water is in force. For evaluation of environmental waters and water sources for preparation of drinking water, highly sensitive methods for the determination of OPs in surface water, ground water, and drinking water are required.

Most analytical methods for pesticide analysis of OPs in aqueous samples are based on chromatographic techniques. Gas chromatography (GC) with nitrogen-phosphorus detection [2] (NPD), mass spectrometry [1] (MS), and flame photometric detection [3] (FPD) has traditionally been the method of choice for the analysis of OPs. FPD is a highly selective and sensitive detector that works by measuring the emission of phosphorus- (or sulfur-) containing species, which will minimize interferences from materials that do not contain phosphorus. Since the chromatograms of extracts were free from interfering peaks, no cleanup was needed. For the OPs analysis, FPD is a potentially efficient detector for monitoring water samples.

OPs are active compounds that can be adsorbed onto active sites throughout the sample flow path, including the injection port, liner, golden seal, capillary column, and any metal detector parts. A capillary column is one of the major sources of active sites owing to its large surface area and the long residence time of an analyte in the column. Peak tailing, response loss, and compound degradation will occur for these active compounds when the column is not inert.

The DB-1701P column was specially designed for the analysis of pesticides [4]. It has a better inertness for active compounds, which offers improved resolution, better selectivity, and higher sensitivity for OPs analysis. This application note presents a sensitive method developed on a DB-1701P column using GC/FPD for the analysis of OPs in drinking water.

Experimental

Instrument

An Agilent 7890 GC equipped with split/splitless capillary inlets and FPD was used for this work. The inlets were fitted with a long-lifetime septa (P/N 5183-4761) and single-taper helix liner (P/N 5188-5397). Injections were done using 10-µL syringes (P/N 9301-0714).

Many analytes will degrade on reactive sites in the chromatographic system. Analysts must ensure that injectors and splitters are free from contamination and are silanized. Columns should be installed and maintained properly.

GC Conditions

Column	DB-1701P, 30 m × 0.32 mm × 0.25 µm (P/N 122-7732)
Carrier gas	Helium, constant pressure mode, 25 psi
Inlet	Split/splitless @ 270 °C, splitless
Oven temperature	60 °C (1 min); 30 °C/min to 180 °C (7 min); 15 °C/min to 220 °C (3 min)
Detector	250 °C, FPD in phosphorus mode
Detector gas	H2 75 mL/min, air 100 mL/min, makeup (N2) 60 mL/min
Injection size	1 µL

Standard Solution

Six OP stock solutions (see Table 1) were purchased from China National Standards Research Center. These six OPs are commonly used in agriculture and are strictly monitored. A mix stock solution (10 mg/L) of OPs was prepared in acetone. Six calibration standards solutions were prepared by diluting the stock solution with acetone. The calibration standard solutions should be stored in tightly sealed bottles at temperatures below 5 °C.

Table 1. Six Organophosphorus Pesticides Solutions

Compound	Molecular formula	Molecular weight	Standard solution (mg/mL) in methanol
1 Dichlorvos	$C_4H_7Cl_2O_4P$	220.98	0.89
2 Dimethoate	$C_5H_{12}NO_3PS_2$	229.28	1.00
3 Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	350.59	1.00
4 Methylparathion	$C_8H_{10}NO_5PS$	263.63	1.00
5 Malathion	$C_{10}H_{19}O_6PS_2$	330.36	1.02
6 Parathion	$C_{10}H_{14}NO_5PS$	291.26	1.00

Sample Preparation

100 mL of water sample was transferred to a 250-mL separatory funnel. After adding 20 mL of methylene chloride, the separatory funnel was sealed and then shaken vigorously for 1 to 2 minutes with periodic venting to release excess pressure. Once the funnel was still for 10 minutes, the extract for the organic layer was collected. The extraction was repeated twice, using fresh portions of solvent. The resulting three portions of the extracts were combined and dried with anhydrous sodium sulfate, then evaporated to near dryness. The residue was dissolved with 1 mL of acetone and transferred into the sample vial for GC analysis.

Results and Discussion

The separation of six OPs is illustrated in Figure 1. As you can see, all OPs can be baseline separated with highly efficient and symmetrical peaks on the DB-1701P column, which demonstrated significantly reduced peak tailing and adsorption for these challenging analytes.

Linearity and Reproducibility

FPD is a selective detector for sulfur and phosphorus compounds in complex mixtures. The response of the FPD is linear in phosphorus mode. Table 2 shows the linearity range, r^2 values for six OPs calculated from the study. The calibration curve was constructed from data obtained by 1-µL injections of standards at six levels. All the OPs exhibit a wide linear range from 0.005 to 0.500 ng, with r^2 values higher than 0.999, suggesting a good linearity range for low-level OP quantification in drinking water.

Table 2. Linearity and Limit of Detection (S/N = 3)

Compound		Linearity (ng)	Correlation coefficient (R^2)
1	Dichlorvos	0.004 ~ 0.445	0.9993
2	Dimethoate	0.005 ~ 0.500	0.9991
3	Chlorpyrifos	0.005 ~ 0.500	0.9994
4	Methylparathion	0.005 ~ 0.500	0.9993
5	Malathion	0.005 ~ 0.510	0.9993
6	Parathion	0.005 ~ 0.500	0.9993

The reproducibility of the method was investigated by replicate analysis of three levels of OPs (0.050, 0.250, and 0.450 ng) in Table 3. The relative standard deviation (RSD) of the retention time (RT) of the six OPs was lower than 0.017%. Peak areas were reproducible with an RSD of less than 4.0%. Good RT and peak area repeatability ensure reliable qualitative and quantitative analyses.

Table 3. Reproducibility of Peak area and Retention time (n ≥ 10)

		RSD (%) (n ≥ 10)					
Compound		0.050 ng		0.250 ng		0.450 ng	
		Area	RT	Area	RT	Area	RT
1	Dichlorvos	3.364	0.011	1.680	0.007	1.620	0.011
2	Dimethoate	3.904	0.015	1.497	0.017	1.752	0.011
3	Chlorpyrifos	1.303	0.008	1.476	0.010	1.196	0.009
4	Methylparathion	1.963	0.011	1.642	0.009	1.169	0.008
5	Malathion	1.084	0.009	1.842	0.005	1.426	0.006
6	Parathion	1.750	0.006	1.666	0.008	1.300	0.007

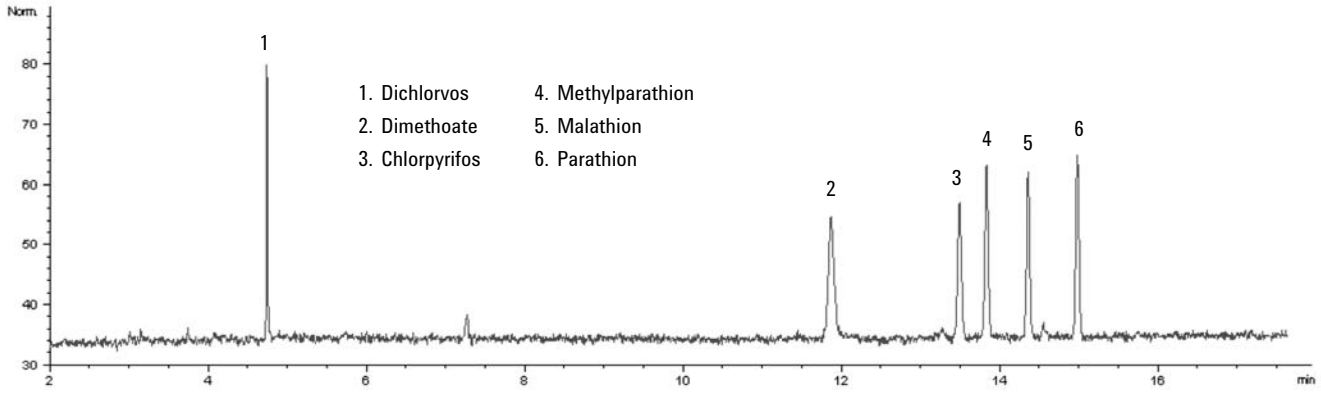

Figure 1. Chromatograms of six OPs standard solution on DB-1701P column.

Recovery and Limit of Detection (LOD)

Table 4 presents the recoveries for spiked water samples. Replicate samples of 100-mL ultrapure water were spiked with OPs at 0.50, 2.50, and 4.50 µg/L. During the study, no target OPs were found in the ultrapure water, so it is regarded as blank water. The spiked samples were treated according to the procedure described in the sample preparation. Excellent recoveries were obtained for all the compounds, ranging from 88 to 104%. Duplicate samples were analyzed and demonstrated that the method has a good repeatability at trace levels (see Figure 2).

Table 4. Recovery of Three Levels of OPs

		Recovery (%)		
Compound		**0.50 µg/L**	**2.50 µg/L**	**4.50 µg/L**
1	Dichlorvos	88.7	90.0	91.2
2	Dimethoate	103.5	98.5	100.7
3	Chlorpyrifos	90.3	90.4	91.0
4	Methylparathion	92.8	92.5	91.6
5	Malathion	92.2	91.6	92.5
6	Parathion	91.8	90.4	91.8

Table 5 lists the LODs of the method and the MRLs of EPA Method 525. The LODs were determined at a signal-to-noise ratio of 3. It demonstrates the high sensitivity of FPD for trace analysis of OPs. The developed method enables quantitative determination of OPs in water solutions at concentration levels lower than 0.03 µg/L, which is about 100 times lower than the MRLs in EPA Method 525. It also meets the requirement of EU limits (0.1 µg/L) in drinking water.

Table 5. Limit of Detection in 100-mL Water Sample

Compound		**LOD (µg/L)**	**MRLs* (µg/L)**
1	Dichlorvos	0.012	1
2	Dimethoate	0.030	80
3	Chlorpyrifos	0.027	30
4	Methylparathion	0.021	20
5	Malathion	0.023	250
6	Parathion	0.020	3

* MRLs in EPA Method 525

Real Sample

In order to check the applicability of the proposed method to real matrices, tap water samples and ultrapure water samples were collected. A 100-mL aliquot of each sample was analyzed following the procedure described in the sample preparation section. Peak areas were used for quantitation. The use of FPD eliminates the interferences that do not contain phosphorus. None of the samples gave peaks that interfered with the determination of the six OPs (Figure 3). In these samples, no OPs were found above the method's LOD.

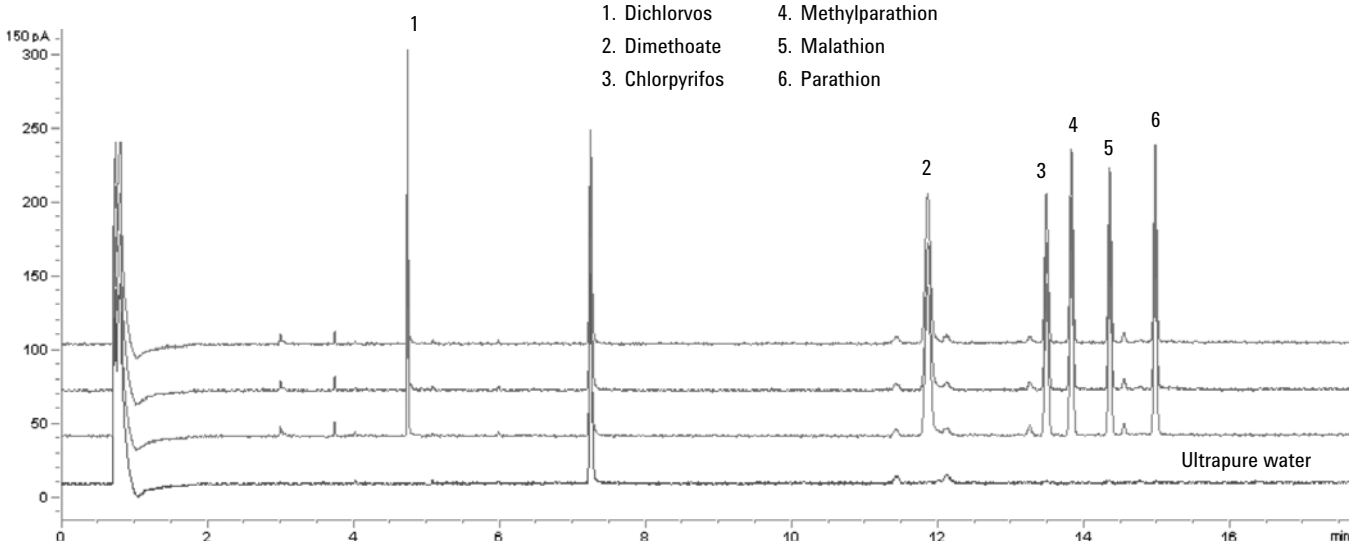

Figure 2. Overlay chromatogram of 0.50-µg/L spiked samples on DB-1701P column.

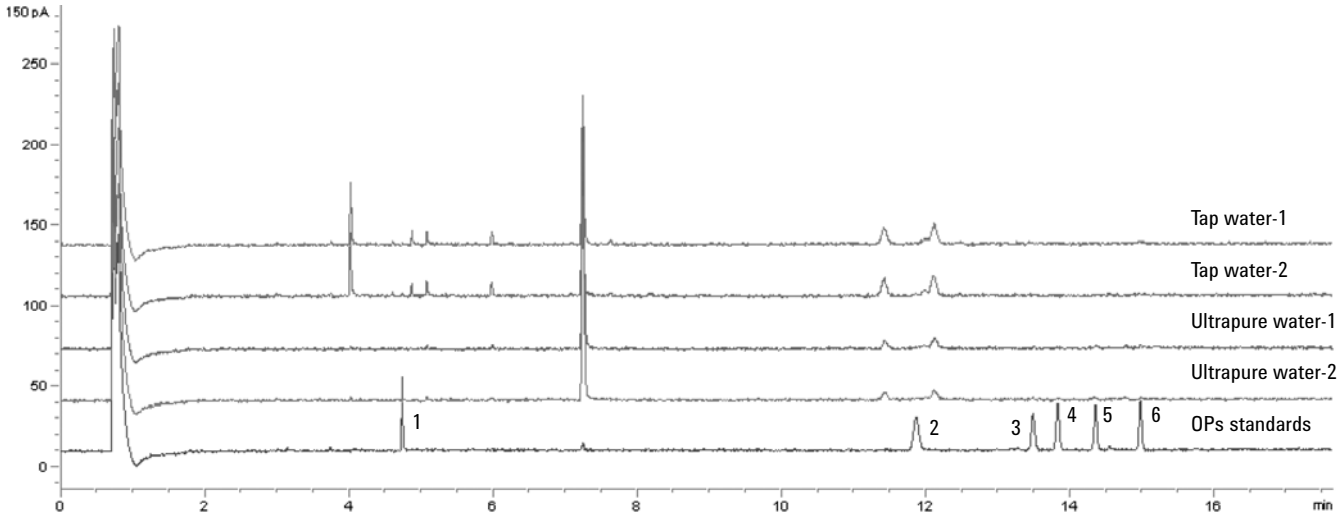

Figure 3. Chromatogram of real water samples on DB-1701P column.

Conclusions

This application note describes a method for the quantification of OPs in drinking water samples. After liquid-liquid extraction and concentration, the sample extracts were directly analyzed on a DB-1701P column using an Agilent 7890 Series GC with FPD. The method provides good linearity, repeatability, and high recovery. It is adequate to determine OPs LODs lower than 0.03 µg/L, which is in full compliance with the MRLs in EPA Method 525 and EU regulations in drinking water. The local drinking water samples were determined to be free of OP contamination.

It is a fast, simple, and economic method to analyze OPs at micro-trace levels. Therefore, it is suitable to control the water quality for OPs according to the MRLs specified in the regulations.

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Published in the USA
November 13, 2008
5990-3158EN

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Analysis of Organochlorine Pesticides in Drinking Water by Agilent 7820 Gas Chromatograph/Micro-Electron Capture Detector

Wenmin Liu and Chunxiao Wang

Application Brief

Environmental

The Agilent 7820 GC configured with a micro-electron capture detector (μECD) provides high sensitivity, good linearity, and stability for the analysis of organochlorine pesticides in drinking water. The results achieved are better than the requirements stated in the Chinese National Standard Method GB/T 5750.9-2006 [1,2].

Experiment

Analytical Conditions

Inlet	260 °C, Split/splitless, Liner (5062-3587)
Injection volume	1 μL
Column	30 m × 0.32 mm × 0.25 μm (Agilent J&W 123-0732)
Carrier gas	N_2, Constant flow: 1 mL/min
Oven	50 °C (3 min) 30 °C/min 210 °C (30 min)
Detector	ECD, 300 °C, Makeup flow (N_2): 60 mL/min
Data analysis system	Agilent EZChrom Elite Compact

Results

The Agilent 7820 μECD's high sensitivity ensures reliable and rugged trace pesticide analysis in drinking water. The method can be easily transferred across geographies and labs. Figure 1 shows the chromatogram of 500 ng/L pesticides using the Agilent 7820 μECD with excellent signal to noise (S/N).

The design of the μECD ensures a wide linear dynamic range for the analytes. Table 1 shows linearity (R^2) for the pesticides.

Highlights

- The Agilent 7820 configured ECD provides high sensitivity for the analysis of organochlorine pesticides.
- ALS and EPC ensure good repeatability and ease of use.
- Using N_2 as the carrier gas significantly lowers analysis cost compared with the use of He.
- Agilent EZChrom Elite Compact software is designed for the Agilent 7820 is used for easy data acquisition and analysis.

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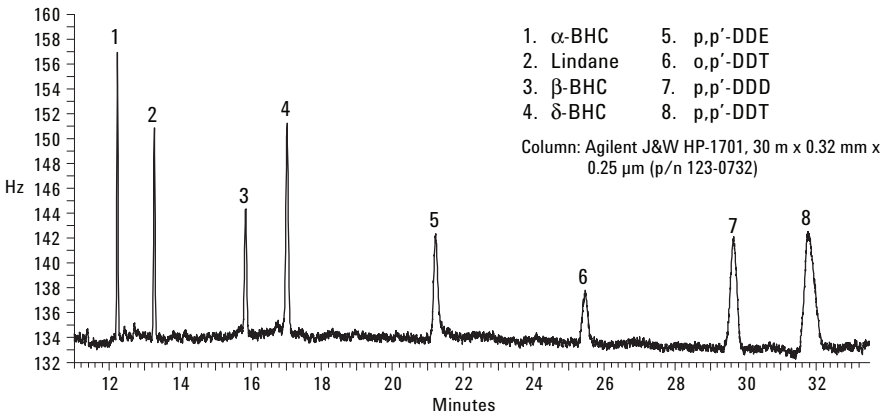

Figure 1. Chromatogram of organochlorine pesticides at 500 ng/L.

Table 1. Pesticide Linearity Data

Peak no.	Compounds	Linear range (ppb)	R^2
1	α-BHC	0.5–500	0.9998
2	β-BHC	0.5–500	0.9998
3	Lindane	0.5–500	1
4	σ-BHC	0.5–500	0.9996
5	p,p'-DDE	1–500	0.9998
6	o,p'-DDT	1–500	0.9998
7	p,p'-DDD	0.5–500	0.9999
8	p,p-DDT	0.5–500	0.9993

The use of an automatic liquid sampler (ALS) and EPC ensure the ease of use of the 7820 and its good repeatability. The response factors (RFs) and their relative standard deviations (RSDs) (n = 10) are less than 20%, much better than the precision requirements for RFs in the contract laboratory program of the United States Environmental Protection Agency (USEPA).

Proven reduced susceptibility to contamination on the µECD improves the analysis of dirty matrix samples. Figure 2 shows the chromatograms of pesticide standards and spiked water sample (10 ppb). The recoveries of each analyte are shown in Table 2. The sample preparation method refers to standard GB/T 5750.9-2006.

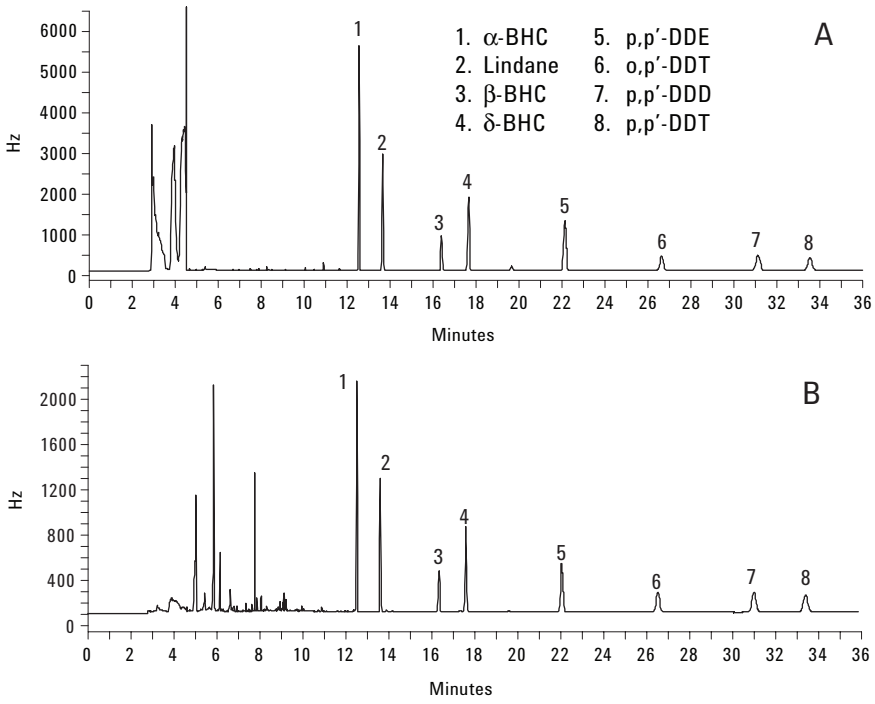

Figure 2. Chromatograms of organochlorine pesticides standard at 10 µg/L (A) and spiked drinking water (B).

Table 2. Recoveries of Analytes

Compounds	α-BHC	β-BHC	Lindane	σ-666	p,p'-DDE	o,p'-DDT	p,p'-DDD	p,p-DDT
Recovery (%)	101.5	96.6	85.7	99.2	92.7	92.2	86.3	97.9

References

1. Chinese National Standard Methods for Drinking Water – Pesticides Parameters, GB/T 5750.9-2006
2. Tu Chuanhong, "Analysis of Organochlorine and Pyrethroid Pesticides with Agilent 6820 Gas Chromatograph/Micro-Electron Capture Detector," Agilent Technologies publication 5989-1333EN

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Printed in the USA
January 23, 2009
5990-3473EN

Agilent Technologies

Achieving the Desired Prescribed Sensitivities of Selected Herbicides by Direct On-Column Aqueous Injection of Potable and Environment Samples Using the Agilent 6410BA LC/QQQ

Application Note

Environmental

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Abstract

Here we describe the analysis of 20 selected herbicides by direct on-column aqueous injection of environmental water samples of several matrices with little pretreatment. We demonstrate that this approach fulfills sufficient sensitivity requirements outlined by the UK Drinking Water Inspectorate. Precision data obtained were typically in the range of 2.2 to 7.0% and analyte recoveries were between 90.2 to 104.7%. Limits of detection were less than 10 ng/L (10 ppt) for all of the compounds in this suite.

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Introduction

Several sample preparation/analysis approaches are available for the determination of herbicides in water samples, typically LC/MS after solid phase extraction (SPE) and even LC/MS employing on-line analyte enrichment [1]. Solid phase extraction is time consuming and adds an expense to the method with consumable materials and additional man-hours. On-line enrichment [ibid], on the other hand, requires the purchase of additional hardware, such as switching valves and an additional LC pump. With the introduction of affordable, reliable and sensitive LC/QQQ instrumentation such as the Agilent 6410BA triple quadrupole LC/MS system, it is now possible to achieve prescribed analysis requirements by injecting aqueous samples directly onto the analytical column using conventional injection volumes of up to 100 µL.

The aim of this application note is to demonstrate a reliable and robust analytical method for the analysis of 20 selected herbicides in potable and environmental water samples, with a performance criteria of < 12.5% analyte precision, analyte recoveries in the range of 90 to 110%, and limits of detection < 10 ng/L (10 ppt).

The method presented here describes the analysis of a mixture of 20 acidic, neutral, and basic herbicides (Figure 1) in different water matrices by direct aqueous injection. An overview of the full validation data is summarized.

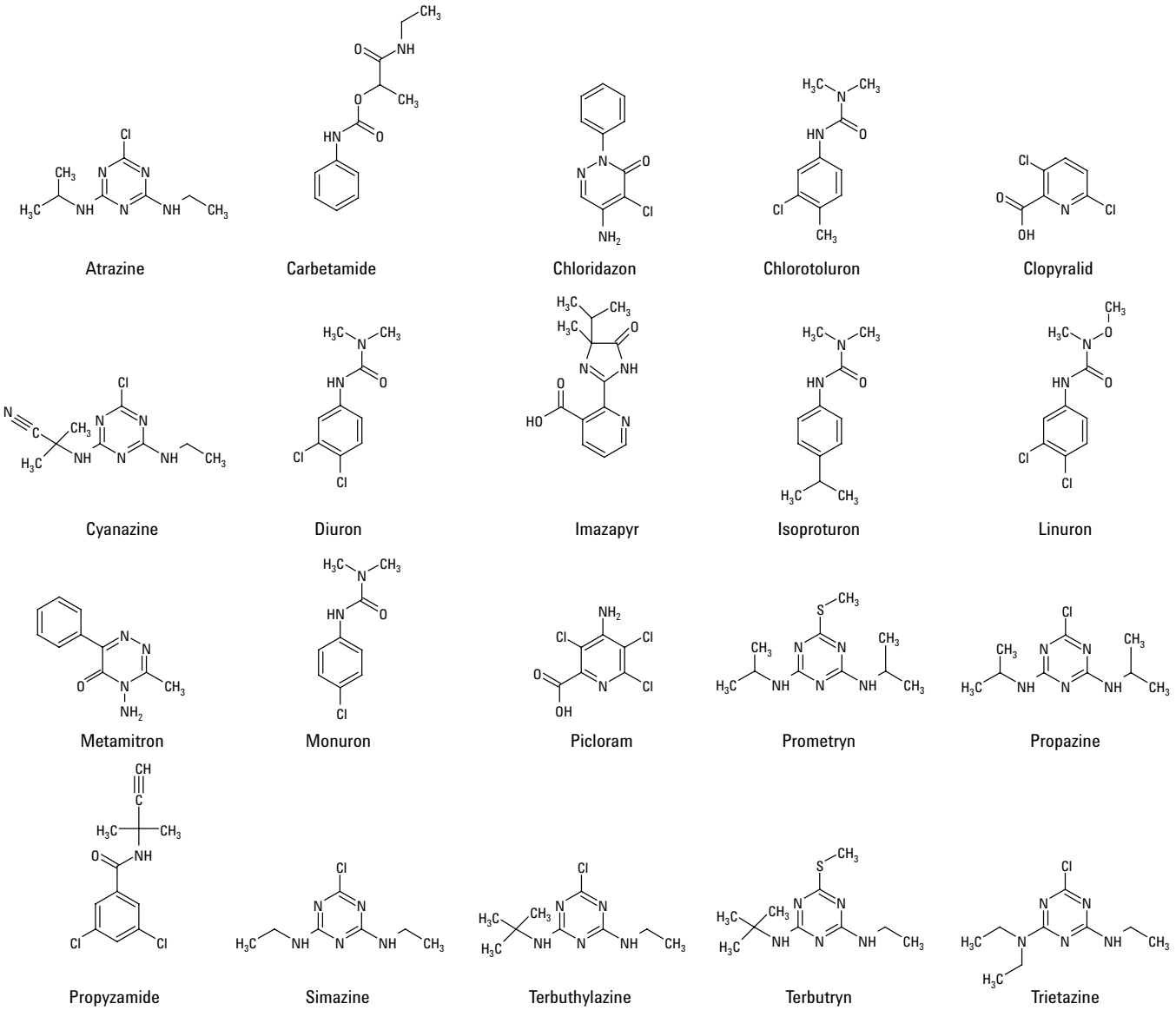

Figure 1. Suite of neutral, acidic, and basic herbicides.

Experimental

This analysis was performed using an Agilent LC/QQQ 6410BA mass spectrometer upgraded with a hotbox kit coupled to an Agilent 1200 Series LC system. The LC system consisted of a binary pump (G1312B), vacuum degasser (G1379B), automatic liquid sampler (G1367C), thermostatted column compartment (G1316B), and MassHunter data system. The hotbox upgrade kit (G2573A) comprised an additional MS turbo-pump with controller and replacement entrance and exit lenses for the collision cell.

Sample Preparation

Minimal sample preparation was required, which was simple acidification of all standards and samples. These were acidified to a concentration of 0.1% formic acid, which was used as the pH modifier.

Instrumentation

LC Conditions

Column:	Agilent ZORBAX SB-C18, 2.1 × 100 mm 1.8 µm thermostatted at 70 °C
Mobile phase	A: 0.1% formic acid in HPLC water B: methanol

Gradient program:

Time (min)	A (%)	B (%)	Flow rate (mL/min)
Initial	95	5	0.3
0.5	95	5	0.3
1.0	80	20	0.3
20.0	20	80	0.3
20.1	95	5	0.3

Injection volume:	100 µL
Total run time:	26.0 min

QQQ MS Conditions

Source Conditions

Electrospray AP-ESI:	Positive ionization polarity
Drying gas temperature and flow:	300 °C, 10 L/min
Nebulizer gas pressure:	40 psi
Capillary voltage:	3000 V
Fragmentor voltage:	See Table 1
MRM parameters:	See Table 1

MRM Parameters

Table 1. MRM Transitions for Herbicide Suite

Time seg	Time (min)	Delta EMV (V)	Compound	Precursor ion (*m/z*)	Product ions (*m/z*)	Fragmentor voltage (V)	Collision energy (V)	Dwell time (msec)
2	0.2	600	Clopyralid	192.0	146.2	75	19	400
			Clopyralid	192.0	174.2 (q)*	75	6	100
3	6.4	600	Picloram	241.0	223.1	95	9	400
			Picloram	241.0	195.0 (q)*	95	18	100
4	7.6	400	Metamitron	203.1	175.1	115	14	90
			Metamitron	203.1	104.1 (q)*	115	22	90
			Imazapyr	262.2	234.3	130	14	90
			Imazapyr	262.2	217.2 (q)*	130	17	90
			Chloridazon	222.1	104.2	135	22	90
			Chloridazon	222.1	92.1 (q)*	135	27	90
5	10.0	400	Carbetamide	237.1	192.3	80	2	70
			Carbetamide	237.1	72.2 (q)*	80	22	70
			Monuron	199.1	72.2	105	16	70
			Monuron	199.1	126.1 (q)*	105	25	70
			Cyanazine	241.2	214.2	125	12	70
			Cyanazine	241.2	104.1 (q)*	125	31	70
			Simazine	202.1	132.2	125	16	70
			Simazine	202.1	104.1 (q)*	125	27	70
6	14.5	400	Chlorotoluron	213.1	72.2	110	21	250
			Chlorotoluron	213.1	140.2 (q)*	110	24	250
7	15.6	400	Diuron	233.1	72.2	110	22	90
			Diuron	233.1	160.3 (q)*	110	26	90
			Atrazine	216.2	174.2	120	15	90
			Atrazine	216.2	104.1 (q)*	120	32	90
			Isoproturon	207.2	72.2	110	22	90
			Isoproturon	207.2	165.3 (q)*	110	10	90
8	17.0	400	Prometryn	242.2	200.3	135	17	30
			Prometryn	242.2	158.2 (q)*	135	24	30
			Terbutryn	242.2	186.2	120	17	30
			Terbutryn	242.2	91.2 (q)*	120	30	30
			Linuron	249.1	182.1	105	18	100
			Linuron	249.1	160.3 (q)*	105	12	100
			Propazine	230.2	188.2	125	15	30
			Propazine	230.2	146.1 (q)*	125	24	30
			Terbuthylazine	230.2	174.2	110	15	30
			Terbuthylazine	230.2	104.1 (q)*	110	30	30
			Propyzamide	256.1	190.1	95	12	30
			Propyzamide	256.1	173.0 (q)*	95	22	30
9	19.6	400	Trietazine	230.2	202.2	130	18	250
			Trietazine	230.2	99.2 (q)*	130	24	250

*(q) = Qualifier ion

Results and Discussion

The total ion chromatogram (TIC) for a 0.5 µg/L (500 ppb) standard consisting of this 20 herbicide suite is shown in Figure 2, which also illustrates the positioning of the time segmentation.

Five levels of calibration standards were used to prepare the calibration curves over the concentration range of 0.0, 0.05, 0.10, 0.30, and 0.50 µg/L. Selected and representative calibration curves are shown in Figures 3a through 3c.

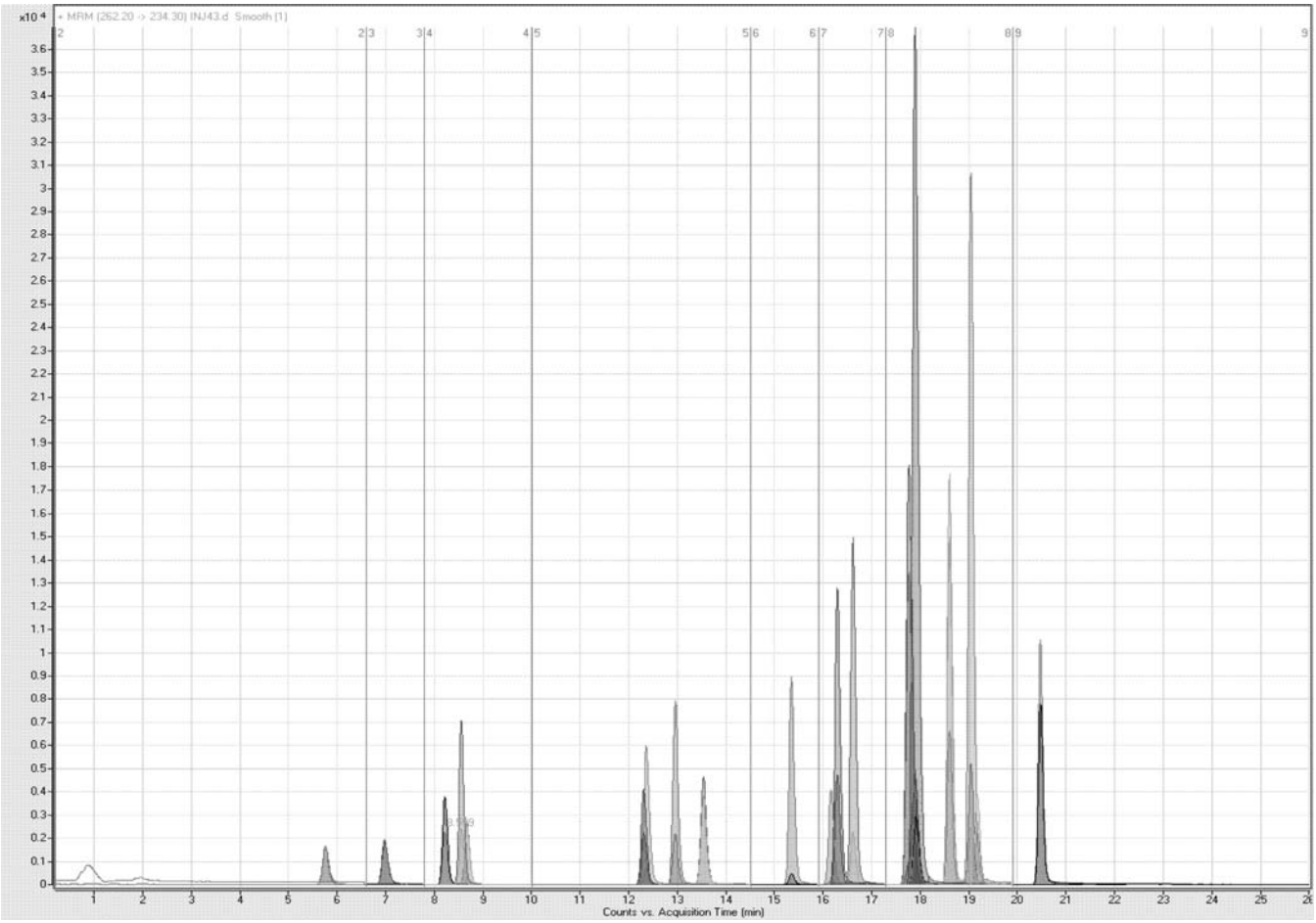

Figure 2. MRM overlay showing 20 herbicides from 0.5 µg/L standard.

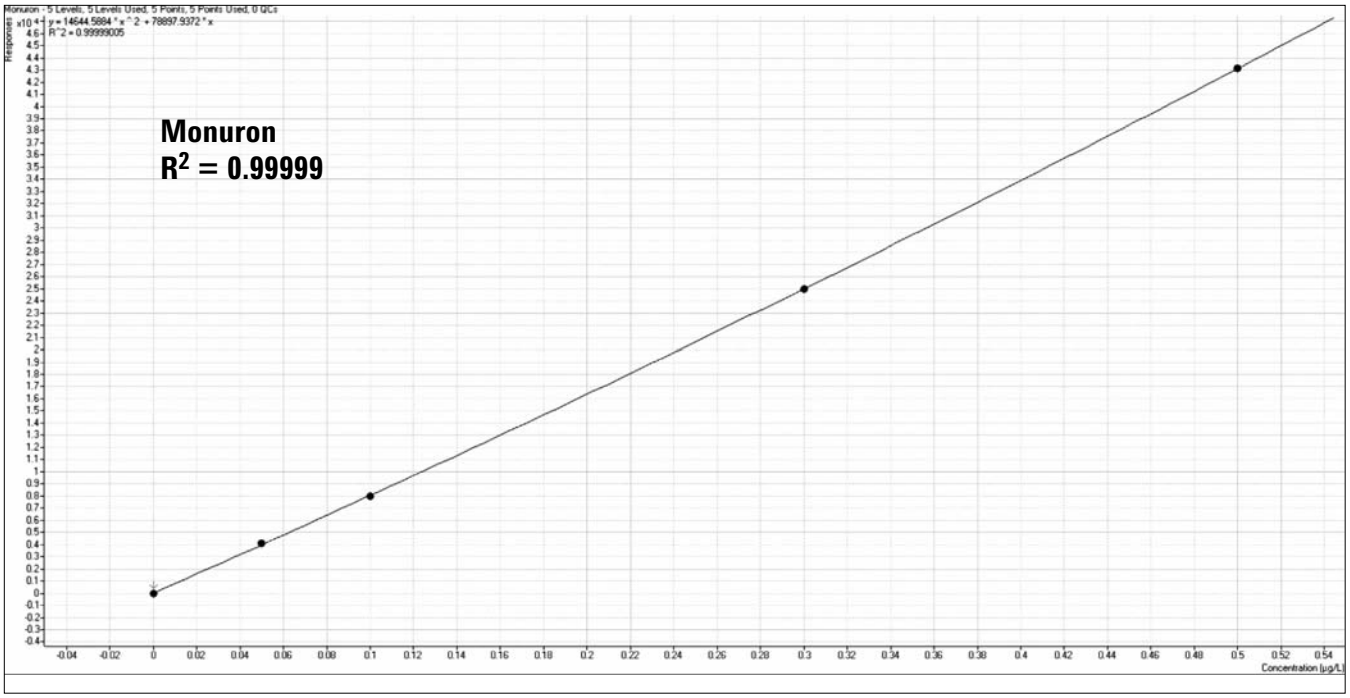

Figure 3a. Monuron calibration curve.

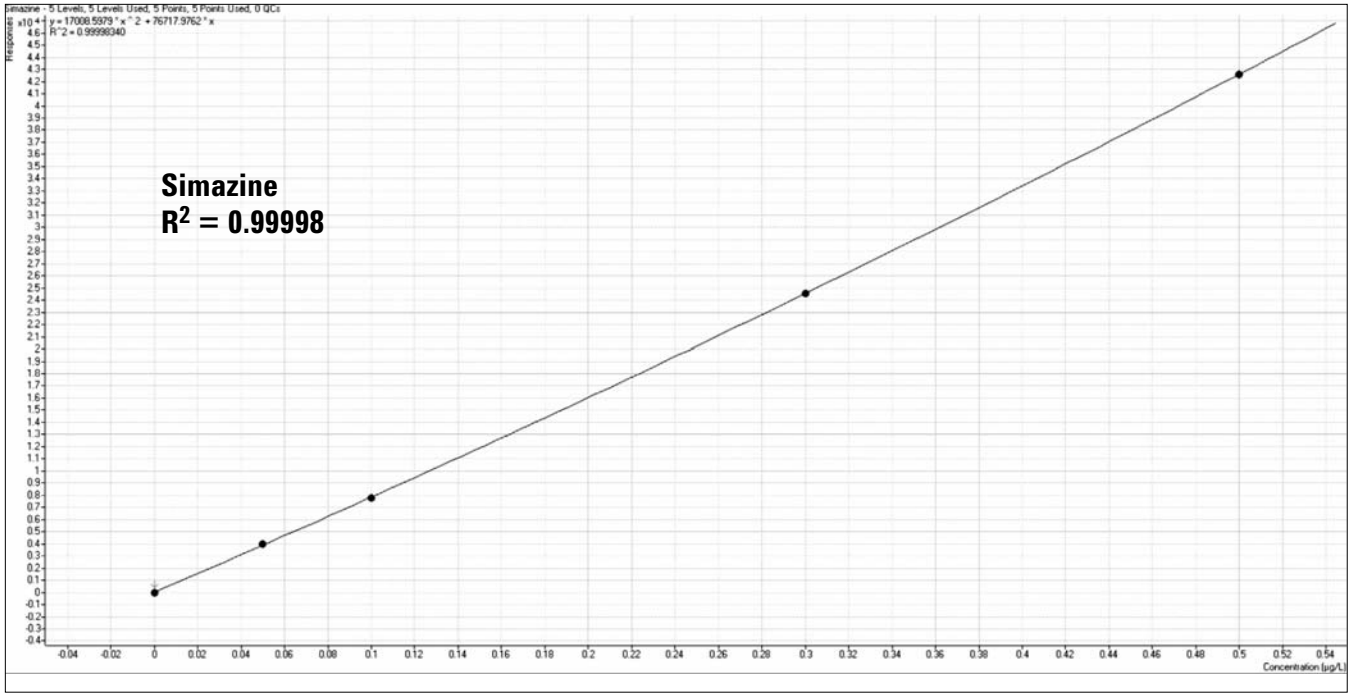

Figure 3b. Simazine calibration curve.

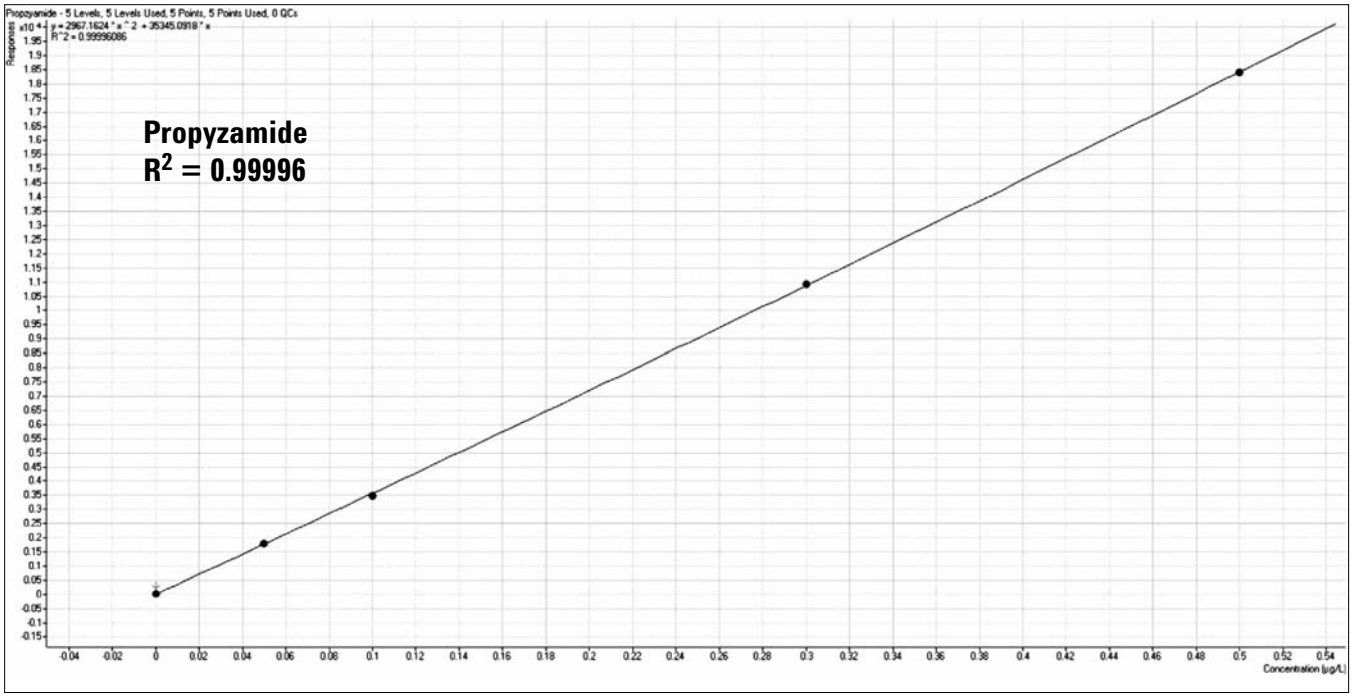

Figure 3c. Propyzamide calibration curve.

Validation of the method was carried out on 11 batches of samples. Borehole groundwater, potable water (which was from a surface water source), and river water were spiked at two levels (0.01 and 0.10 µg/L). Deionized water was spiked at three levels with analytical quality control material at 0.01, 0.10, and 0.40 µg/L. Each batch of samples was analyzed in duplicate and in a random order. The limit of detection (LOD) for each herbicide was calculated from the within-batch standard deviation (5 x sw) of the deionized water spiked at 0.01 µg/L. Recovery for the groundwater, potable water, and river water was calculated from the 0.1 µg/L spike.

Experimental results are shown in Table 2.

Table 2. Validation Data: %Recovery, ±%RSD, and Limit of Detection (LOD)

Compound	Borehole groundwater %Rec	Potable water %Rec	River water %Rec	LOD (µg/L)
Clopyralid	100.8 ± 5.7	104.7 ± 5.7	101.4 ± 7.0	0.007
Picloram	99.7 ± 4.0	94.2 ± 4.0	94.3 ± 5.5	0.005
Metamitron	100.5 ± 4.3	96.2 ± 3.9	97.1 ± 3.4	0.003
Imazapyr	101.7 ± 3.4	97.9 ± 3.2	97.3 ± 3.9	0.005
Chloridazon	99.7 ± 3.5	93.0 ± 4.5	92.9 ± 4.5	0.004
Carbetamide	98.0 ± 5.2	90.2 ± 4.7	93.8 ± 3.9	0.009
Monuron	99.8 ± 3.3	92.5 ± 3.8	90.8 ± 3.5	0.005
Cyanazine	99.4 ± 4.5	91.0 ± 4.5	92.7 ± 3.2	0.004
Simazine	100.1 ± 2.9	98.9 ± 2.9	98.2 ± 3.1	0.004
Chlorotoluron	99.5 ± 3.2	99.9 ± 3.8	99.7 ± 3.7	0.003
Diuron	98.3 ± 3.7	100.2 ± 5.0	98.9 ± 5.3	0.006
Atrazine	99.4 ± 2.2	99.4 ± 2.9	100.5 ± 3.5	0.002
Isoproturon	99.1 ± 3.8	99.7 ± 3.7	99.0 ± 3.9	0.003
Prometryn	99.7 ± 2.9	100.1 ± 3.0	100.5 ± 3.5	0.003
Terbutryn	99.0 ± 2.9	99.1 ± 3.4	99.7 ± 3.3	0.002
Linuron	99.3 ± 5.8	100.2 ± 3.3	102.4 ± 6.4	0.003
Propazine	99.6 ± 3.2	99.9 ± 3.3	99.4 ± 2.9	0.002
Terbuthylazine	99.8 ± 3.8	99.0 ± 3.0	100.6 ± 2.9	0.003
Propyzamide	101.4 ± 4.4	99.4 ± 3.3	99.8 ± 3.8	0.004
Trietazine	99.9 ± 2.8	100.0 ± 2.7	101.0 ± 2.5	0.002
Overall suite	**99.7 ± 3.8**	**97.8 ± 3.7**	**98.0 ± 4.0**	**0.004**

Selected and representative examples of MRM chromatograms derived from real sample matrices are shown in Figure 4.

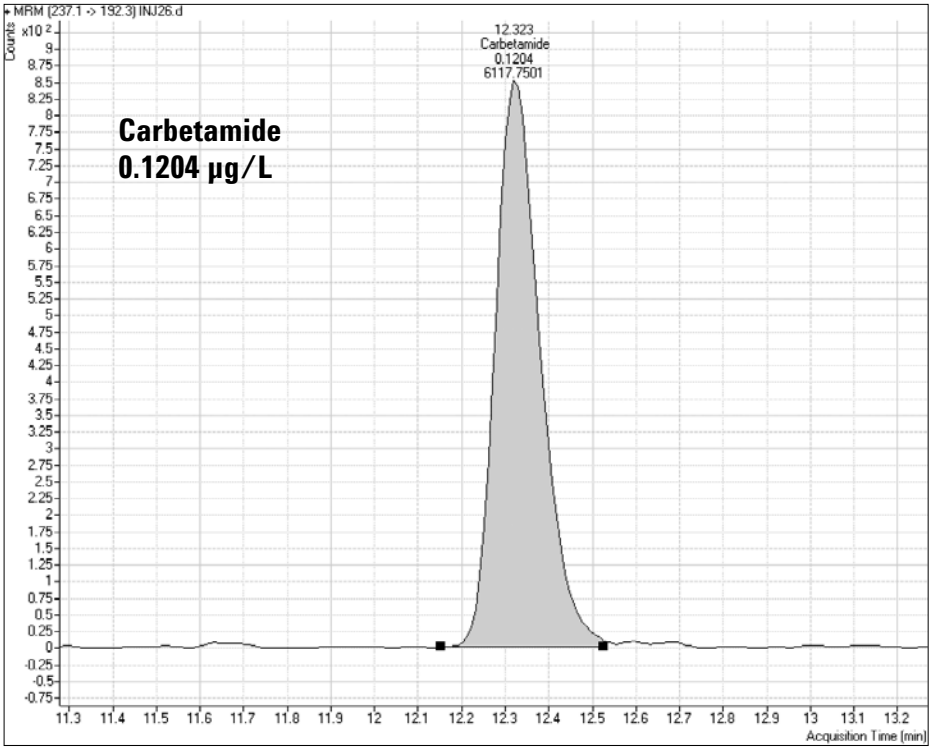

Figure 4a. MRM chromatogram of carbetamide (river water matrix).

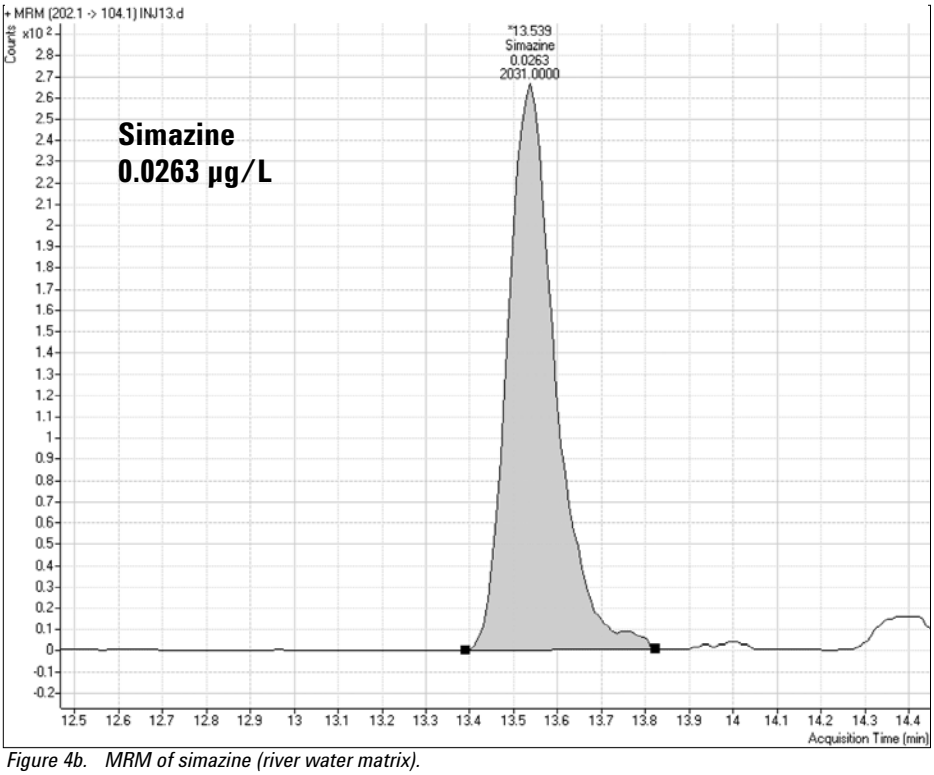

Figure 4b. MRM of simazine (river water matrix).

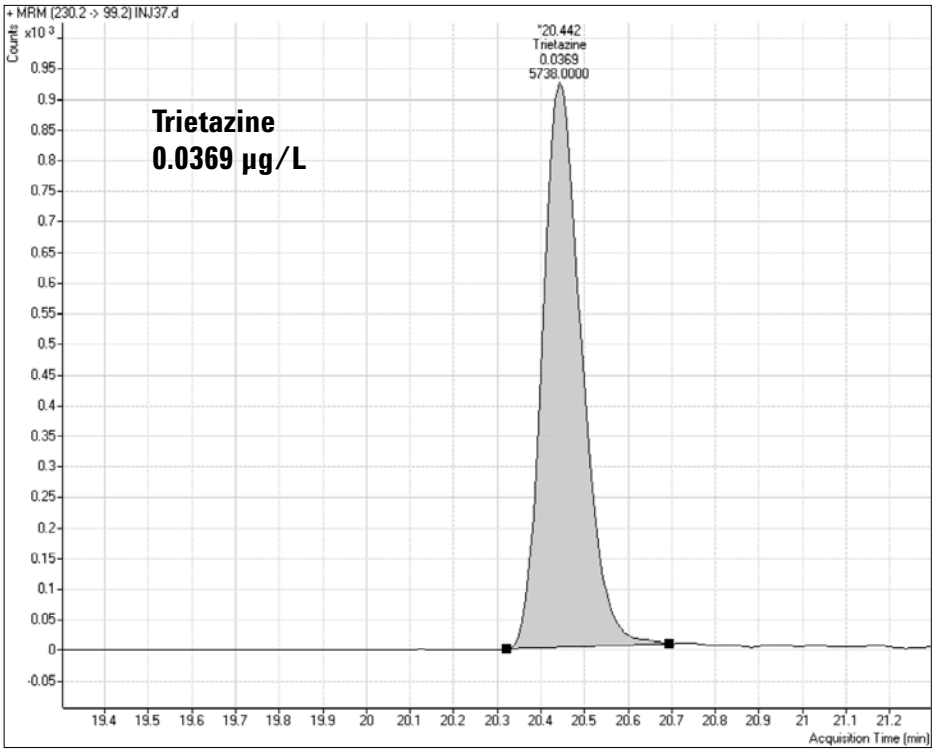

Figure 4c. MRM of trietazine (river water matrix).

Conclusions

The data show that the method herein presented is capable of sensitive and quantitative analysis for the 20 herbicides in a single analytical suite by a direct aqueous injection of 100 µL sample volumes onto the analytical column. Only sample acidification was undertaken as a preparation stage. All the method performance criteria are met, which are < 12.5% analyte precision, recoveries in the range of 90 to 110%, and limits of detection < 10 ng/L (10 ppt).

We demonstrate in this application note that direct aqueous injection of 100 µL samples onto the analytical column achieves the required method performance levels and is possible due to the sensitivity and selectivity of the Agilent LC/QQQ 6410BA instrumentation. The net benefit of such an approach to this methodology is a direct cost reduction in the form of consumable items (solid phase cartridges), which are no longer required, together with significant man-hour cost reductions since only minimal sample preparation is undertaken (acidification).

Reference

1. "Determination of Phenyl Urea and Triazine Herbicides in Potable and Groundwaters by LC/MS Using AP-ESI Selective Ion Monitoring and Direct Large Volume Aqueous Injection," Agilent Technologies publication 5989-0813EN.

For More Information

For more details concerning this application note, please contact Neil Cullum at Anglian Water Services Laboratory, Huntingdon, Cambridgeshire, UK.

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Printed in the USA
March 31, 2009
5990-3762EN

Agilent Technologies

The Use of Accurate Mass, Isotope Ratios, and MS/MS for the PPCPs in Water

Application

Environmental

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Abstract

An Agilent 6510 Quadrupole Time-of-Flight Mass Spectrometer (QTOF) is used to analyze several surface water samples for the presence of pharmaceutical compounds. A simple gradient elution is carried out on a Rapid Resolution High Throughput Extend C18 column (particle size 1.8 µm). Of 54 potential compounds, as many as 11 are identified in one of the samples using an algorithm known as the Agilent Molecular Feature Extractor (MFE). To make comparisons among several samples, another algorithm, known as Mass Profiler, is applied to the data processed by the MFE. Since the MFE may generate thousands of potential compounds known as features, Mass Profiler makes statistical comparisons of the features between two different samples to determine what is unique and what is common. All of this work is done with the full-scan mass spectral data. When compounds of interest are determined, accurate mass full-scan MS/MS can be invoked for structural elucidation. The results of full-scan MS/MS applied to caffeine are included as an example and are relevant because many medications include caffeine as an ingredient.

Introduction

During the three decades prior to the year 2000, the study of chemical pollution was confined primarily to pesticides. Following a seminal article by C. Daughton [1], this focus began to shift to the emerging environmental concern for pharmaceuticals and personal care products (PPCPs). Many of these pharmaceuticals, including estrogen, have been known as endocrine disruptors, or chemicals that disrupt the physiological function of hormones in organisms. In 2004 a report from the United States Geological Survey [2] was made as a result of discovering a high preponderance of intersex (male fish exhibiting female characteristics) in smallmouth bass of the Potomac River.

The USGS has found pesticides, flame retardants, and personal-care products containing known or suspected endocrine-disrupting compounds in the Potomac River. Many of these compounds continue to be known as emerging contaminants because they are still being discovered and don't exist on any currently regulated target lists. As such, it is important to use adequate techniques to help identify these compounds and possible metabolites.

Using accurate mass in full-scan (mass range) mass spectrometry (MS), compound empirical formulas can be determined for purposes of identification. Furthermore, the high degree of spectral resolution allows for selective identification among co-eluting compounds. Isotope ratios are an additional tool because they help identify com-

pounds with high carbon numbers as well as those that contain elements like chlorine and sulfur. Although these tools do a lot to confirm chemical formula, it may still be left to the user to decide which of the possible structures of isobaric compounds apply.

To assist in the analytical need for structural elucidation, selective MS/MS by using the quadrupole time-of-flight mass spectrometer (QTOF) is implemented. Because the Agilent QTOF also has very accurate mass at the MS/MS level, it is easier to determine the structures of the product ions, which correspond as substructures of the precursor ion and thereby reduce the number of possible structures pertaining to the derived empirical formulas from several to one.

The list of pharmaceuticals to look for in the environment is ever-increasing and many of them are metabolites with unknown structures. Identifying these compounds requires the technology of the QTOF. Furthermore, the fast scanning capability is necessary for identifying 10s to 100s of these compounds in samples with relatively short run times. The Agilent QTOF is capable of acquiring full-scan MS data at the rate of 20 spectra/sec. The resulting large amount of data representing a possibly large number of compounds needs to be converted into useful information. The Agilent Molecular Feature Extractor (MFE), which is a standard part of the MassHunter Qualitative Analysis software, carries out the following steps:

- Persistent chemical background removed
- Co-eluting interferences resolved
- Isotopic clusters recognized and grouped
- 2D/3D data visualization
- Chemical identification (accurate mass, isotope matching)
- Database searching (NIST, ChemIDPlus)

In addition to applying the algorithm Mass Feature Extractor to pull out the features from the chromatographic data, which could be compounds, another algorithm, known as Mass Profiler, is applied to the list of features among different samples to determine differences and commonalities. Each sample is injected three times, or multiple samples from the same source could be used to determine what is statistically consistent in terms of the features derived for the sample by MFE. The result is called a group. Two groups representing two different sample sources can then be compared to see what features differ, are unique, or are common, and, if common, whether they differ in abundance.

A batch of water samples is filtered and extracted using solid-phase extraction, which resulted in an approximate 1,000-fold increase in concentration. Samples analyzed in this work are believed to contain compounds at the 10 to 100 ppb level, which corresponds to the 10 to 100 ppt range in the original water sample. The compounds that may be in these samples are included with their exact neutral masses in Table 1.

Table 1. List of Compounds with Corresponding Neutral Masses That May Be in a Given Sample

Compound	Neut. mass	Compound	Neut. mass	Compound	Neut. mass
Acetaminophen	151.06333	Diphenhydramine	255.16231	Paroxetine	329.14272
Albuterol	239.15214	Duloxetine	297.11873	Ranitidine	314.14126
Aspirin	180.04226	Enalaprilat	348.16852	Sertraline	305.07380
Buproprion	239.10769	Erythromycin	573.51210	Simvastatil	418.27192
Caffeine	194.08038	Fluoxetine	309.13405	Sulfachloropyridazine	284.01347
Carbamazepine	236.09496	Fluvoxamine	318.15551	Sulfadimethoxine	310.07358
Cimetidine	252.11572	Furosemide	330.00772	Sulfamethazine	278.08375
Clofibric acid	214.03967	Gemifrozil	250.15698	Sulfamethizole	270.02452
Citalopram	324.16379	HCTZ	296.96447	Sulfamethoxazole	253.05211
Codeine	299.15215	Ketoprofen	254.09429	Thiabendazole	201.03607
Cotinine	176.09496	Miconazole	413.98602	Triclocarban	313.97805
Dehydronifedipine	344.10084	Naproxen	230.09429	Triclosan	287.95116
Diclofenac	295.01668	Norfluoxetine	295.11840	Trimethoprim	274.14298
Diltiazem	414.16133	Norsertraline	293.05000	Venlafaxine	267.12593
		1,7-dimethylxanthine	180.06473	Warfarin	308.10486

Experimental

Sample Preparation

Prepared samples are provided by the United States Geological Service National Water Quality Laboratory (USGS/NWQL) in Denver, Colorado. The details of the procedure used are not included in this application, but are available upon request. Pharmaceuticals are typically extracted from surface water by using disposable polypropylene syringe cartridges that contain 0.5 g of polymeric sorbent. One liter of sample is pumped through the solid-phase extraction (SPE) cartridge. The analyte material is later eluted into 1 mL of methanol, resulting in a concentration increase of three orders of magnitude.

LC/MS Method Details

LC Conditions

Agilent 1100 Series binary pump, degasser, wellplate sampler, and thermostatted column compartment

Column	Agilent ZORBAX RRHT Extend C18 2.1 mm × 50 mm, 1.8 µm Agilent p/n: 727700-902
Column temperature	40 °C
Mobile phases	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile
Flow rate	0.3 mL/min
Injection volume	5 µL
Gradient	

Time (min)	%B	
0.0	0	
10.0	67	Stop time: 15 min
11.0	100	Post run: 10 min

MS Conditions

Mode	Positive electrospray ionization using the Agilent G3251A Dual ESI source
Nebulizer pressure	40 psig
Drying gas flow	9 L/min
Drying gas temp.	350 °C
V_{cap}	3500 V
Scan range	*m/z* 50–1000
Scan speed	1 scan/sec

MS/MS Conditions

Collision energy	30 V
Scan range	*m/z* 50–1000
Scan speed	1 scan/sec

Results and Discussion

Of the several samples analyzed, results for Samples 4 and 10 will be considered here. To get an idea of the task at hand, an overlay of the total ion and base peak chromatograms for the first injection of Sample 4 is shown in Figure 1. The base peak chromatogram is generated to help the analyst identify peaks in the chromatogram corresponding to real compounds. Figure 2 shows the spectrum at the apex of one such peak. Note the complexity of this spectrum and the difficulty involved in not only determining which spectral peaks are of value, as they may pertain to co-eluting compounds, but then having to apply this reasoning to several peaks in the chromatogram.

Applying the algorithm of the Molecular Feature Extractor program to this data file results in the display of the processed chromatogram and the corresponding contour plot shown in Figure 3. The upper left-hand chromatogram is the unprocessed TIC, same as shown in Figure 1. On the right is the processed chromatogram after applying the steps listed in the Introduction. Random background noise has been removed. Below each of these chromatograms are shown the corresponding contour plots, which are the presentations of spectral data points in an *m/z* versus chromatographic retention time plots. The contour plot at the lower left-hand corner of the display shows a very dense distribution of data points, most of which correspond to random noise.

The contour plot at the lower right-hand corner is the result of processing the data so that a significant amount of molecular features are derived for closer examination. In fact, using the following settings for filtering the data, some 5,431 features are derived for this sample in the first injection:

- Spectral S/N > 2
- Mass range: *m/z* 150 to 800
- $[M + Na]^+$ and $[M + NH_4]^+$ adducts considered
- Relative intensity in the spectrum > 0.1%
- Each feature must contain at least 2 ions

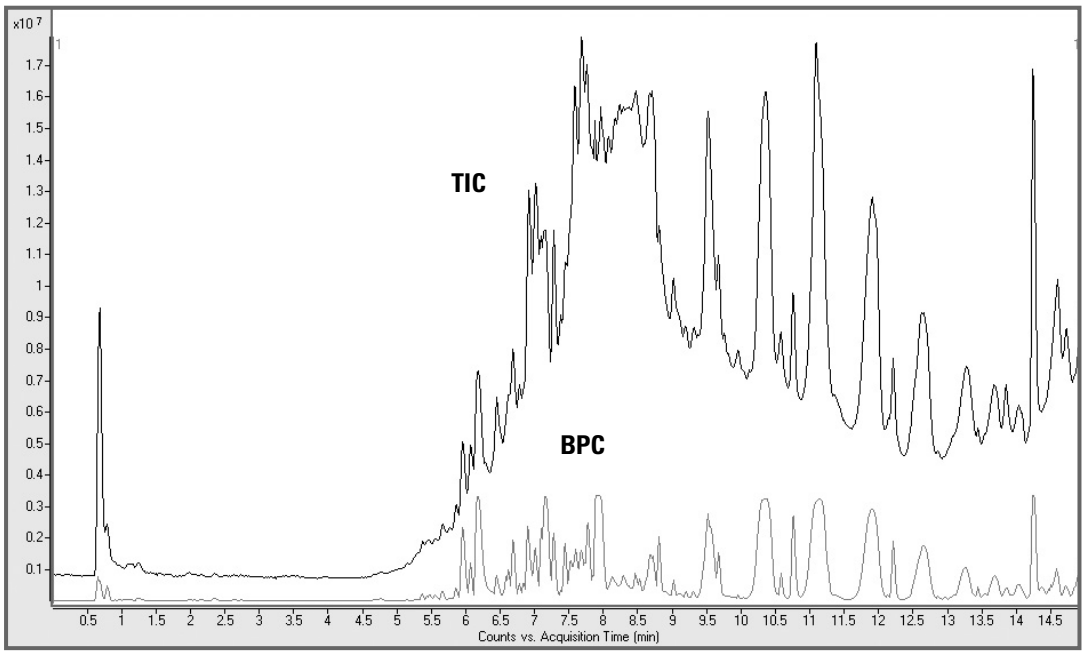


Figure 1. Overlay of total ion chromatogram (TIC) and base peak chromatogram (BPC) for Sample 4.

If we now investigate some of the features that have been found we can begin with the peak apex spectrum examined in Figure 2. The retention time is 6.445 minutes and MFE has derived features at 6.448 minutes as shown in Figure 4. The unprocessed spectrum at the top of the figure matches that of Figure 2. However, removing random noise and using the filtering rules above a processed spectrum containing 12 features is derived and shown at the bottom. A subset of the list of features is shown at right.

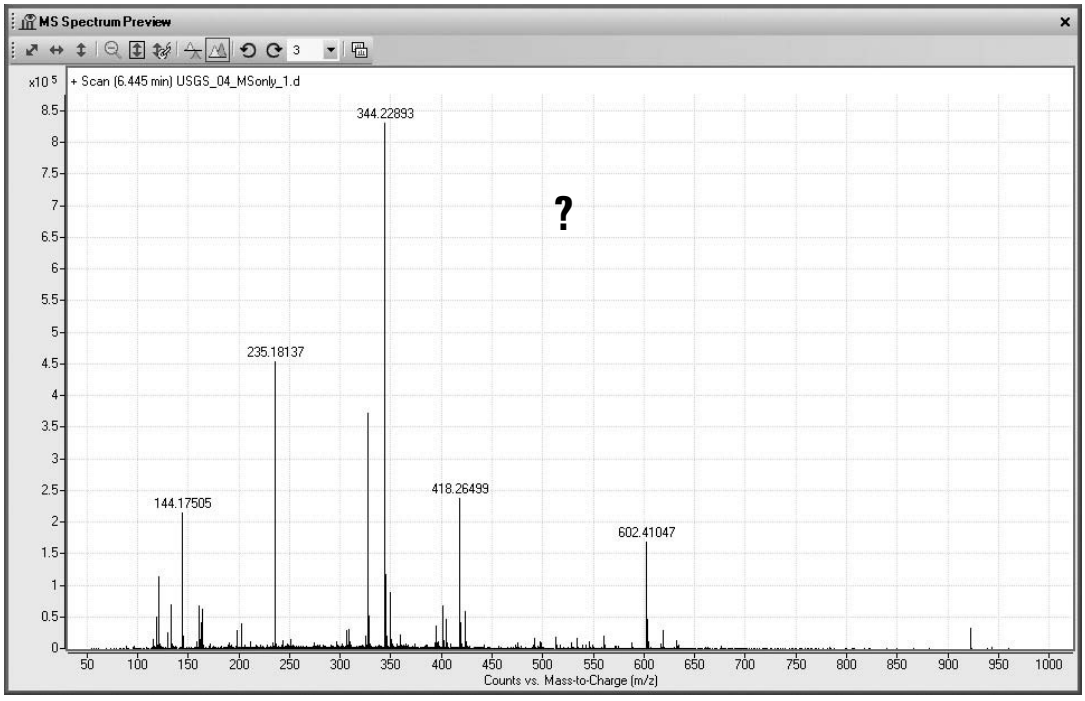


Figure 2. Spectrum at apex of base peak with retention time of 6.445 minute.

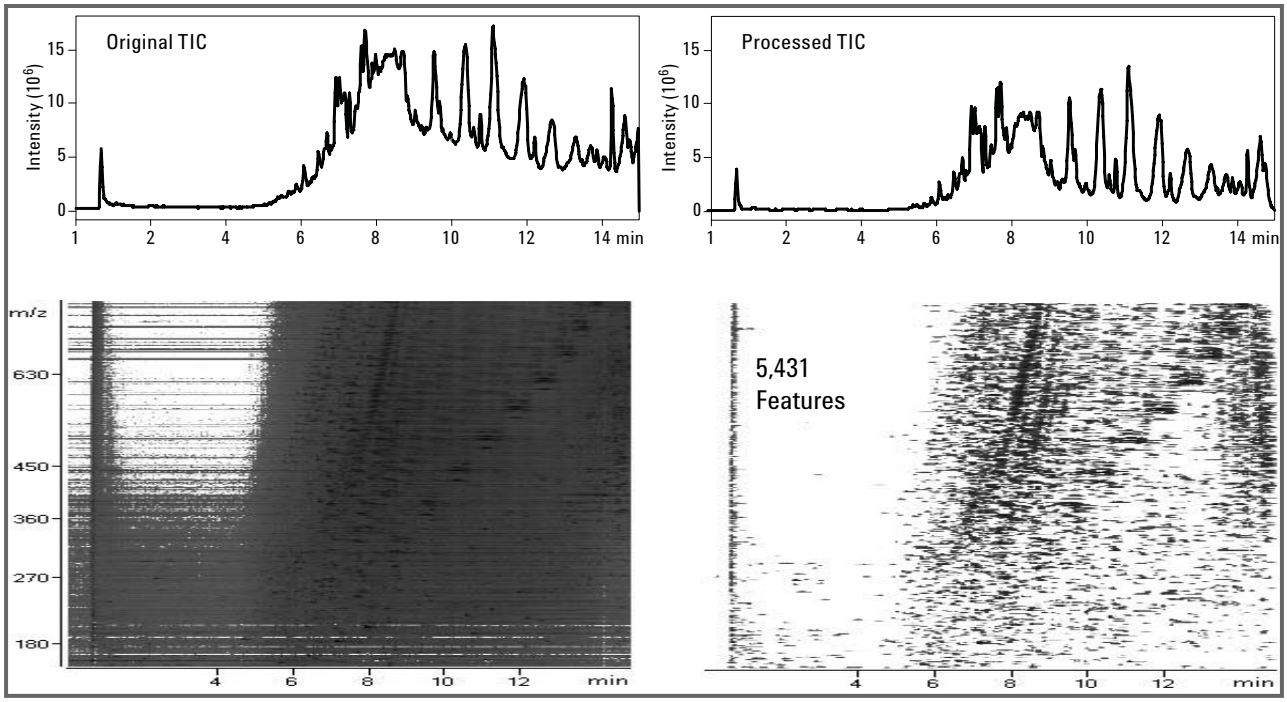
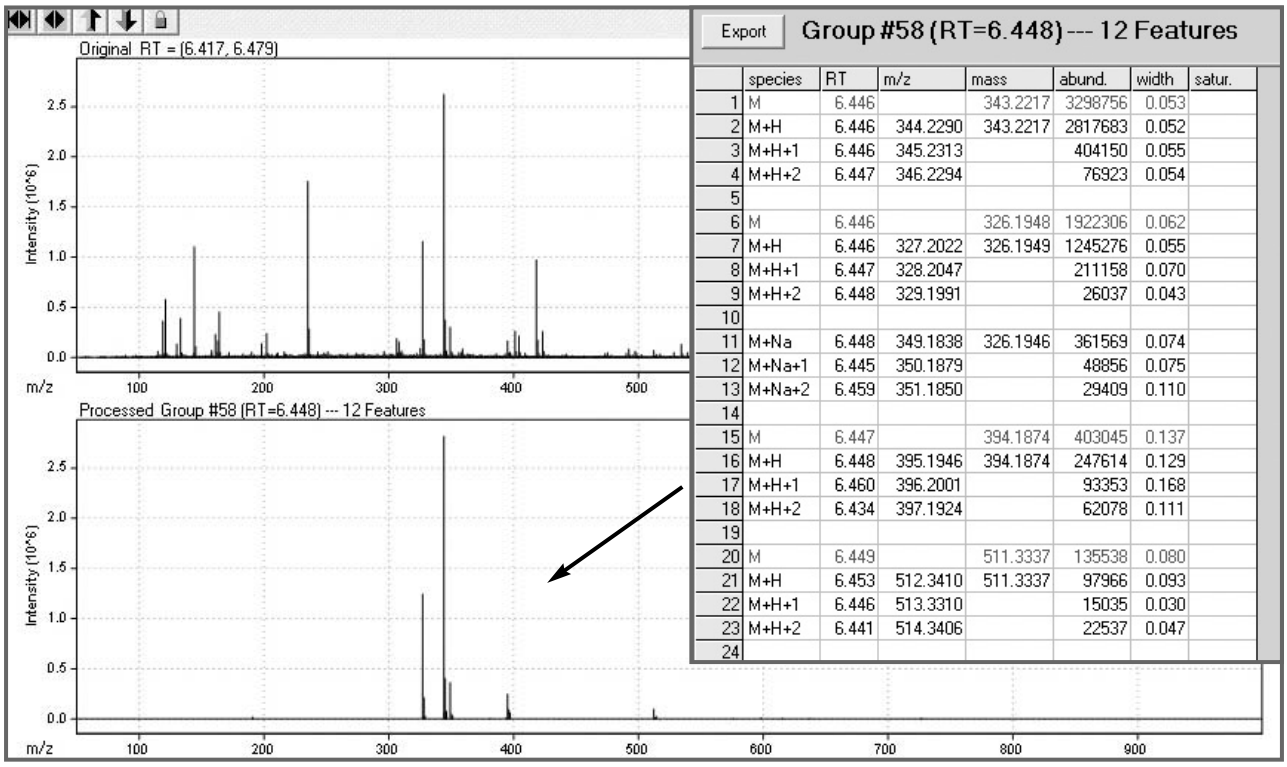


Figure 3. Both unprocessed and processed data of Sample 4 using MFE.



	species	RT	m/z	mass	abund.	width	satur.
1	M	6.446		343.2217	3298756	0.053	
2	M+H	6.446	344.2290	343.2217	2817683	0.052	
3	M+H+1	6.446	345.2313		404150	0.055	
4	M+H+2	6.447	346.2294		76923	0.054	
5							
6	M	6.446		326.1948	1922306	0.062	
7	M+H	6.446	327.2022	326.1949	1245276	0.055	
8	M+H+1	6.447	328.2047		211158	0.070	
9	M+H+2	6.448	329.1991		26037	0.043	
10							
11	M+Na	6.448	349.1838	326.1946	361569	0.074	
12	M+Na+1	6.445	350.1879		48856	0.075	
13	M+Na+2	6.459	351.1850		29409	0.110	
14							
15	M	6.447		394.1874	403045	0.137	
16	M+H	6.448	395.1946	394.1874	247614	0.129	
17	M+H+1	6.460	396.2001		93353	0.168	
18	M+H+2	6.434	397.1924		62078	0.111	
19							
20	M	6.449		511.3337	135538	0.080	
21	M+H	6.453	512.3410	511.3337	97966	0.093	
22	M+H+1	6.446	513.3310		15035	0.030	
23	M+H+2	6.441	514.3406		22537	0.047	
24							

Figure 4. Twelve features shown at derived retention time of 6.448 minutes.

If we want to filter the data to only show compounds corresponding to the list at the beginning of this article, we can place the neutral masses into an inclusion list of MFE as shown in Figure 5. We also assume that the compounds of interest do not elute until after 4 minutes and the mass range of interest is 150 to 600, which corresponds to the compounds of Table 1.

After applying the filtering of data with the compound list shown in Figure 5, eight features appear to be found in Sample 4 as shown in Figure 6. The corresponding chromatogram containing these eight features is also shown.

Before looking more closely at any one of these compounds, the data of Sample 4 is now going to be compared with data from another sample, Sample 10. The comparison will be carried out using an algorithm known as Mass Profiler. In order to use Mass Profiler, at least three injections of each sample must be made to determine what is consistently there and what is random and should be disregarded. In this work each sample is injected three times. The data is first processed by MFE to generate features. Mass Profiler filters out features that are inconsistent among the three injections for each sample. The resulting data is called a Group. Therefore, in comparing Samples 4 and 10 Mass Profiler will be referring to them as Group 4 and Group 10.

In Figure 7, Mass Profiler shows a plot of features common to both Groups 4 and 10 and displays them as mass versus retention time. By clicking on any one of the feature points in the display, one can see the common feature for both Groups along with possible empirical formulas for the derived neutral mass.

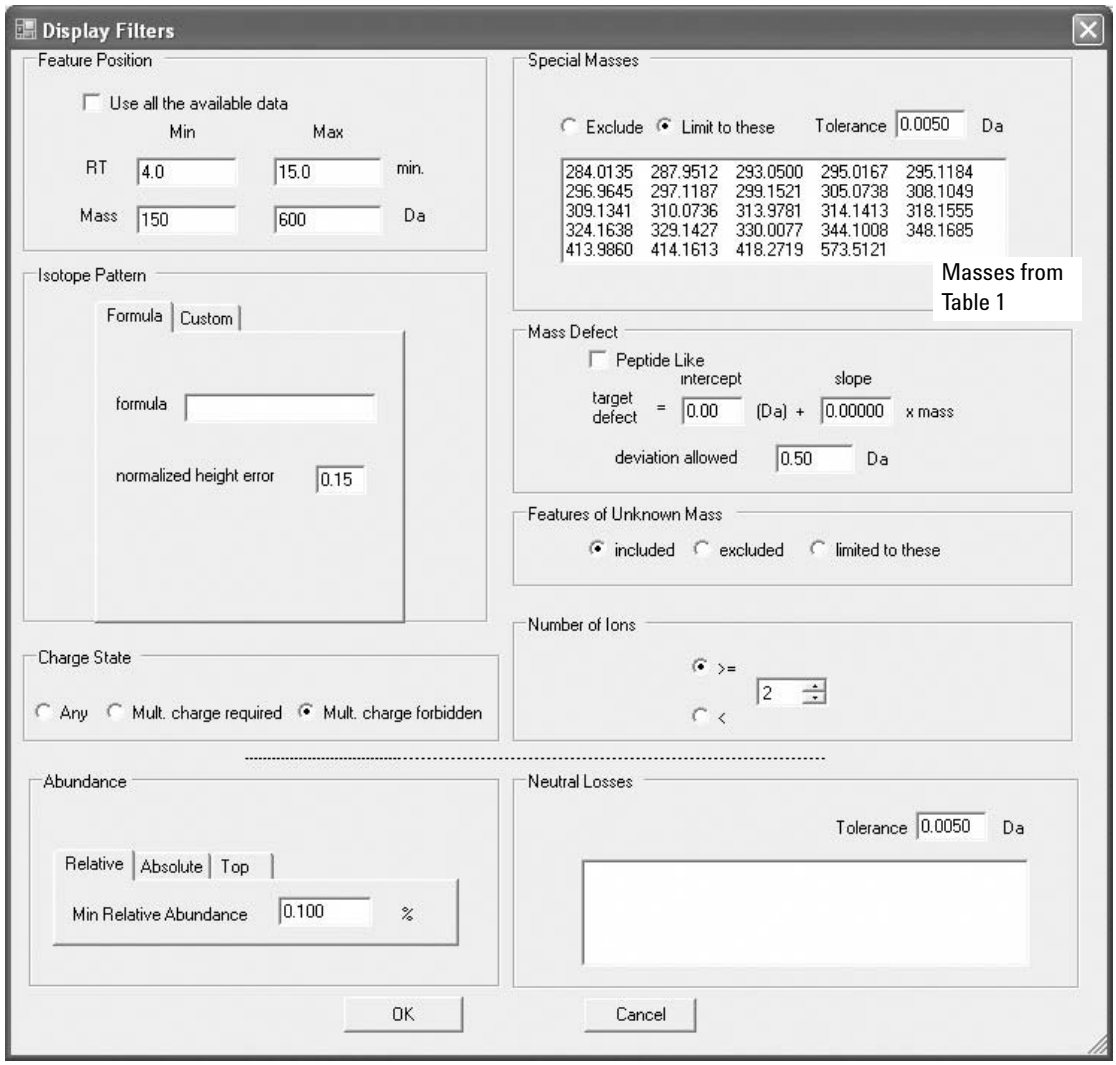


Figure 5. Display filter settings for finding features that match compound list of Table 1.

Export features:8 / groups:8

	Group	RT	mass	mass SD	abund.	satur.	height	#ions	minZ	maxZ	#z	width	#features
1	1	9.629	236.0961		915731		267469	2	1	1	1	0.045	1
2	2	5.678	299.1536		431858		91085	2	1	1	1	0.083	1
3	3	8.095	236.0961		382835		96590	2	1	1	1	0.052	1
4	4	8.671	299.1527		229538		23883	3	1	1	1	0.121	1
5	5	6.064	299.1525		146722		26146	2	1	1	1	0.076	1
6	6	11.598	250.1607		134252		19366	2	1	1	1	0.128	1
7	7	8.262	252.1204		63528		12743	2	1	1	1	0.058	1
8	8	5.307	296.9660	0.0019	28147		3522	4	1	1	1	0.072	1

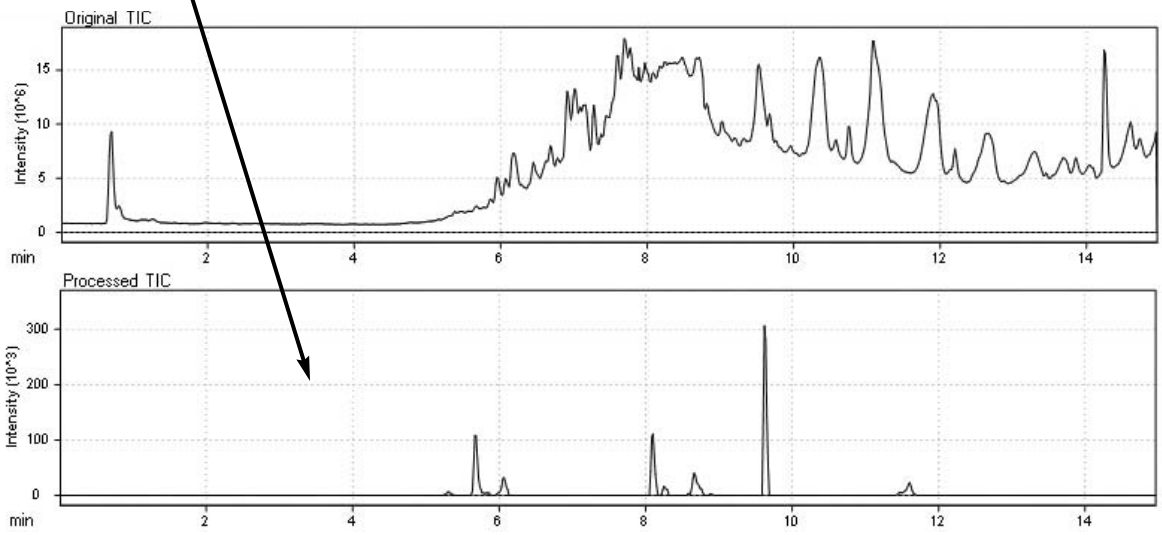


Figure 6. Eight features found corresponding to the neutral masses of Table 1. Corresponding processed chromatogram also shown.

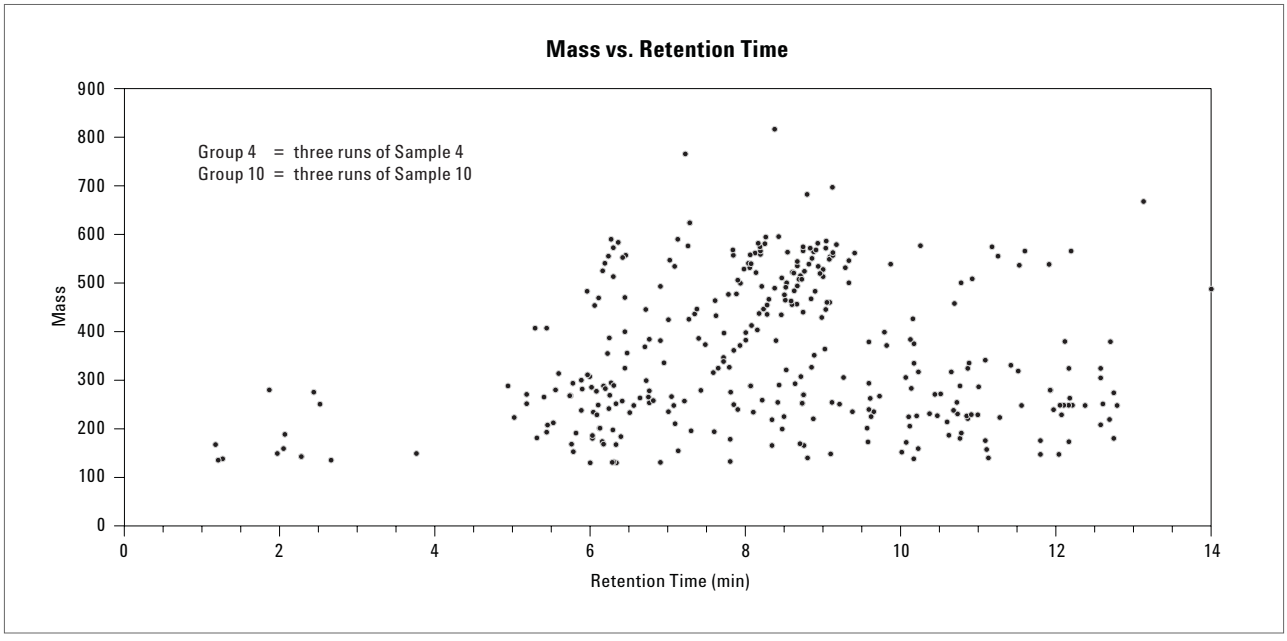


Figure 7. Features present in both Groups 4 and 10 total 346.

For example, in comparing features between the two sample groups a differential analysis plot can be generated as shown in Figure 8. In this plot, the features of Group 10 that are more or less abundant than the corresponding features in Group 4 are represented. More specifically, at a retention time of 8.495 minutes there is a data point in Figure 8 that corresponds to a feature in Group 10 that is approximately 4× intensity over the corresponding feature in Group 4, which corresponds to a log 2 ratio of 2. By clicking on this data point in the display of Figure 8 one can see that this feature is identified as diphenhydramine, with a chemical formula of $C_{17}H_{21}NO$ and accurate mass of 0.7 ppm. See Figure 9.

With Mass Profiler it is also possible to compare two samples in terms of what features are in one sample that are not in the other. In Figure 10 we see such a comparison.

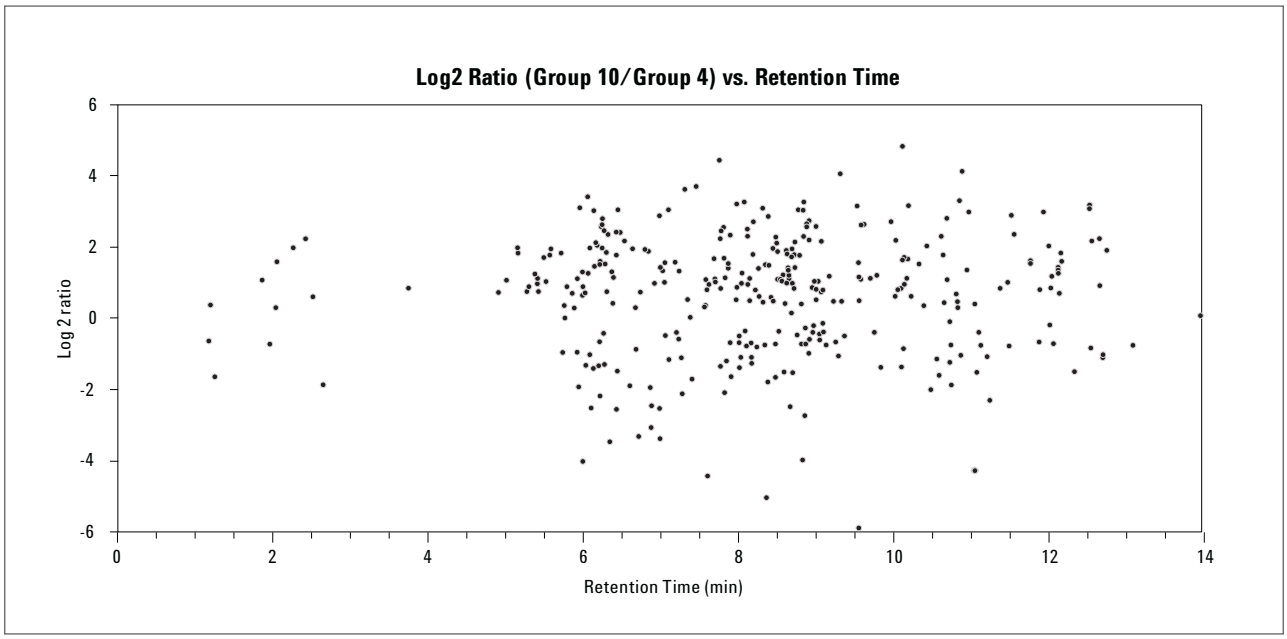


Figure 8. Features common to Groups 4 and 10 but differing in magnitude.

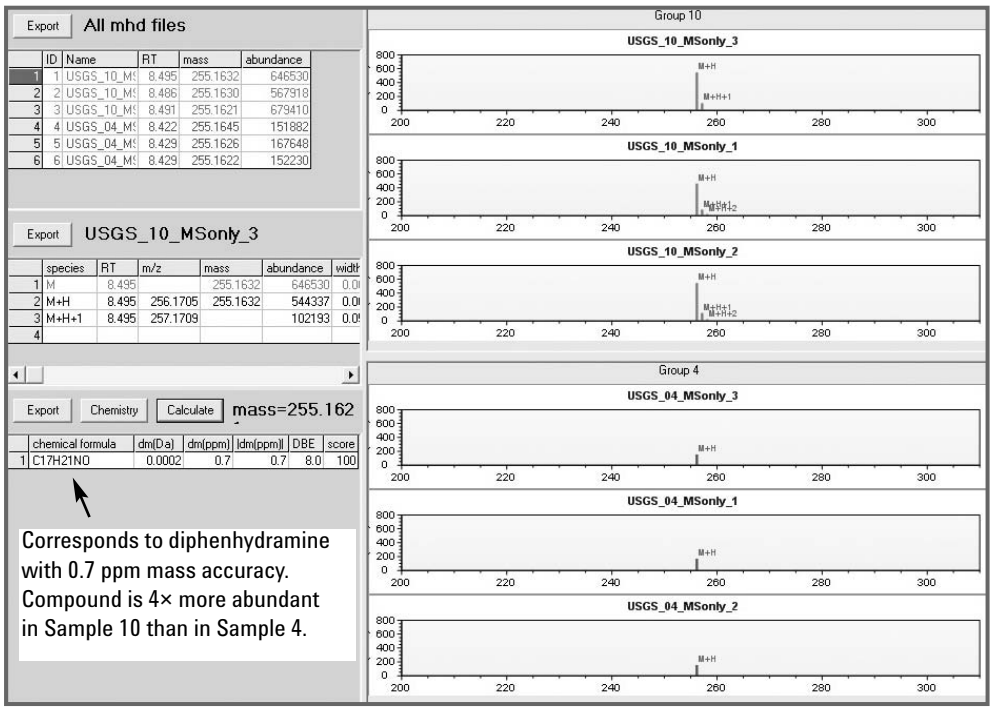


Figure 9. Feature at 8.495 minutes retention time corresponds to diphenhydramine.

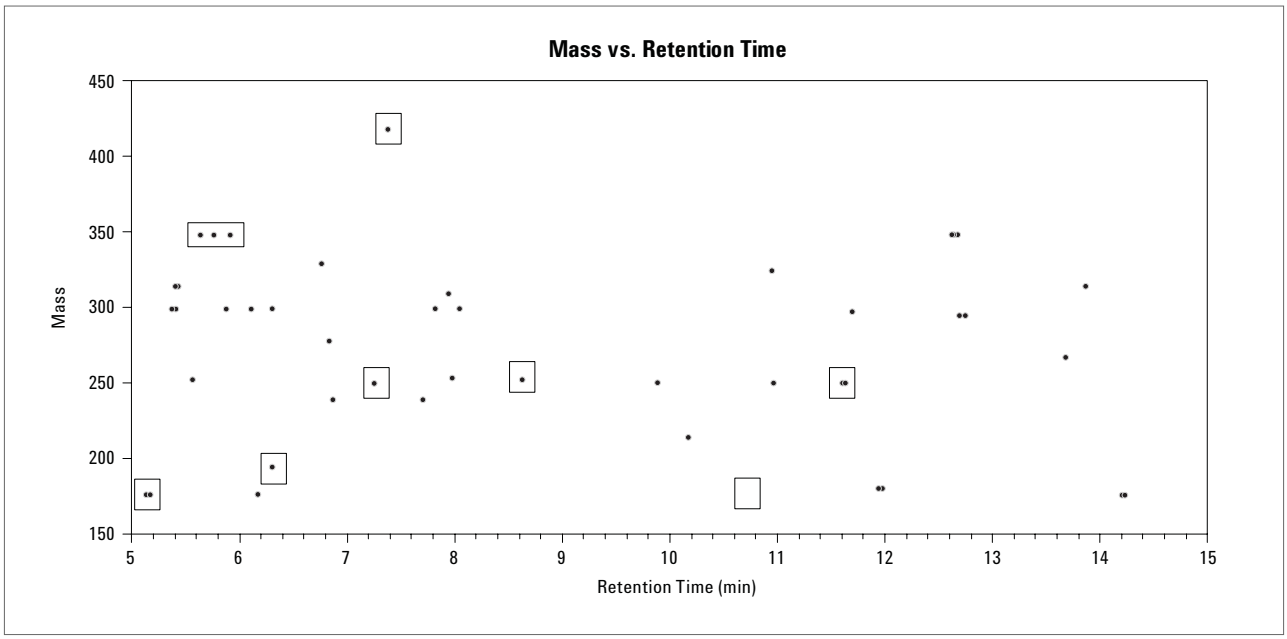


Figure 10. Features only in Group 4 (highlighted with boxes) or in Group 10.

Mass Profiler has determined that there are 33 features only in Group 4 or in Group 10 and are not common to the two samples. Since the display in Mass Profiler is in color the features exclusive to Group 4 are blue and the features exclusive to Group 10 are red. Since Agilent applications are normally published in black and white, boxes have been placed around the blue features for Group 4 for viewing convenience.

So far, all of the data have been collected in full-scan MS mode. Once features are identified as compounds needing more structural information, or it is of interest to perform some quantitation, a targeted MS/MS analysis can be performed in which the ion mass of the feature is considered as precursor ion and fragmented to form accurate mass product ions. The accurate mass of these product ions can determine their chemical formula and possible structures. Because the QTOF also has a high degree of spectral resolution in MS/MS mode, very narrow extracted ion chromatograms may be generated for each ion and then summed together for quantitation signal.

In Figure 11 we see the accurate mass MS/MS fragmentation of caffeine using the MS/MS settings noted in the LC/MS Method Details. Caffeine is of environmental interest because many medications contain it as an ingredient. Chemical formulas for each product ion is derived based on the possible arrangements of C, H, N, and O. Knowing the structure of caffeine, structures of the fragment ions can be proposed using their corresponding chemical formula. The fragment structures are generated using ACD/MS Fragmentor (ACD Labs Release v. 10, Advanced Chemistry Development, Inc., Toronto, ON, Canada).

Conclusions

The QTOF is an excellent instrument for identifying compounds using accurate mass in full-scan MS and MS/MS. Accurate mass leads to chemical formula, which can also give structural information when forming product ions in MS/MS. As a lot of data is acquired by this type of instrument to look at samples that may contain large amounts of known and unknown compounds, it is important to have algorithms like Molecular Feature Extractor that can filter usable features out of the chemical background. These features are generated from spectra as a result of removing random background signal and finding clusters of isotopes that make sense.

While this analysis is useful for one sample it may also be important to make comparisons among multiple samples as well. Another algorithm known as Mass Profiler makes such comparisons. More specifically, comparisons such as what is common to two samples and how they differ in amount. Or, what features are in one sample that aren't in the other. Once the feature is considered for more investigation, targeted MS/MS may be carried out on that feature to get structural information based on the generation of product ions.

Calculate chemical formula given accurate mass measurement and using elements C, H, N, and O

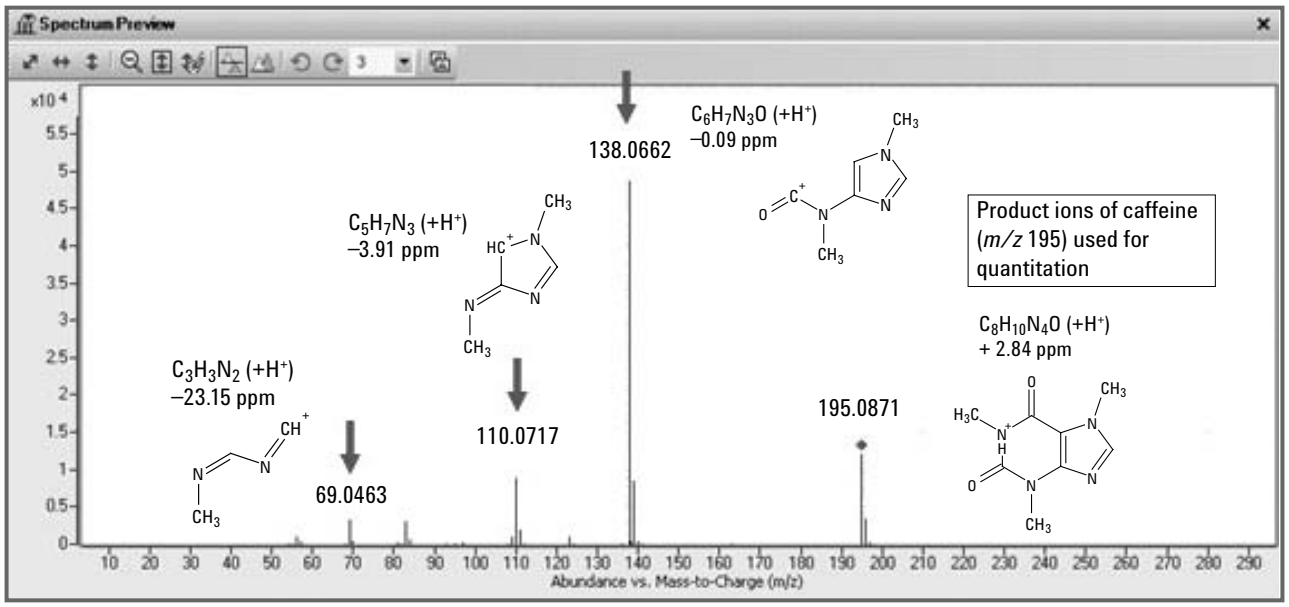


Figure 11. Targeted MS/MS mode for caffeine-producing product ions that may be used for structural elucidation as well as quantitation.

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1. C. G. Daughton and T. A Ternes, "Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?," *Environmental Health Perspectives*, 107, Suppl. 6, Dec 1999.

2. D. B. Chambers and T. J. Leiker, "A Reconnaissance for Emerging Contaminants in the South Branch Potomac River, Cacapon River, and Williams River Basins, West Virginia, April–October 2004," Open File Report 2006-1393, United States Geological Survey, http://pubs.usgs.gov/of/2006/1393/.

Acknowledgments

The authors gratefully acknowledge the assistance of Stephen Werner and Ed Furlong of the National Water Quality Lab – United States Geological Survey (Lakewood, CO) for providing the samples analyzed in this work.

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For more details concerning this application, please contact Michael Zumwalt at Agilent Technologies, Inc.

Printed in the USA
October 12, 2007
5989-7339EN

Rapid Analysis of Herbicides by Rapid Resolution LC with Online Trace Enrichment

Application

Environmental

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Abstract

Environmental and Food Safety agencies are constantly updating methods to improve detection limits and to resolve interfering compounds. One particular method, EPA 555, is used for the analysis of chlorinated phenoxy acid herbicides in drinking water. A mandated trace enrichment step significantly impacts the ease of use and reliability of the method. The method uses 5-µm analysis columns and online trace enrichment. The variation here uses small ZORBAX 3.5- and 1.8-µm RRHT columns and an autoSPE (Solid Phase Extraction) cartridge with an automated switching valve mounted in the column compartment. Combined with sample introduction via direct injection to the autoSPE cartridge, instead of the loading pump specified in the EPA method, we dramatically reduce the overall analysis time and virtually eliminate the potential of sample cross-contamination.

Introduction

Trace analyte detection in relatively clean matrices is an excellent application for online SPE procedures. Compared to manually loading samples with disposable SPE cartridges, which require an elution step into a vial prior to analysis, online SPE assures 100% sample transfer to the analysis column and dramatically increases sensitivity by increasing the analyte mass delivered to the column. In EPA Method 555, 20 mL of drinking water is loaded through a pump to an SPE cartridge mounted on a high-pressure switching valve on the HPLC system. Because few, if any, autosamplers can inject this large volume, the sample must be pumped onto the cartridge. Contamination of the loading pump with prior samples is always a concern, and adequate flushing and blank runs become an important part of the overall method procedure.

To reduce the sampling volume sufficient for available automatic preparative samplers, without losing sensitivity in the method, it is necessary to reduce the analysis column size while preserving resolving power. Ancillary benefits of using smaller columns generally include reduced analysis time and solvent consumption, and greater compatibility with ionization sources in mass spectrometers.

If the ratios of their column length to particle size are equal, columns are considered to have equal resolving power. Significant reductions in column volume can be made by reducing the length and/or internal diameter of the column. In the latter case, the flow rate would normally be reduced as in Equation 1.

$$\text{Flow}_{\text{col. 1}} \times \left[\frac{\text{Diam.}_{\text{column2}}}{\text{Diam.}_{\text{column1}}}\right]^2 = \text{Flow}_{\text{col. 2}} \quad \text{(eq. 1)}$$

The combined effect of reduced length and diameter contributes to a reduction in solvent consumption. We normally scale the injection mass to the size of the column and a proportional injection volume would be calculated from the ratio of the void volumes of the two columns, multiplied by the injection volume on the original column, as in Equation 2 below.

$$\text{Inj. vol.}_{\text{col. 1}} \times \left[\frac{\text{Volume}_{\text{column2}}}{\text{Volume}_{\text{column1}}}\right] = \text{Inj. vol.}_{\text{col. 2}} \quad \text{(eq. 2)}$$

Short columns packed with small particle sizes are typically operated at high linear velocities. The increase in elution speed will decrease absolute peak width and may require the user to adjust data acquisition rates and reduce signal filtering parameters. This will ensure that the chromatographic separation is accurately recorded in the acquisition data file.

For gradient elution separations, where the mobile phase composition increases through the initial part of the analysis until the analytes of interest have been eluted from the column, successful method conversion to smaller columns requires that the gradient slope be preserved. We can express the gradient slope as in Equation 3.

$$\%\ \text{Gradient slope} = \left[\frac{(\text{End\%} - \text{Start\%})}{\#\text{Column volumes}}\right] \quad \text{(eq. 3)}$$

Note that the use of % change per column volume rather than % change per minute frees the user to control gradient slope by altering gradient time and/or gradient flow rate. A large value for gradient slope yields very fast gradients with minimal resolution, while lower gradient slopes produce higher resolution at the expense of increased solvent consumption and somewhat reduced sensitivity. Longer analysis time may also result unless the gradient slope is reduced by increasing the flow rate, within acceptable operating pressure ranges, rather than by increasing the gradient time.

Resolution increases with shallow gradients because the effective capacity factor, k*, is increased. Much like in isocratic separations, where the capacity term is called k', a higher value directly increases resolution. The effect is quite dramatic up to a k value of about 5–10, after which little improvement is observed. In the subsequent examples, we will see the results associated with the calculations discussed above.

Experimental Conditions

See figure 1 for configuration.

System

Agilent 1200 Series Rapid Resolution LC consisting of:
G1379B micro degasser
G1312B binary pump SL
G1312A binary pump with solvent selection valve option, or
G1354A quaternary pump
G1367C HiP ALS autosampler SL, and
G2258A Dual Loop Prep autosampler 5 ml
G1316B Thermostatted column compartment SL with 6- or 10-port 2-position switching valve
G1315C UV/VIS diode array detector (DAD) SL, flow cell as indicated in individual chromatograms
ChemStation 32-bit version B.02.01

Columns

Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm
Agilent ZORBAX SB-C18, 3.0 × 150 mm, 3.5 µm
Agilent ZORBAX SB-C18, 2.1 × 80 mm, 1.8 µm
Agilent ZORBAX SB-Aq, 4.6 × 12.5 mm, 5 µm

Mobile phase conditions

Organic solvent:	Acetonitrile
Aqueous solvent:	25 mm phosphoric acid in Milli-Q water

Gradient conditions

Gradient slope:	7.8 or 2.3% per column volume, as indicated. See individual chromatograms for flow rate and time

Sample

EPA 555 Group A chlorinated phenoxy acid herbicides (picloram, chloramben, dicamba, bentazon, 2,4-D, dichlorprop, 2,4,5-TP, acifluorfen), 100 µg/mL in methanol or diluted to 20 ng/L (20 ppb) in reagent water acidified with 25 mm phosphoric acid.

Results

The separation was initially performed via direct injection of concentrated standard on a 4.6 × 250 mm, 5-µm ZORBAX SB-C18 column, thermostatted to 25 °C, using conditions referenced in US EPA method 555 (Figure 2). The described trace enrichment procedure using pump A as the loading pump was performed (Figure 3). The method was then scaled in flow and time for exact translation to a 3.0 × 150 mm 3.5-µm column (Figure 4) using 5-mL trace enrichment injection. Finally, a 2.1 × 80 mm 1.8-µm configuration (50-mm plus 30-mm columns in series) is used to demonstrate the feasibility of this separation under conditions for trace enrichment requiring less than 1.5-mL injection. (Figure 5)

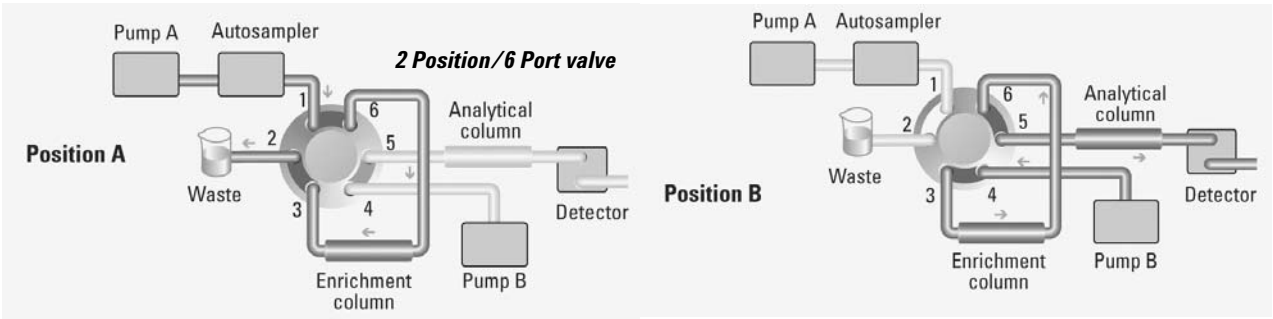


Figure 1. Trace enrichment autoSPE scheme.

Figure 1 shows the schematic placement of modules and columns in the system. The A pump is the loading pump in case of volumes exceeding the 5-mL capacity of the G2258A Dual Loop Autosampler, thus pump A uses one line for sample and a second line for the aqueous eluent, 25 mm phosphoric acid. If direct injection from the autosampler is used, pump A is delivering 25 mm phosphoric acid. If the A pump is fitted with a degasser, the sampling line should bypass the degasser module to minimize contamination with sample solutions. To conduct sampling through the A pump, the valve should be in position B while the new sample is flushed through the A pump. Then switch the valve to the A position and load the required 20 mL sample volume. The analysis begins when the valve is returned to the B position, at which time the sampling line on the A pump would be flushed with reagent water or the next sample, as appropriate.

Figures 2 and 3 show the standard separation by direct injection and pumped trace enrichment, respectively. With column regeneration steps, this results in a total analysis time of 60 minutes. Translation of the gradient to the 3.0- × 150-mm column requires a reduction in flow rate, due to the smaller diameter, and a reduction in gradient time because of the shorter column length. The resulting analysis is reduced from 60 to 36 minutes and solvent consumption is proportionately reduced from 60 mL to 15.5 mL.

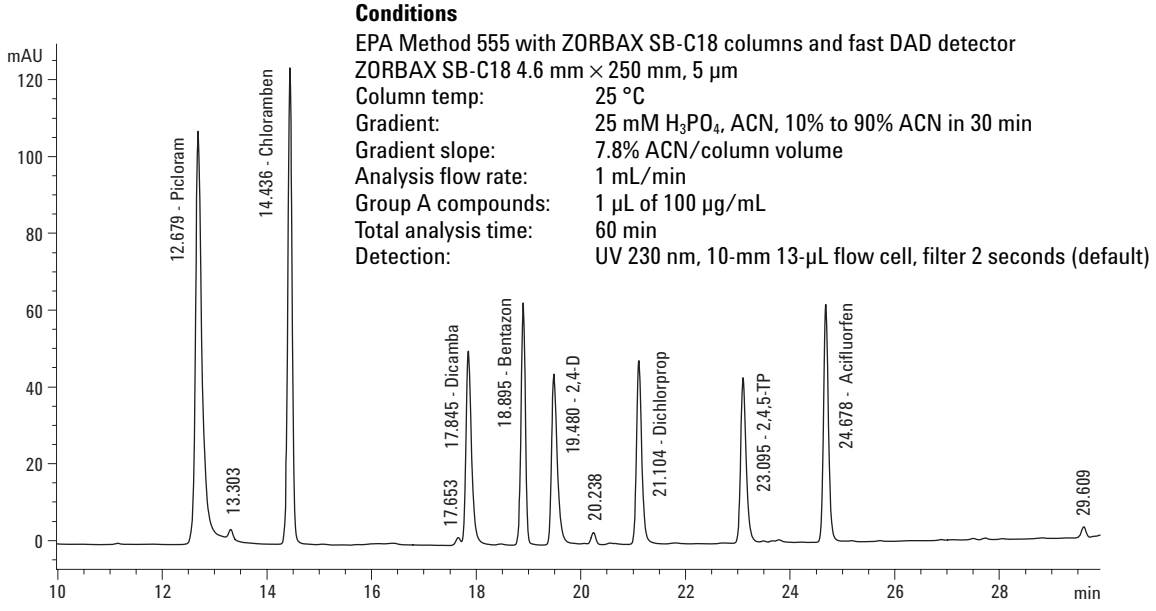
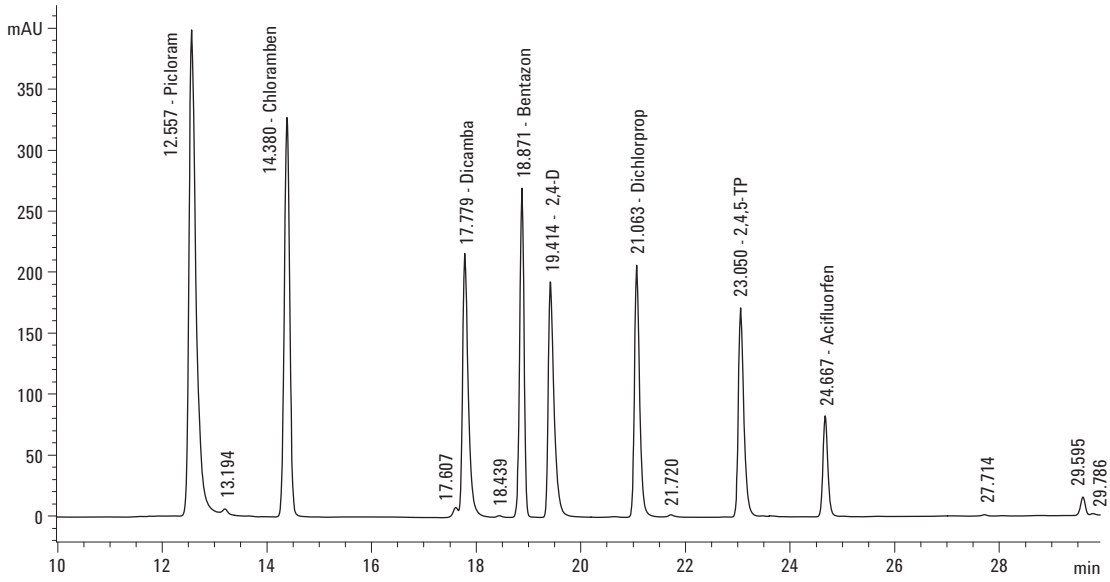


Figure 2. Gradient separation of herbicides on 4.6 mm × 250 mm, 5 µm ZORBAX SB-C18.



Conditions

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector

ZORBAX SB-C18 4.6 mm × 250 mm, 5 µm	
Column temp:	25 °C
Gradient:	25-mM H_3PO_4, ACN, 10% to 90% ACN in 30 min
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	1 mL/min
Group A compounds:	20 mL of 20 µg/L trace enrichment
Total analysis time:	60 min
Detection:	UV 230 nm, 10-mm 13-µL flow cell, filter 2 seconds (default)

Figure 3. Trace enrichment (20 mL) of 20-ppb solution on 4.6 × 250 mm 5-µm ZORBAX SB-C18.

Conditions:

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector

ZORBAX SB-C18 3 mm × 150 mm, 3.5 µm	
Column temp:	25 °C
Gradient:	25 mm H_3PO_4, ACN, 10% to 90% ACN in 18 min
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	0.43 mL/min
Group A compounds:	5 mL of 20 µg/L (20 ppb)
Detection:	UV 230 nm, 3-mm 2-µL flow cell, filter 0.2 seconds
Total analysis time:	36 min

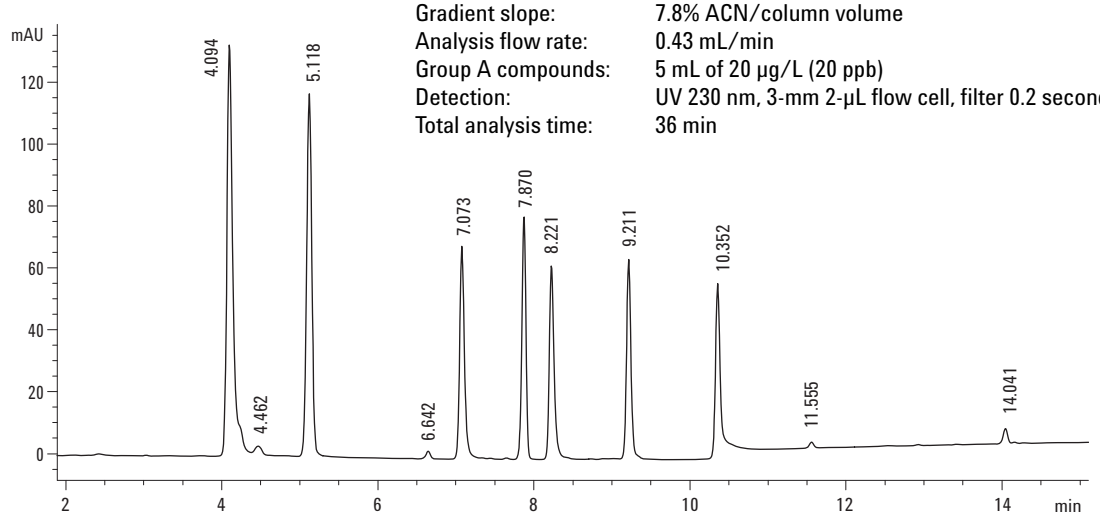


Figure 4. Trace enrichment (5 mL)of 20-ppb solution on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

The last peak in Figure 4 is missing due to a valve timing error that was not detected until sometime after the lab work was completed. Peak 8 was not eluted from the trace enrichment column before the valve switched offline for regeneration and equilibration. Note the baseline shift that occurs after peak 7, not seen in other autoSPE examples.

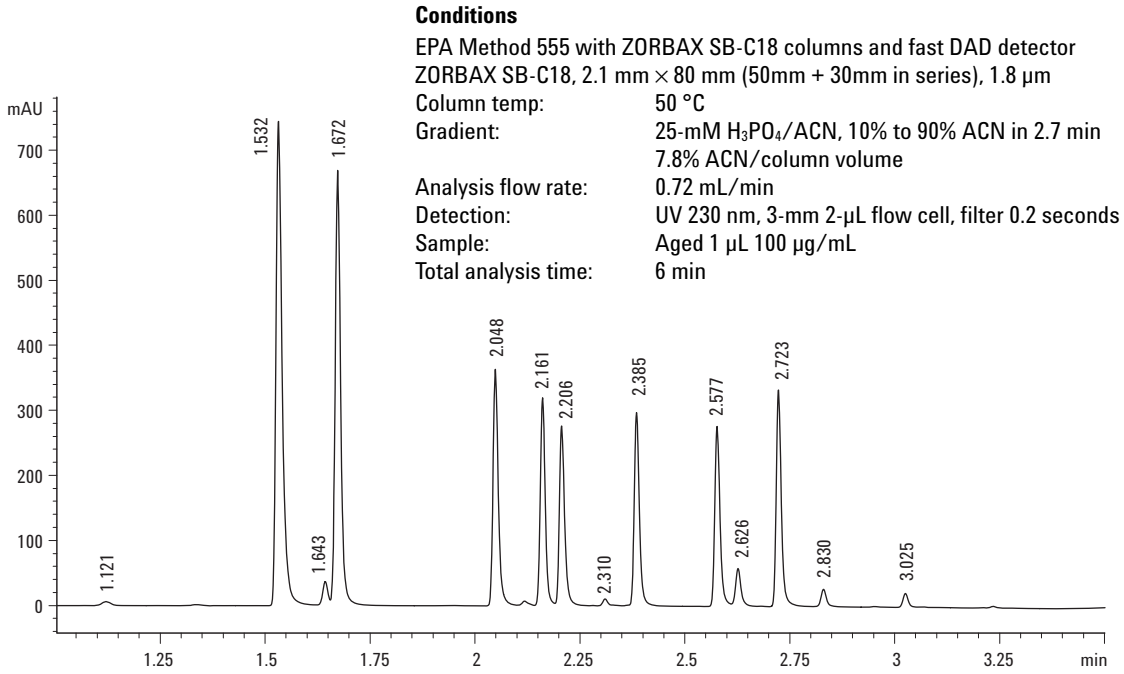


Figure 5. High-speed gradient separation of herbicides on 2.1 × 80 mm, 1.8-µm ZORBAX SB-C18.

In Figure 5 we see the combination of highest speed and resolution, using the full capability of the 1200 Rapid Resolution LC. Operating pressure was, at the maximum point, about 520 bar. We maintain comparable resolution to the original 4.6 × 250 mm, 5-µm method, a 60-minute run time, with an analysis time of only 6 minutes.

Conclusion

As is the case for many existing methods, it is both possible and practical to modernize this method to improve throughput and overall performance. Here we see the potential for a 10-fold increase in analysis speed and elimination of the loading pump scheme found in the original method. Approximately 1.3 mL of sample solution, injected to the autoSPE setup using the 2.1 × 80 mm configuration, is all that is needed to replace the 20-mL injection previously loaded through the pump. This approach can greatly improve productivity and ensure minimal sample cross-contamination.

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Printed in the USA
March 30, 2007
5989-5176EN

Agilent Technologies

Determination of Pesticides in Water by SPE and LC/MS/MS in Both Positive and Negative Ion Modes

Application

Environmental

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Abstract

Using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS), 46 pesticides in positive ion mode and 14 pesticides in negative ion mode were analyzed at low pg level on column without any derivatization. Good linearity was observed for all analytes from 5 pg to 1 ng on column.

Introduction

To monitor trace pesticide residues in surface and ground water, an effective sample preparation and analysis method is required. In 1996, US Geological Survey National Water Quality Laboratory (NWQL) developed and implemented a graphitized carbon-based SPE and high-performance liquid chromatography (HPLC) method to determine polar pesticide concentrations [1].

Subsequently, the NWQL developed an HPLC-mass spectrometry (MS) method to improve the sensitivity and selectivity. This method is capable of quantifying pesticides and pesticide metabolites in filtered water at concentrations as low as 10 ng/L.

Taking advantage of the Multiple Reaction Monitoring (MRM) technique, any interference and matrix signal from organic matter in the water can be minimized from the target compound signals for better confirmation and quantitation. In this application, SPE and LC/MS/MS methods are described to analyze 46 pesticides in positive ion mode and 14 pesticides in negative ion mode.

Experimental

Sample Preparation Procedure
See reference 1 for more information

1. Filter water samples in the field or laboratory using 0.7-µm glass fiber filters.
2. Pump 1 L of the filtered water sample, at a flow rate of 20 mL/min, through a Carbopak-B SPE cartridge containing 0.5 g of graphitized carbon sorbent.
3. Elute the compounds with 1.5 mL methanol, followed by 13 mL of an 80:20 methylene chloride:methanol mixture that has been acidified with trifluoroacetic acid anhydride (0.2%).
4. Reduce the two fractions to near dryness and then combine. The final volume of the extract is 1 mL.

Calibration Samples

Separate stock solutions of either positive ion mode or negative ion mode analytes were diluted 1 to 10 to make the calibration standard solutions. The concentrations of the seven calibration solutions were 5, 10, 50, 100, 200, 500, and 1,000 pg/µL (ppb).

Instrumentation

Positive Ion Mode

LC:	1200 LC
Column:	ZORBAX Extend-C-18, RRHT, 2.1 mm × 100 mm, 1.8 µm
Column temperature:	40 °C
Mobile phases:	A: 0.1% formic acid in water, add NH_4OH buffer to pH 5.5 B: Acetonitrile (ACN)
Flow rate:	0.3 mL/min

Gradient:

Time	%B
0	0
15	100
20	100
21.5	0

Injection volume:	1.0 µL
MS:	G6410A QQQ
Ionization:	ESI (+)
Mass range:	100–500 amu
Scan time:	300 ms
Capillary:	3500 V
Nebulizer P:	40 psi
Drying gas:	9 L/min
Gas temperature:	350 °C
Skimmer:	35 V

Negative Ion Mode

LC:	1200 LC
Column:	ZORBAX Extend-C-18, RRHT, 2.1 mm × 100 mm, 1.8 µm
Column temperature:	60 °C
Mobile phases:	A: 0.04% glacial acetic acid in water B: Acetonitrile (ACN)
Flow rate:	0.3 mL/min

Gradient:

Time	%B
0	0
1	40
2	52
3	60
4	100
8	100
9	0

Injection volume:	1.0 µL
MS:	G6410A QQQ
Ionization:	ESI (–)
Mass range:	120–400 amu
Scan time:	300 ms
Capillary:	3500 V
Nebulizer P:	40 psi
Drying gas:	9 L/min
Gas temperature:	200 °C
Skimmer:	35 V

The MRM parameters for positive ion mode and negative ion mode are listed in Tables 1 and 2, respectively.

Table 1. Positive Ion Mode MRM Method Parameters

Name	RT	Precursor	Quant ion	Qual ion	Collision V	Dwell	Segment
3(4 chlorophenyl) methyl/urea	8.05	185	128	93	10	30	4
3-Keto Carbofuran	8.24	236	179	151	10	30	4
3-OH Carbofuran	6.90	238	163	181	10	40	3
Aldicarb	8.20	116	89	70	5	30	4
Aldicarb sulfone	5.52	223	76	86	5	50	2
Aldicarb sulfoxide	4.99	207	89	132	5	150	1
Atrazine	9.86	216	174	96	20	40	5
Bendiocarb (Ficam)	9.32	224	167	109	5	40	5
Benomyl	6.61	192	160	132	30	40	3
Bensulfuron	10.86	411	149	182	15	60	7
Bromacil	8.44	261	205	162	20	30	4
Caffeine	5.48	195	138	110	15	50	2
Carbaryl	9.69	202	145	127	15	40	5
Carbofuran	9.36	222	165	123	10	40	5
Chlorimuron ethyl	11.57	415	186	213	10	60	8
Cycloate	14.52	216	83	154	15	60	8
Desethyl atrazine	7.06	188	146	79	15	40	3
Desisopropyl atrazine	5.94	174	68	104	30	50	2
Desisopropyl desethyl atrazine	1.76	142	86	57	15	150	1
Diphenamid	10.82	240	134	167	20	60	7
Diuron	10.02	233	72	160	20	75	6
Fenuron	6.90	165	72	92	15	40	3
Flumetsulam	7.49	326	129	262	20	30	4
Fluometuron	9.70	233	72	168	20	40	5
Hydroxy-atrazine	6.82	198	156	114	20	40	3
Imazaquin	7.68	312	267	252	20	30	4
Imazethapyr	6.99	290	177	69	30	40	3
Imidacloprid	7.01	256	175	209	15	40	3
Linuron	11.45	249	160	182	15	60	7
Metalaxyl (Apron)	10.15	280	220	192	10	75	6
Methiocarb	11.28	226	169	121	5	60	7
Methomyl	5.77	163	88	106	5	50	2
Metsulfuron methyl	8.43	382	167	199	15	30	4
Neburon	12.99	275	57	88	20	60	8
Nicosulfuron (Accent)	7.97	411	182	213	15	30	4
Norflurazon	10.51	304	284	160	30	75	6
Oryzalin	12.58	347	288	305	10	60	8
Oxamyl (Vydate)	5.59	237	72	90	10	50	2
Propham	10.68	138	120	92	10	75	6
Propiconazole (Tilt)	12.89	342	156	69	20	60	8
Propoxur (Baygon)	9.26	210	111	168	5	40	5
Siduron	11.12	233	137	94	15	60	7
Siduron isomer	11.28	233	137	94	15	60	7
Sulfometuron, methyl ester	9.25	365	150	199	15	40	5
Tebuthiuron	8.14	229	127	116	15	30	4
Terbacil	8.69	161	144	88	15	30	4

Table 2. Negative Ion Mode MRM Method Parameters

Name	RT	MW	Quant	Qual	Frag V	Collision V	Dwell	Segment
Clopyralid	3.47	191	190 > 146	192 > 148	80	5	70	1
Picloram	3.69	240	239 > 195	241 > 198	80	5	70	1
Dicamba	4.31	220	219 > 175	219 > 145	60	0	50	2
DCPA	4.49	330	273 > 215	271 > 213	100	5	50	2
Bentazone	4.69	240	239 > 132	239 > 197	120	25	50	2
2,4-D	5.02	220	219 > 161	221 > 163	80	15	25	3
Bromoxynil	5.06	275	274 > 79	274 > 81	120	25	25	3
MCPA	5.09	200	199 > 141	201 > 143	100	10	25	3
Triclopyr	5.26	255	254 > 196	256 > 198	80	10	25	3
2,4-DP	5.42	234	233 > 161	235 > 163	80	5	25	3
2,4-DB	5.66	248	247 > 161	249 > 163	80	10	40	4
MCPB	5.70	228	227 > 141	229 > 143	80	5	40	4
Acifluorofen	5.89	361	360 > 316	360 > 286	60	5	40	4
Dinoseb	6.50	240	239 > 193	239 > 163	120	25	40	4

Results and Discussion

Figure 1 shows the total ion chromatogram (TIC) for the positive ion mode. As seen in Figure 1, the analysis time is less than 15 minutes for the 46 analytes. Using a 1.8 µm particle size column, the peak widths of these analytes are about 0.1 minute. The narrower peak width helps to achieve a higher signal-to-noise (s/n) ratio.

Analysis time in negative ion mode is less than 7 minutes for the 11 analytes, as seen in Figure 2.

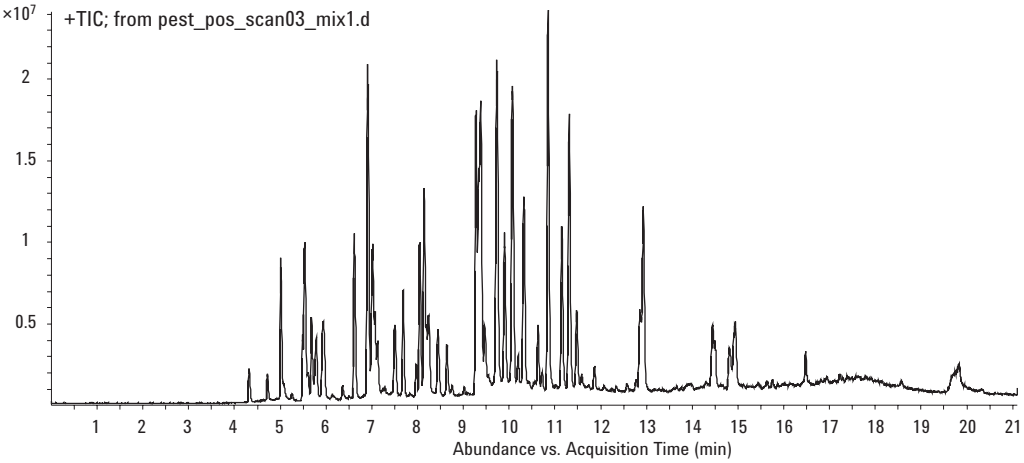

Figure 1. Positive ion mode TIC of 46 pesticides.

A few compounds, for example, Dicamba, MCPB and 2,4-DB, are sensitive to heat from the drying gas. Higher drying gas temperature (350 °C) will lower the intensity of the precursor ion.

Therefore, in the negative ion mode, the drying gas temperature was set to 200 °C. Figure 3 shows the overlaid chromatograms of all 14 pesticides, each at 5 pg on column, from the negative ion mode MRM analysis.

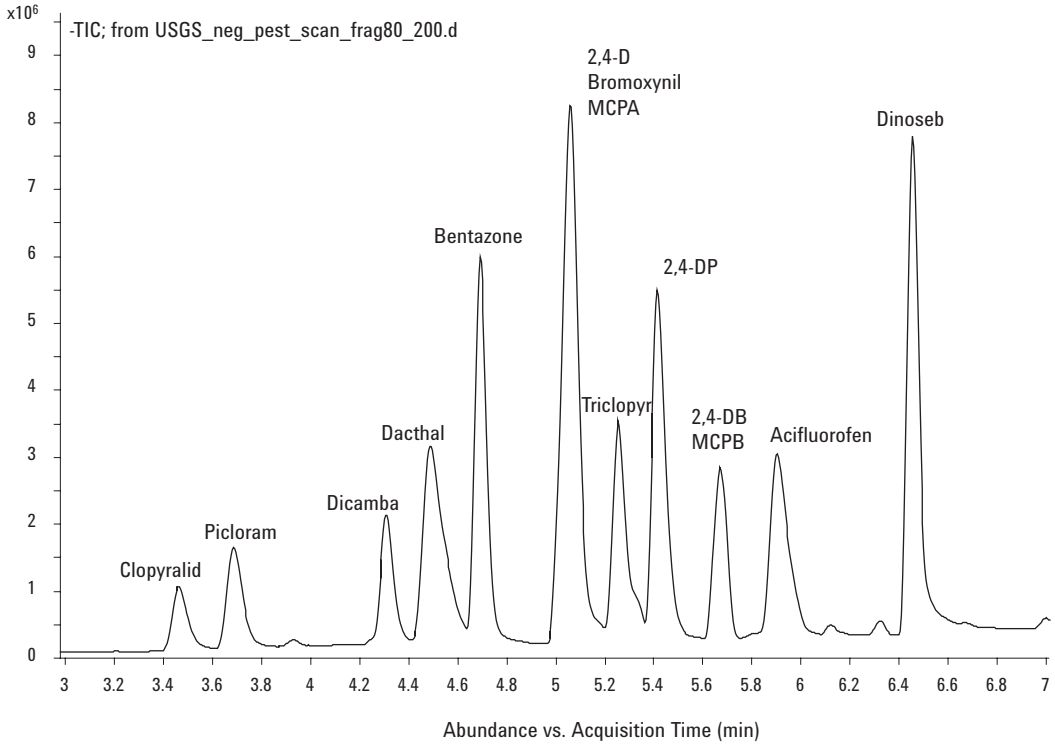

Figure 2. Negative ion mode TIC of 14 pesticides.

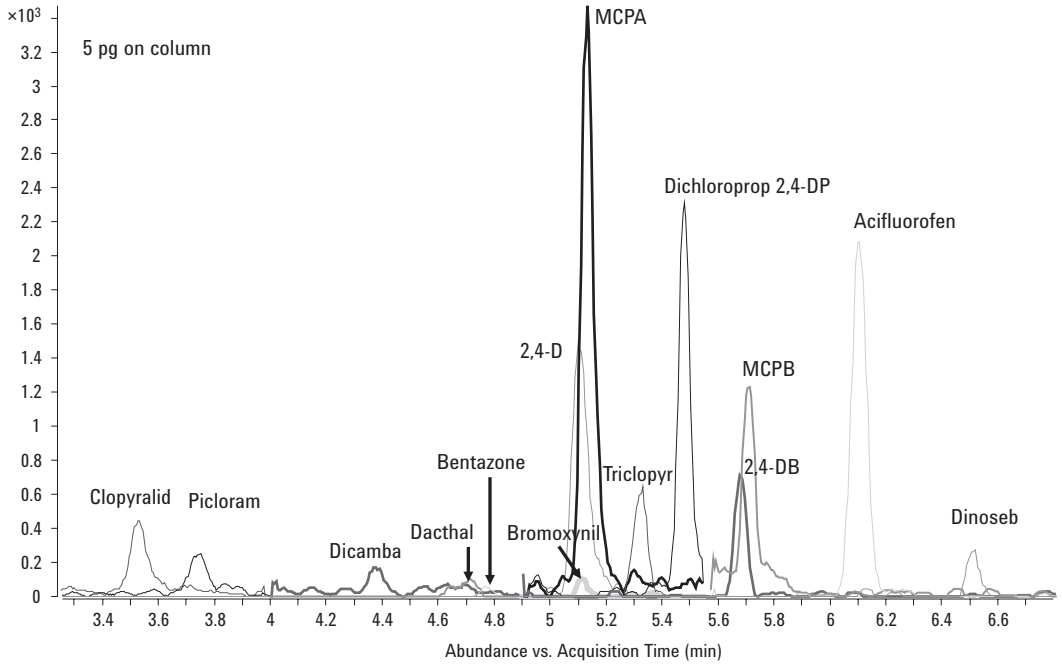

Figure 3. Overlay of the MRM results from 14 pesticides in negative ion mode.

Table 3 shows the linearity results for 14 pesticides over the range of 5, 10, 50, 100, 200, 500, and 1,000 pg on column. The calibration model used was a linear model that included origin with no weighting. All analytes showed excellent linearity.

Table 3. Pesticide linearity: 5, 10, 50, 100, 200, 500, 1,000 pg on column

Pesticide	R^2 (linear fit, include origin, no weighting)
Clopyralid	0.9976
Picloram	0.9993
Dicamba	0.9975
DCPA	0.9994
Bentazone	0.9975
2,4-D	0.9990
Bromoxynil	0.9999
MCPA	0.9980
Triclopyr	0.9990
2,4-DP	0.9948
2,4-DB	0.9887
MCPB	0.9847
Acifluorofen	0.9969
Dinoseb	0.9905

Conclusions

Using SPE and LC/MS/MS, 46 pesticides in positive ion mode and 14 pesticides in negative ion mode were analyzed at low pg level on column without any derivatization. Good linearity of responses was observed from 5 pg to 1 ng of analytes on column.

Reference

1. U.S. Geological Survey Water-Resources Investigations Report 01-4134, http://nwql.usgs.gov/Public/pubs/WRIR01-4134.html

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Printed in the USA
July 25, 2006
5989-5320EN

Agilent Technologies

Determination of Pharmaceuticals in Water by SPE and LC/MS/MS in Both Positive and Negative Ion Modes

Application

Environmental

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Abstract

Using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS), 19 pharmaceuticals in positive ion mode and 11 pharmaceuticals in negative ion mode were analyzed at low picogram level on column without any derivatization. Good linearity was observed for analytes from 1 pg to 1 ng on column.

Repeatability from six injections of analytes at 5 pg on column showed RSDs below 15%, for all target compounds except for fluoxetine at 23%.

Introduction

Many articles in leading medical journals and newspapers reported sexual development and reproductive problems in animals and humans, for example, low sperm counts, genital deformities, male fish making eggs, and others. Scientists suggested that man-made chemicals (for example, pesticides and pharmaceuticals) are disrupting the endocrine system.

Compounds like antibiotics, over-the-counter medicines, and caffeine drain through the sewage system largely unaltered into rivers and streams, and even get into the drinking water supply in very small amounts. In order to monitor the trace pharmaceuticals in surface and ground water, an effective sample preparation and analysis method is required.

In 1999, the US Geological Survey National Water Quality Laboratory (NWQL) developed and implemented an OASIS HLB, solid-phase extraction (SPE), and a high-performance liquid chromatography (HPLC)-mass spectrometry (MS) method to analyze pharmaceuticals.

Using the Multiple Reaction Monitoring (MRM) technique, any interference and matrix signal from organic matters in the water can be minimized from the target compound signals for better confirmation and quantitation. In this application, SPE and LC/MS/MS methods are described to analyze 19 pharmaceuticals in positive ion mode and 11 pharmaceuticals in negative ion mode.

Experimental

Sample Preparation Procedure

See Reference 1 for more information.

1. Filter water samples in the field or laboratory using 0.7-µm glass fiber filters.
2. Pump 1 L of the filtered water sample, at a flow rate of 10 mL/min, through an Oasis HLB (SPE) cartridge containing 0.5 g of sorbent.
3. Elute the HLB column with 6 mL of methanol followed by 4 mL of 0.1% TFA (trifluoroacetic acid) in methanol.
4. The resulting solvent extract is then concentrated to approximately 100 µL.
5. Add internal standard (ISTD). The extract is reconstituted to 1 mL.

Calibration Standards

For positive ion mode, nine calibration solutions were prepared: 1, 5, 10, 20, 40, 100, 200, 400, and 1,000 pg/µL. For negative ion mode, six levels were used: 10, 20, 40, 80, 400, and 800 pg/µL.

Instrumentation

Positive Ion Mode

LC:	1200 LC
Column:	ZORBAX Extend-C-18, RRHT, 2.1 mm × 100 mm, 1.8 µm
Column temperature:	40 °C
Mobile phases:	A: 0.1% formic acid in water, add NH_4OH buffer to pH 5.5 B: Acetonitrile (ACN)
Flow rate:	0.3 mL/min

Gradient:

Time	**%B**
0	0
15	100
20	100
21.5	0

Injection volume:	1.0 µL
MS:	G6410A QQQ
Ionization:	ESI–(+)
Mass range:	125 to 800 amu
Scan time:	300 ms
Capillary:	3500 V
Nebulizer P:	40 psi
Drying gas:	9 L/min
Gas temperature:	350 °C
Skimmer:	35 V

Negative Ion Mode

LC:	1200 LC
Column:	ZORBAX Extend-C-18, RRHT, 2.1 mm × 100 mm, 1.8 µm
Column temperature:	60 °C
Mobile phases:	A: 0.04% Glacial acetic acid in water B: Acetonitrile (ACN)
Flow rate:	0.3 mL/min

Gradient:

Time	**%B**
0	0
1	40
2	52
3	70
6	100
13	100
14	0

Injection volume:	1.0 µL
MS:	G6410A QQQ
Ionization:	ESI (–)
Mass range:	120–800 amu
Scan time:	300 ms
Capillary:	3500 V
Nebulizer P:	40 psi
Drying gas:	9 L/min
Gas temperature:	200 °C
Skimmer:	35 V

The MRM parameters for positive ion mode and negative ion mode are listed in Tables 1 and 2, respectively.

Table 1. Positive Ion Mode MRM Method Parameters

Name	RT	MW	Precursor	Quant ion	Collision V	Dwell	Segment
Metformin HCl	0.856	129	130.4	71.5	15	300	1
Acetaminophen	4.591	151	152.3	110.3	18	30	2
Salbutamol	4.717	239	240.4	148.4	15	30	2
Cimetidine	4.815	252	253.4	94.9	17	30	2
1,7,-Dimethylxanthine	4.89	180	181.3	123.9	20	30	2
Cotinine	5.24	176	177.3	118.3	29	30	2
Codeine	5.321	299	300.4	164.9	30	30	2
Caffeine	5.493	194	195.3	137.9	22	30	2
Trimethoprim	5.935	290	291.4	122.8	25	30	2
Thiabendazole	7.194	201	202.3	131.3	35	100	3
Sulfamethoxazole	7.309	253	254.3	156.0	15	100	3
Azithromycin	7.326	749	375.5	157.9	16	100	3
Diphenhydramine	8.446	255	256.5	167.1	5	100	4
Diltiazem HCl	8.693	414	415.4	177.6	18	100	4
Carbamazepine	8.912	236	237.4	194.0	20	100	4
Fluoxetine HCl	9.71	309	310.4	148.5	0	100	5
Dehydronifedipine	10.635	344	345.4	283.9	27	100	5
Warfarin	11.152	308	309.4	163.3	15	100	5
Miconazole nitrate salt	12.865	416	417.2	159.3	30	300	6

Table 2. Negative Ion Mode MRM Method Parameters

Name	RT	MW	Precursor	Quant ion	Frag. V	Collision V	Dwell	Segment
Hydrochlorothiazide	3.42	297	296	269	140	20	70	1
Aspirin	3.49	180	179	122	120	15	70	1
Enalaprilat	3.71	348	347	114	120	10	70	1
Furosemide	4.51	330	329	285	140	15	70	1
Ketoprofen	5.17	254	253	209	80	5	70	2
Clofibric acid	5.20	214	213	127	80	10	70	2
Napoxen	5.20	230	229	170	80	10	70	2
Diclofenac sodium salt	5.84	294	294	250	100	10	100	3
Ibuprofen	6.03	206	205	161	80	0	100	3
Ibuprofen-d3	6.03	209	208	164	80	0	100	3
Gemfibrozil	6.49	250	249	121	120	25	150	4
Triclocarban	6.66	314	313	160	140	15	150	4

Results and Discussion

The total ion chromatogram (TIC) in negative ion mode is shown in Figure 1. The analysis time in negative ion mode is less than 7 minutes for the 11 analytes. Their peak widths are about 0.1 minute, using a 1.8-µm particle size column. The narrower peak width gives a higher signal-to-noise (s/n) ratio compared to a 3.5-µm or larger particle size column.

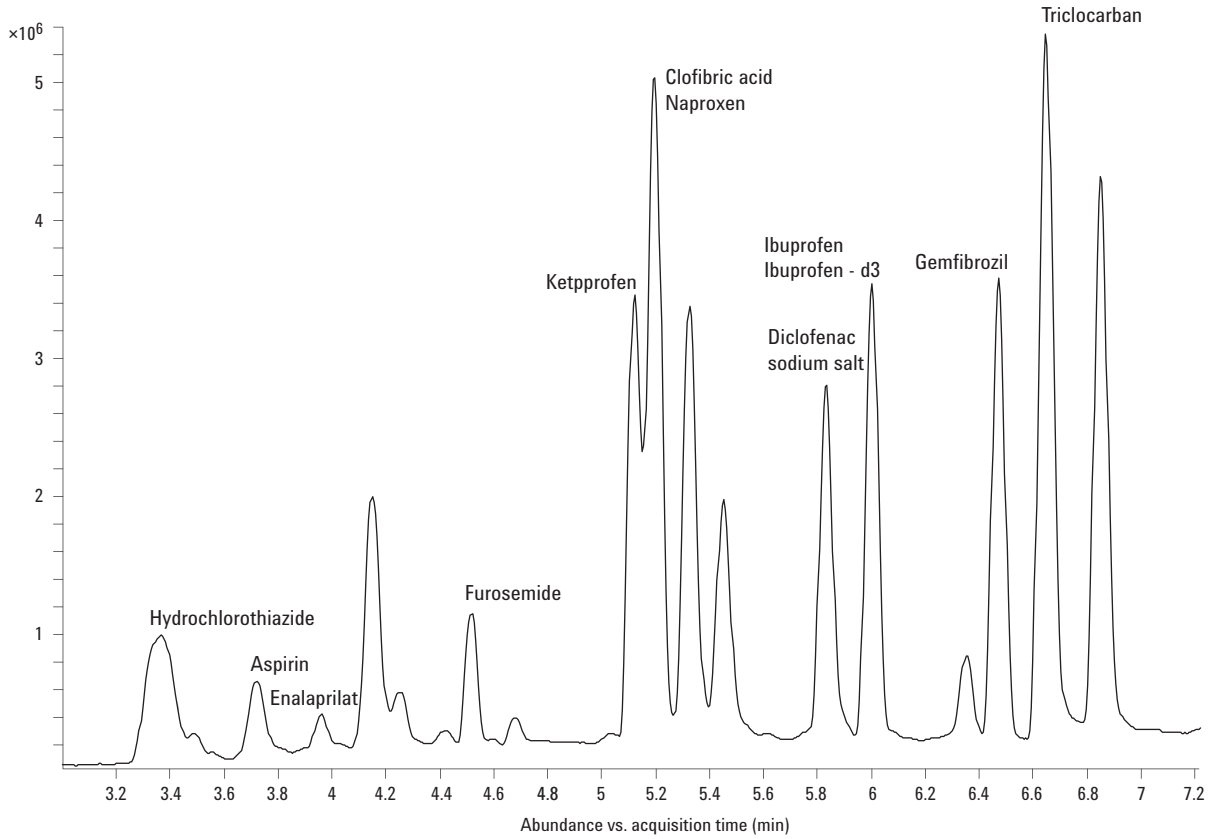

Figure 1. Negative ion mode TIC of 11 pharmaceuticals.

A few compounds, for example, ketoprofen (Figure 2), are sensitive to heat from the drying gas. Higher drying gas temperature (350 °C) lowers the intensity of the precursor ions. Therefore, in the negative ion mode, the drying gas temperature was set to 200 °C.

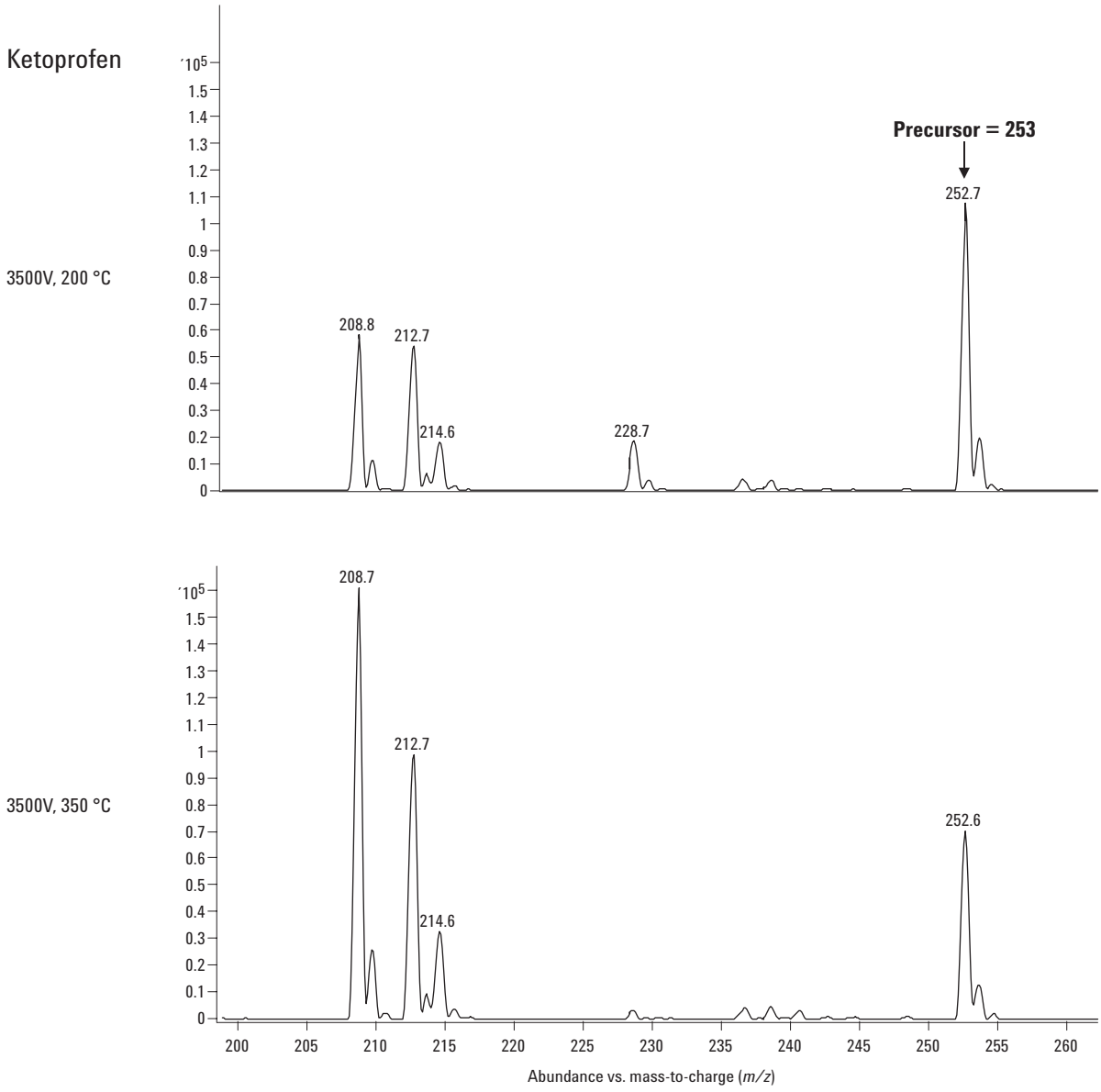

Figure 2. Higher drying gas temperature lowers precursor intensity for certain compounds.

In Figure 3, it was interesting to see that the fragment ion actually had a higher *m/z* value than the precursor ion. For azithromycin, the doubly charged ion showed higher intensity than the singly charged ion and was chosen as the precursor. Therefore, depending on the precursor chosen, it is sometimes necessary to set the upper mass of the product ion scan to be higher than the precursor ion.

Figure 4 shows the overlaid chromatograms of 19 pharmaceuticals, each at 5 pg on column, from the positive ion mode MRM analysis.

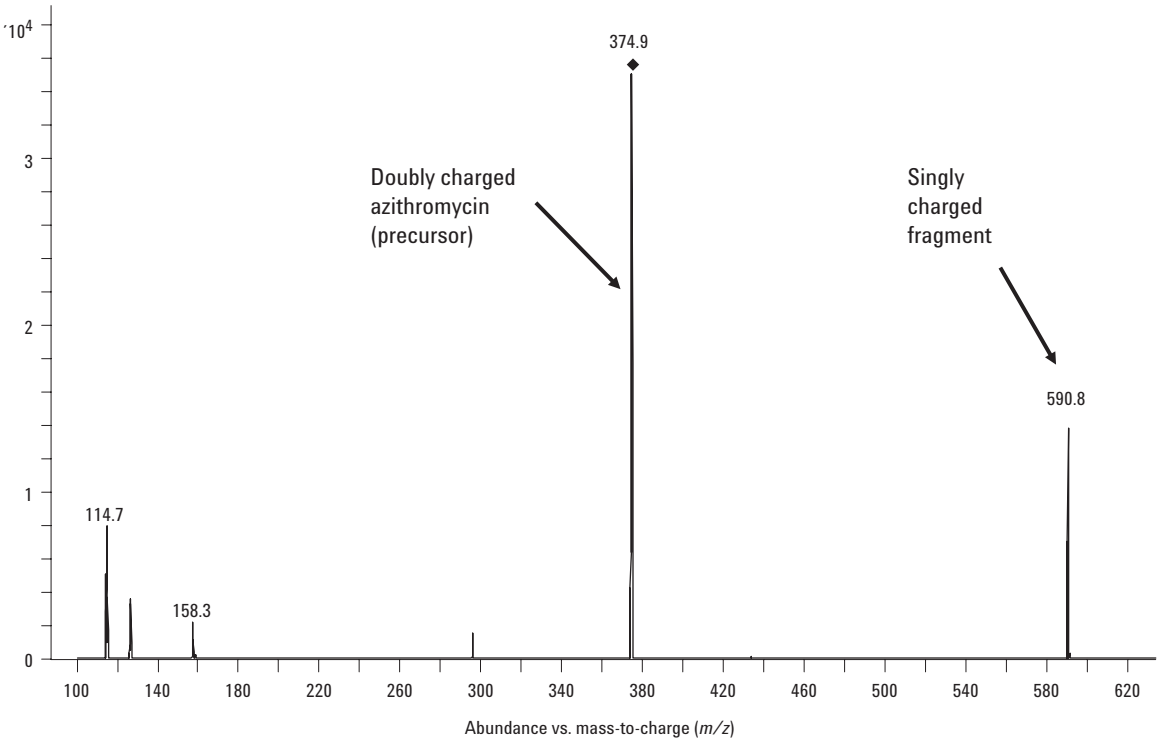

Figure 3. Doubly charged precursor results in a fragment at higher *m/z*.

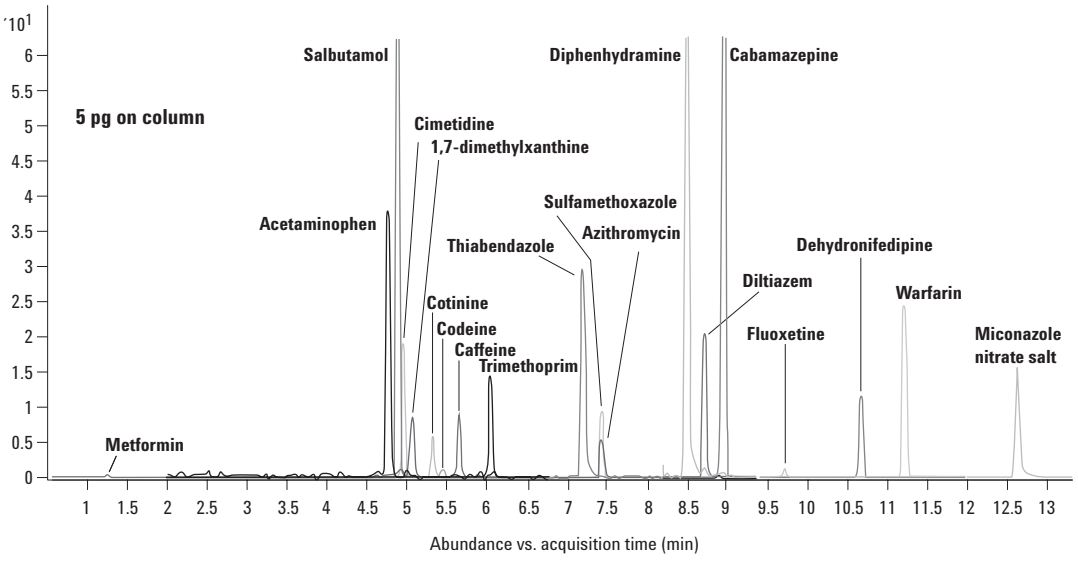

Figure 4. Overlaid MRM chromatograms of the 19 pharmaceuticals in positive ion mode.

Figure 5 shows the overlaid chromatograms of 10 pharmaceuticals, each at 10 pg on column, from the negative ion mode MRM analysis. In both Figures 4 and 5, the analysis times were relatively short and s/n ratios were high.

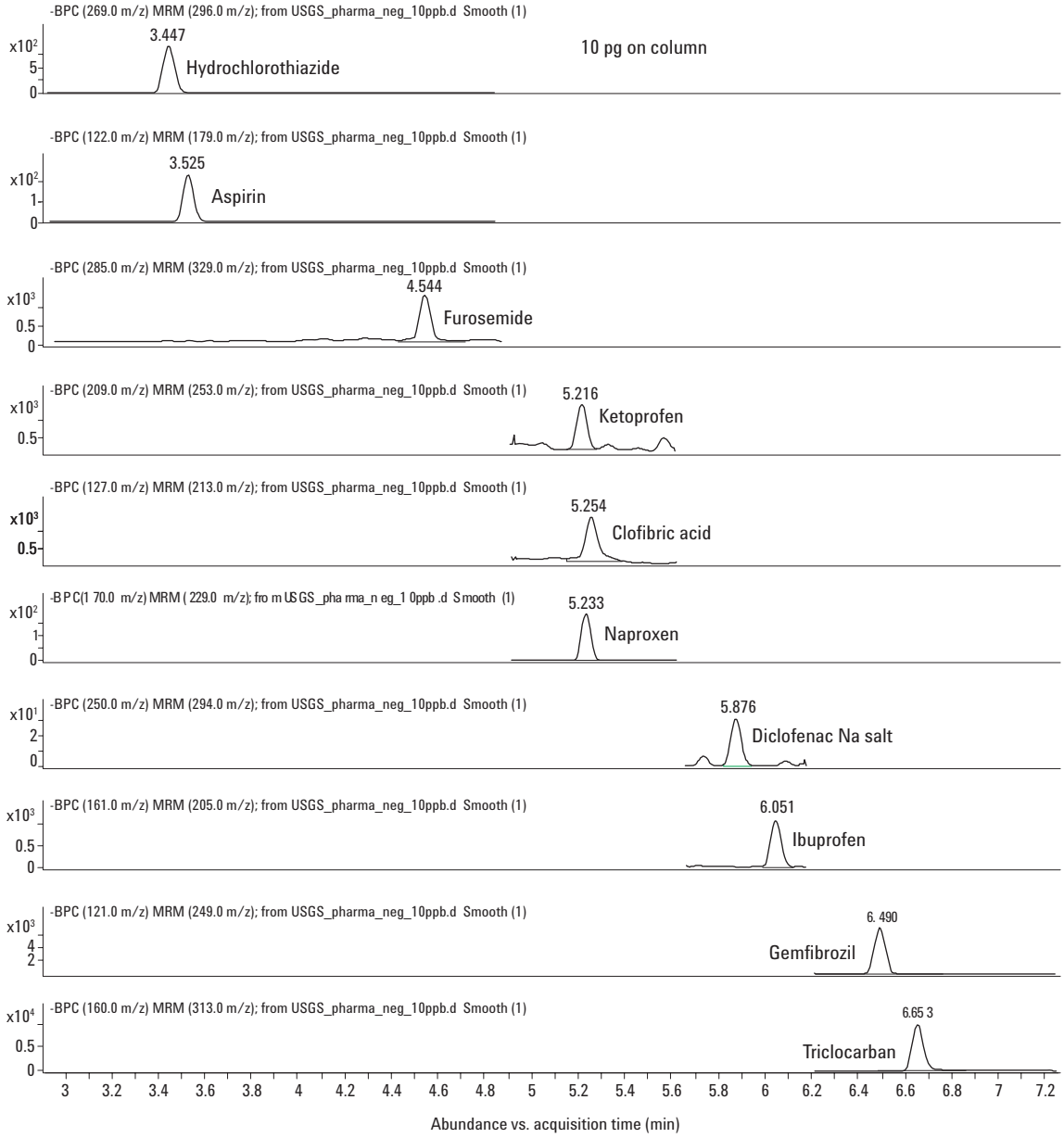

Figure 5. Overlay of MRM results from the 10 pharmaceuticals in negative ion mode.

Table 3 shows the linearity results of all 19 pharmaceuticals (ESI+) over the range of 1, 5, 10, 20, 40, 100, 200, 400, and 1,000 pg on column. Two calibration models were used: a linear model and a quadratic model that both included origin with no weighting. Some of the compounds showed significant fitting improvement from the linear model to the quadratic model. This is the nature of these compounds.

Table 3. Linearity: 1, 5, 10, 20, 40, 100, 200, 400, and 1,000 pg on Column (ESI+), Origin Included, No Weighting

Compound	R^2 (linear fit)	R^2 (quadratic fit)
Metformin HCl	0.9975	0.9999
1,7,-Dimethylxanthine	0.9998	0.9998
Acetaminophen	0.9852	0.9999
Caffeine	0.9992	0.9997
Cimetidine	0.9968	0.9998
Codeine	0.9989	0.9997
Cotinine	0.9971	0.9998
Salbutamol	0.9850	0.9994
Trimethoprim	0.9980	0.9999
Azithromycin	0.9633	0.9998
Sulfamethoxazole	0.9998	0.9999
Thiabendazole	0.9997	0.9998
Carbamazepine	0.9926	0.9999
Diltiazem HCl	0.9997	0.9997
Diphenhydramine	0.9975	0.9998
Dehydronifedipine	0.9985	0.9993
Fluoxetine HCl	0.9984	0.9997
Warfarin	0.9989	0.9997
Miconazole nitrate salt	0.9989	0.9995

Table 4 shows the repeatability results from six injections of 5 pg of each analyte on column. In general, the RSDs are below 15%, except for fluoxetine, which was at 23%.

Table 4. Repeatability from Six Injections at 5 pg/µL (5 pg on column), ESI(+)

Compound	%RSD
Metformin HCl	12.4
1,7,-Dimethylxanthine	8.6
Acetaminophen	6.1
Caffeine	5.7
Cimetidine	4.1
Codeine	16.2
Cotinine	10.5
Salbutamol	3.7
Trimethoprim	3.6
Azithromycin	9.4
Sulfamethoxazole	10.7
Thiabendazole	5.3
Carbamazepine	2.8
Diltiazem HCl	4.7
Diphenhydramine	3.7
Dehydronifedipine	5.4
Fluoxetine HCl	23.4
Warfarin	4.4
Miconazole nitrate salt	2.9

Table 5 shows the linearity results of all 11 pharmaceuticals (ESI–) over the range of 10, 20, 40, 80, 400, and 800 pg on column. All the R^2 values were above 0.99, except triclocarban, which was about 0.97.

Table 5. Linearity: 10, 20, 40, 80, 400, and 800 pg on Column (ESI–), Origin Included, No Weighting

Compound	R^2 (linear fit)
Hydrochlorothiazide	0.9999
Aspirin	0.9977
Enalaprilat	0.9981
Furosemide	0.9997
Ketoprofen	0.9988
Clofibric acid	0.9997
Naproxen	0.9994
Diclofenac Na salt	0.9993
Ibuprofen	0.9997
Ibuprofen-d3	0.9998
Gemfibrozil	0.9993
Triclocarban	0.9655

Once the method is established, one can screen and quantitate target analytes in water. Figures 6, 7, and 8 are MRM analyses of actual water sample extracts in positive and negative ion modes.

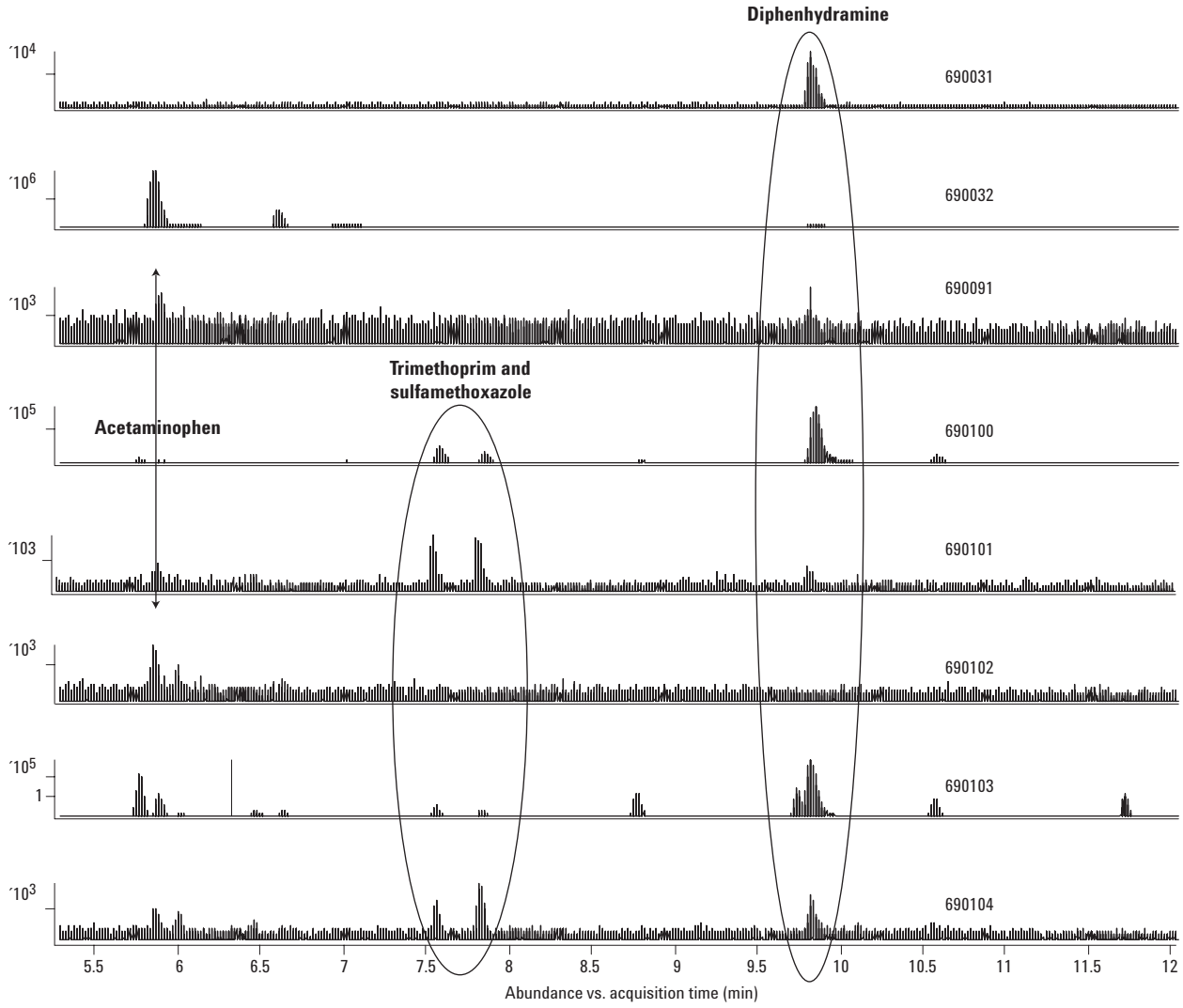

Figure 6. Pharmaceuticals screening in positive ion mode.

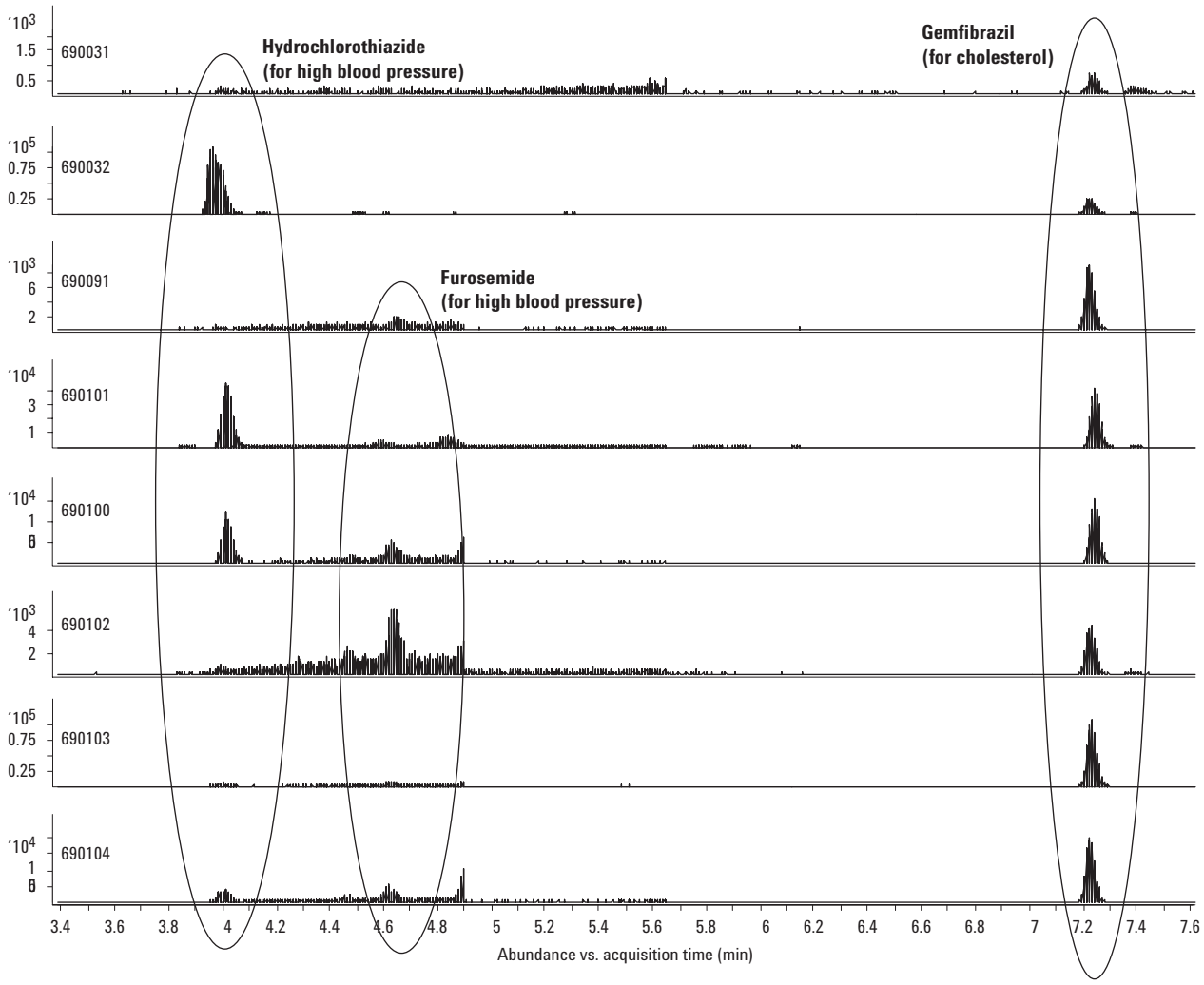

Figure 7. Pharmaceuticals screening in negative ion mode.

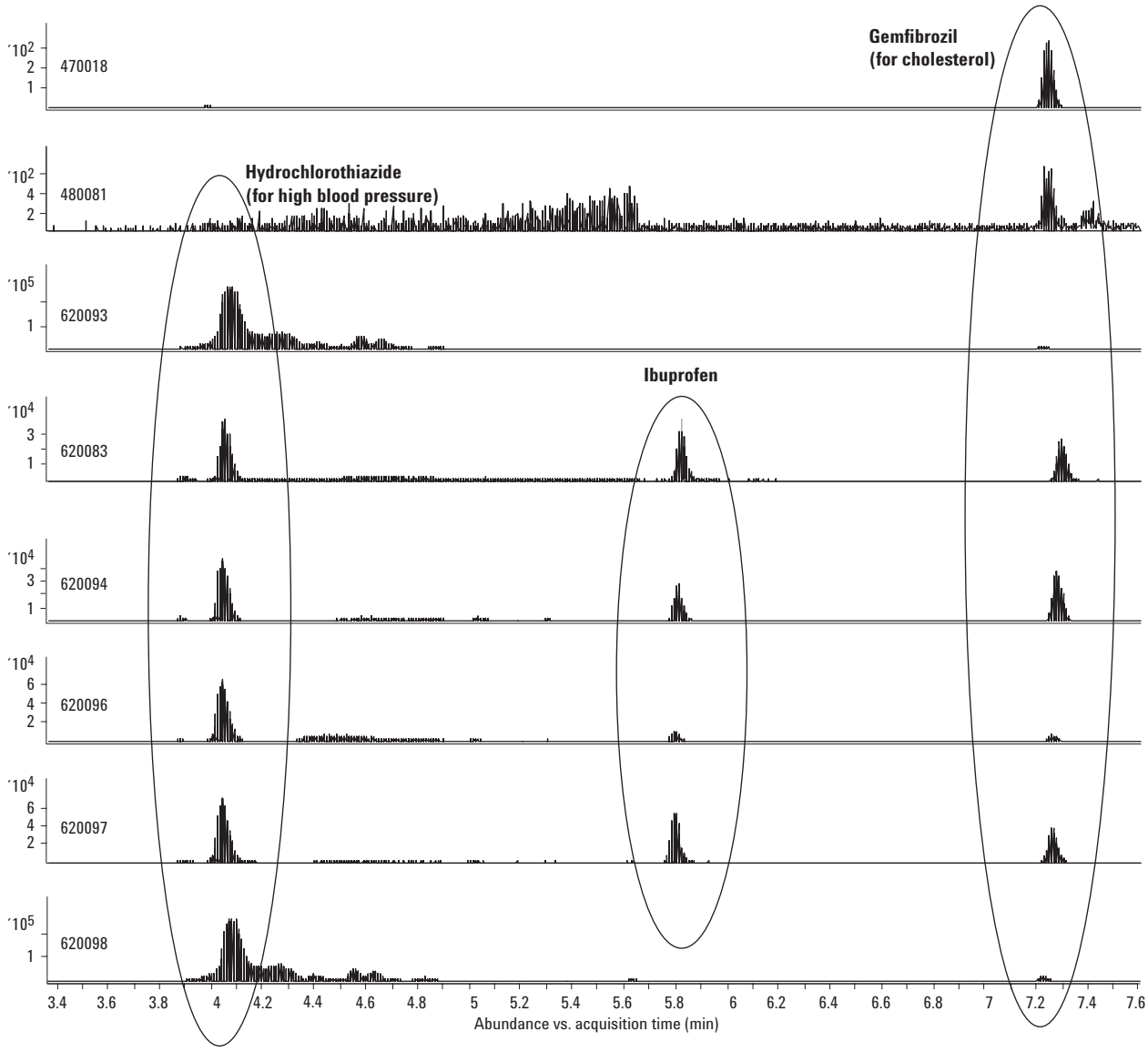

Figure 8. Pharmaceuticals screening in negative ion mode.

Figure 6 shows several of the pharmaceuticals, for example, diphenhydramine and acetaminophen, that were common to several of the water samples. Some of the antibiotics were also found in the samples. Interestingly enough, in Figures 7 and 8, the most common pharmaceuticals in the water samples were related to high blood pressure and cholesterol medications.

Conclusion

Using SPE and LC/MS/MS, 19 pharmaceuticals in positive ion mode and 11 pharmaceuticals in negative ion mode were analyzed at low picogram level on column without any derivatization. Good linearity was observed for analytes from 1 pg to 1 ng on column.

Repeatability study from six injections of target analytes at 5 pg on column showed RSDs below 15%, except for fluoxetine at 23%.

This method was applied to water sample extracts, finding that several target pharmaceutical drugs were commonly found among the analyzed samples.

Reference

1. USGS SOP: Instrumental Analysis for Determination of Human Health Pharmaceuticals in Water by Chemically Modified Styrene-Divinylbenzene Resin-Based Solid-Phase Extraction and High-Performance Liguid Chromatography/Mass Spectrometry, by Steve Werner, 2006.

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Printed in the USA
July 25, 2006
5989-5319EN

Agilent Technologies

Screening for 926 Pesticides and Endocrine Disruptors by GC/MS with Deconvolution Reporting Software and a New Pesticide Library

Application Note

Food and Environmental

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Abstract

An updated and greatly expanded collection of mass spectral libraries has been introduced, replacing Agilent's RTL Pesticide Library and DRS pesticide solution. The new library contains 926 pesticides, endocrine disruptors, and related compounds – 359 more than the original library. Included are all compounds specified for GC/MS analysis in the new Japanese "Positive List" regulations. All compounds have locked retention times that can be accurately reproduced using an Agilent GC/MS system with the ChemStation's Retention Time Locking software. The new Database can be used as a standard GC/MS library for compound identification or with Agilent's Screener software for identifications based upon retention time and mass spectral matching. The greatest benefit accrues when these libraries are used with Agilent's new version of Deconvolution Reporting Software (part number G1716AA version A.03.00). This solution allows one to screen GC/MS files for all 926 pesticides and endocrine disrupters in about two minutes per sample. Deconvolution helps identify pesticides that are buried in the chromatogram by co-extracted materials. The new database was compared to the smaller one for the DRS analysis of 17 surface water samples. With the new database, DRS found 99 pesticides, metabolites, fire retardants, and related contaminants that were not contained in the original RTL Pesticide and Endocrine Disruptor Library.

Introduction

Several years ago Agilent Technologies introduced Retention Time Locking (RTL) for gas chromatography (GC) and GC with mass spectral detection (GC/MS). RTL software makes it possible to reproduce retention times from run-to-run on any Agilent GC or GC/MS, in any laboratory in the world, so long as the same nominal method and GC column are used (1). Since any laboratory can reproduce retention times generated in another, it is possible to create mass spectral libraries that contain locked retention times. By locking their method to the published database, users can screen GC/MS files for all of the library's compounds. "Hits" are required to have the correct retention time as well as the correct spectrum, which eliminates many false positives and gives more confidence in compound identifications (2).

More recently, Agilent introduced Deconvolution Reporting Software (DRS) that incorporates mass spectral deconvolution with conventional library searching and quantification. DRS results from a marriage of three different GC/MS software packages:

1) The Agilent GC/MS ChemStation,
2) The National Institute of Standards and Technology (NIST) Mass Spectral Search Program with the NIST '05 MS Library, and
3) The Automated Mass Spectral Deconvolution and Identification System (AMDIS) software, also from NIST.

The original DRS software was intended to be a comprehensive solution for pesticide analysis and, therefore, included the mass spectra (in AMDIS format) and locked retention times for 567 pesticides and suspected endocrine disrupters (3).

Recently, Agilent introduced an updated and greatly expanded Pesticide and Endocrine Disruptor Database (part number G1672AA) that now contains 926 entries. This represents the addition of 359 new compounds to the original library. At the same time, Agilent introduced a new version of the DRS software (part number G1716AA version A.03.00) that can be used with any Agilent-provided or user-developed DRS library.

Pesticide and Endocrine Disruptor Database Contents

The G1672AA Pesticide and Endocrine Disruptor Database contains virtually all GC-able pesticides, including those introduced very recently. In addition, the database includes numerous metabolites, more endocrine disruptors, important PCBs and PAHs, certain dyes (for example, Sudan Red), synthetic musk compounds, and several organophosphorus fire retardants.

This new database includes:

- A conventional mass spectral library for use with Agilent GC/MS ChemStations
- A screener database for use with Agilent's powerful screener software that is integrated into the GC/MS ChemStation
- Locked Retention Times for all 926 compounds that any Agilent 5975 or 5973 GC/MS user can reproduce in their laboratory
- Files for use with Agilent's G1716AA (A.03.00) Deconvolution Reporting Software
- An e-method that can be loaded into Agilent's G1701DA (version D.02.00 SP1 or higher) with instrument parameters for acquiring GC/MS files and analyzing the data with DRS. These parameters are listed in Table 1.
- Example files
- Application notes

On November 29, 2005, the Japanese Government published a "Positive List" system for the regulation of pesticides, feed additives, and veterinary drugs. Maximum Residue Limits (MRL) have been set for 758 chemicals while 65 others have been exempted from regulation. Fifteen substances must have no detectable residues. Other agricultural chemicals not mentioned have a uniform MRL of 0.01 ppm (4). This new regulation is scheduled to take effect on May 29, 2006.

Of the pesticides in the Japanese Positive List, 265 are to be analyzed by GC/MS. The new G1672AA Pesticide library contains mass spectra and locked retention times for all of these compounds. Thus, a laboratory could screen for all 265 "positive list" compounds and several hundred more pesticides in just 1–3 minutes after the GC/MS run.

Experimental

Table 1 lists the instrumentation, software, and analytical parameters used by Agilent for pesticide analysis. Depending upon the desired injection volume, a PTV inlet or split/splitless inlet can be used.

Table 1. Instrumentation and Conditions of Analysis

Gas Chromatograph	**Agilent 6890N**
Automatic Sampler	Agilent 7683 Injector and AutoSampler
Inlet	Agilent PTV operated in the solvent vent mode or Split/Splitless
Column	Agilent 30 m × 0.25 mm × 0.25 µm HP-5MSi (part number 19091S-433i)
Carrier gas	Helium in the constant pressure mode
Retention time locking	Chlorpyrifos-methyl locked to 16.596 min (nominal column head pressure = 17.1 psi)
Oven temperature program	70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C /min to 200 °C (0 min), 8 °C /min to 280 °C (10–15 min)
PTV inlet parameters	Temp program: 40 °C (0.25 min), 1600 °C/min to 250 °C (2 min); Vent time: 0.2 min; Vent flow: 200 mL/min; Vent pressure: 0.0 psi; Purge flow: 60.0 mL/min; Purge time: 2.00 min
Injection volume	15 µL (using a 50-µL syringe)
Mass Selective Detector	**Agilent 5975 inert**
Tune file	Atune.u
Mode	Scan (or SIM with SIM DRS library)
Scan range	50–550 u
Source, quad, transfer line temperatures	230, 150, and 280 °C, respectively
Solvent delay	4.00 min
Multiplier voltage	Autotune voltage
Software	
GC/MSD ChemStation	Agilent part number G1701DA (version D02.00 sp1 or higher)
Deconvolution Reporting Software	Agilent part number G1716AA (version A.03.00) Deconvolution Reporting Software
Library searching software	NIST MS Search (version 2.0d or greater) (comes with NIST '05 mass spectral library – Agilent part number G1033A)
Deconvolution software	Automated Mass Spectral Deconvolution and Identification Software (AMDIS_32 version 2.62 or greater; comes with NIST '05 mass spectral library – Agilent part number G1033A)
MS Libraries	NIST '05 mass spectral library (Agilent part number G1033A) Agilent RTL Pesticide and Endocrine Disruptor Libraries in Agilent and NIST formats (part number G1672AA)

Results and Discussion

DRS, which has been described in preceding papers (3,5,6), can be summarized as follows:

Three separate, but complimentary, data analysis steps are combined into the DRS. First, the GC/MS ChemStation software performs a normal quantitative analysis for target pesticides using a target ion and up to three qualifiers. An amount is reported for all calibrated compounds that are detected. For other compounds in the database, an estimate of their concentration can be reported based upon an average pesticide response factor that is supplied with the DRS software. The DRS then sends the data file to AMDIS, which deconvolutes the spectra and searches the Agilent RTL Pesticide Library using the deconvoluted full spectra. A filter can be set in AMDIS, which requires the analyte's retention time to fall within a user-specified time window. Because RTL is used to reproduce the RTL database retention times with high precision, this window can be quite small – typically 10–20 seconds. Finally, the deconvoluted spectra for all of the targets found by AMDIS are searched against the 147,000-compound NIST mass spectral library for confirmation; for this step, there is no retention time requirement.

This approach was rapidly adopted by many laboratories because of its ability to identify pesticides in complex chromatograms containing high levels of co-extracted interferences. Indeed, the solution proved to be so useful that users began to create their own DRS libraries (7). Therefore, the DRS was unbundled from the pesticide database so that it could be used with any agilent-provided or user-created database.

The original 567-compound RTL Pesticide Library (G1049A) included pesticides, a few metabolites, and most of the GC-amenable endocrine disruptors that were known at the time. The new version of the library includes many more pesticides, endocrine disruptors, and metabolites. This update also contains important compounds from other classes of contaminants that have been found in food and water supplies. Included are eighteen polychlorinated biphenyls (PCBs), four polybrominated biphenyls (PBBs), several polynuclear aromatic hydrocarbons (PAHs), several organophosphorus fire retardants, three important toxaphene congeners, and three Sudan dyes.

Advantages of Deconvolution

Figure 1 shows a screen from AMDIS that illustrates the power of this deconvolution software. The white trace in Figure 1A is the total ion chromatogram while the other three are extracted ions of a deconvoluted peak (a "component" in AMDIS terminology). Note that the TIC and extracted ions are not scaled to each other and this component is actually obscured by co-eluting compounds. Figure 1B juxtaposes the deconvoluted component spectrum (white) with the complete "undeconvoluted" spectrum (black). Clearly, this component is buried under co-eluting peaks that would ordinarily obscure the analyte. Figure 1C shows that the deconvoluted peak (white spectrum) is a good library match for norflurazon (black spectrum). The locked retention time for norflurazon in the RTL Pesticide Database is 26.933 min, which is just 2.3 seconds away from its observed RT in this chromatogram. Confidence in peak identifications is greatly enhanced by the combination of spectral deconvolution and locked retention time filtering.

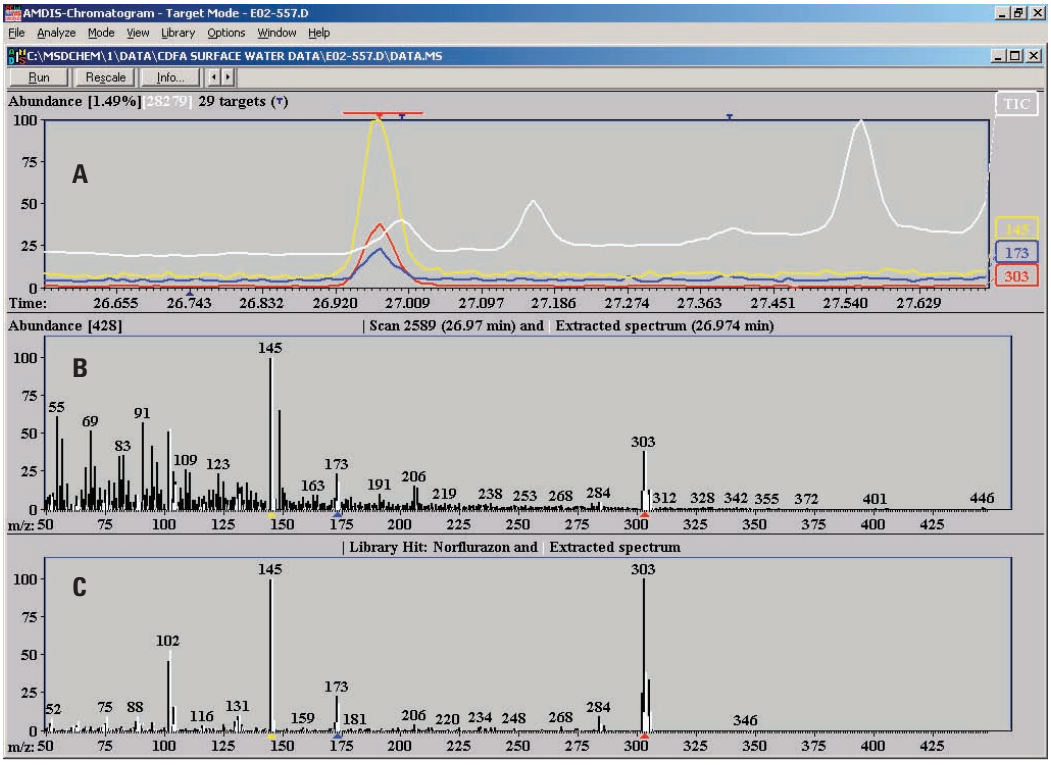

Figure 1. AMDIS screen showing the identification of norflurazon.
A) The total ion and extracted ion chromatograms where norflurazon elutes.
B) The deconvoluted component spectrum (white) juxtaposed with the spectrum at 26.972 min (black).
C) The deconvoluted component matched to the library spectrum of norflurazon.

Surface Water Analysis - Revisiting an Earlier Study

In an earlier study, a comparison was made between Agilent's DRS and conventional pesticide analysis (3). The California Department of Food and Agriculture (CDFA) provided data files for 17 surface water extracts that had been analyzed in their laboratory. Since the GC/MS chromatograms were locked to the Agilent pesticide method, it was possible to analyze these data files using DRS without having to re-run the samples. The original DRS analysis was made using the 567-compound RTL Pesticide Database. For comparison, these same data files were re-analyzed using the new 926-compound RTL Pesticide Database. The chromatogram (Figure 2) and the DRS report (Figure 3) from one of these samples are shown below.

Excluding phthalates, seven new compounds (shown with bold type in Figure 3) were identified using the 926-compound database: 4-chlorophenyl isocyanate (a phenylurea herbicide metabolite); 3,4-dichlorophenyl isocyanate (diuron metabolite); tris(2-chloroethyl) phosphate (a fire retardant); caffeine (a stimulant); Cyprodinil (a fungicide); desmethyl-norflurazon (a metabolite of norflurazon, an herbicide); and tris(2-butoxyethyl) phosphate (a fire retardant). Although caffeine is not generally considered to be dangerous, it is included in the database because it has been found frequently in sewage effluent and in numerous waterways together with a various pharmaceuticals and pesticides (8).

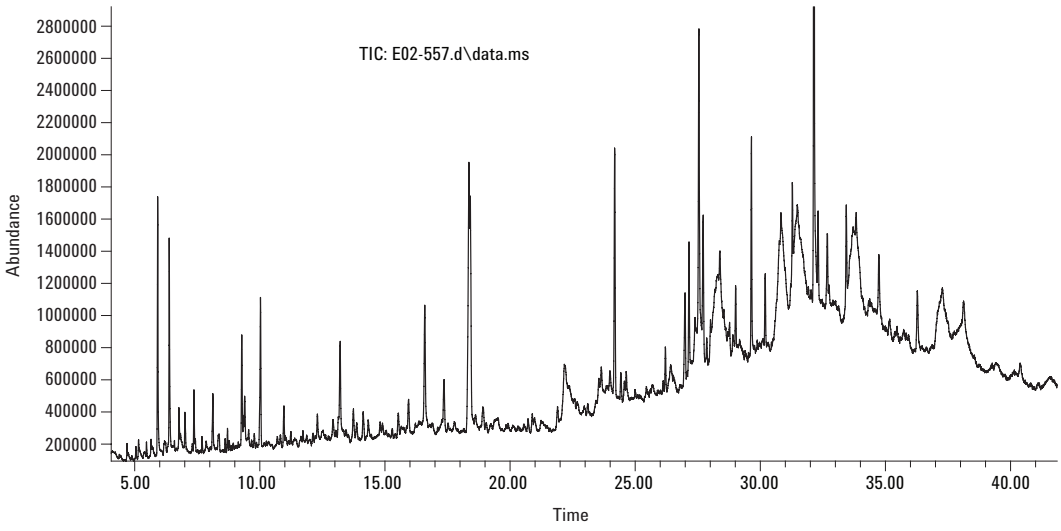

Figure 2. Chromatogram of a surface water extract that was analyzed by DRS using the new RTL Pesticide and Endocrine Disrupter Database. The results of this analysis are shown in Figure 3.

MSD Deconvolution Report
Sample Name: **E02-557**
Data File: **C:\MSDChem\1\DATA\CDFA surface water data\E02-557.d**
Date/Time: **11:24 AM Tuesday, Apr 4 2006**

The NIST library was searched for the components that were found in the AMDIS target library.

RT	Cas #	Compound name	Agilent ChemStation amount (ng)	AMDIS match	RT Diff (sec.)	NIST reverse match	Hit number
4.4689	106445	4-Methylphenol		62	3.2		
4.4689	0000	3-Carbobenzyloxy-4-ketoproline				48	1
4.8840	104121	**4-Chlorophenyl isocyanate**		84	–1.8	86	2
6.3879	102363	**Diuron Metabolite [3,4-Dichlorophenyl isocyanate]**		99	3.1	95	1
6.8357	759944	EPTC		84	2.0	85	1
7.6988	95761	3,4-Dichloroaniline		93	2.1	89	2
7.9342	131113	Dimethylphthalate		67	1.7	84	2
8.1112	25013165	Butylated hydroxyanisole		63	–7.7		
8.1112	0000	7-Methoxy-2,2,4,8-tetramethyltricyclo [5.3.1.0(4,11)]undecane				62	1
8.941	29878317	Tolyltriazole [1H-Benzotriazole, 4-meth-]	1.29				
9.7903	134623	N,N-Diethyl-m-toluamide		85	2.2	84	2
10.0019	84662	Diethyl phthalate		98	2.6	92	1
10.7109	119619	Benzophenone		86	2.6	88	2
10.9684	126738	Tributyl phosphate		96	3.0	90	1
11.6491	1582098	Trifluralin		83	0.7	74	1
12.9326	122349	Simazine		88	1.4	86	2
13.4309	115968	**Tris(2-chloroethyl) phosphate**		79	1.0	78	1
13.7478	1517222	Phenanthrene-d10		95	1.3	83	1
15.4048	58082	**Caffeine**		80	1.6	74	1
15.9474	84695	Diisobutyl phthalate		90	3.2	88	4
16.5988	5598130	Chlorpyrifos Methyl		97	0.4	90	1
17.3653	7287196	Prometryn		90	1.5	84	1
18.4213	84742	Di-n-butylphthalate		99	0.4	94	1
18.9214	51218452	Metolachlor		90	0.7	87	1
20.5633	121552612	**Cyprodinil**		69	–0.1		
20.5633	76470252	9,9-Dimethoxy-9-sila-9, 10-dihydroanthracene				70	1
26.4247	23576241	**Norflurazon, Desmethyl-**		87	–4.5	69	2
26.9700	27314132	Norflurazon		87	1.5	79	1
26.9992	85687	Butyl benzyl phthalate		94	–0.5	94	1
27.3984	51235042	Hexazinone		89	0.8	83	1
28.0127	78513	**Tris(2-butoxyethyl) phosphate**		75	3.3	83	1
29.6537	117817	Bis(2-ethylhexyl)phthalate		98	0.3	90	3
33.9298	84764	Di-n-nonyl phthalate		65	–1.9		
33.9298	0000	Phthalic acid, 3,4-dichlorophenyl propyl ester				71	1
13.739		Phenanthrene-d10	10				

Figure 3. **DRS report from the analysis of a surface water sample.** The compounds shown in bold type were found by the new RTL Pesticide Database but not the original one because these compounds were not included.

For this sample, the ChemStation identified only tolyltriazole at 8.941 min, but AMDIS did not confirm this assignment, nor could it be confirmed manually. Butylated hydroxyanisole was tentatively identified by AMDIS with a low match value, but the retention time is off by –7.7 seconds which is considerably more than most other hits. This compound is not in the NIST library so it could not be confirmed. The ChemStation method used for this analysis required that all three qualifier ions fall within ±20% (relative) which is a rigorous requirement for such a complex sample. This explains why so few compounds were found by the ChemStation.

Cyprodinil (20.563 min) was identified by AMDIS but the NIST library search failed to confirm its presence. The next line shows that the best NIST library match is an anthracene derivative that is nothing like cyprodinil. This result was obtained when AMDIS was configured to "use uncertain peaks" as shown in Figure 4. When this feature is turned off in DRS Compound Identification Configuration, the best NIST library hit for this spectrum is, indeed, cyprodinil. When a compound's identity is ambiguous, as with cyprodinil, it may be useful to perform the DRS search both ways and compare the results.

In the comparison described earlier (3), DRS was able to identify all 37 pesticides found by the CDFA chemist. However, DRS completed the task for all 17 samples in about 20 minutes compared to ~8 hours for the manual procedure (Table 2). Moreover, DRS identified one false positive in the CDFA report and found 34 additional pesticides and related compounds.

Using the new 926-compound Database, it took 32 minutes to analyze all of the samples and DRS was able to find an additional 99 pesticides, metabolites, fire retardants, and related compounds (Table 2).

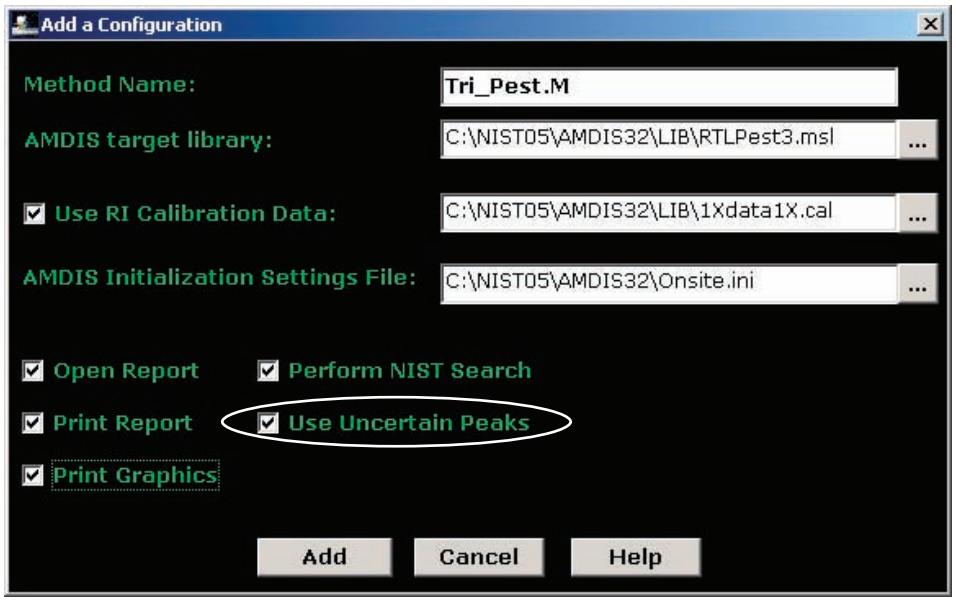

Figure 4. DRS configuration screen for the method called Tri_Pest. When the box labeled "Use Uncertain Peaks" is checked, AMDIS will use uncertain peaks for library searches. When unchecked, AMDIS ignores uncertain mass spectral peaks. Sometimes, this can affect the quality of a library match.

Table 2. Comparison of the Results Obtained by Screening 17 Surface Water Extracts Using Traditional Methods (CDFA) and Using DRS With Two Different Databases – the G1049A With 567 Compounds and the G1672AA With 926 Entries

	CDFA	Agilent DRS (Original G1049A database)	Agilent DRS (G1672 AA database)
Targets found (not counting ISTD)	37	Same 37 +34 more	Same 37 +99 more
False positives	1	0	0
Processing time	~8 hrs (ChemStation only)	20 minutes	32 min

Handling Stereoisomers

Many pesticides have multiple stereoisomers with virtually identical mass spectra. For example, cyfluthrin has four diastereomers arising from its three chiral centers. It is very difficult and sometimes impossible to determine the elution order of these isomers and most analysts report them as a sum of the isomer amounts. Agilent's G1049A RTL Pesticide database arbitrarily assigned each isomer a Roman numeral with I for the earliest eluting isomer, II for the next, and so on. The same Chemical Abstracts Service number (CAS #) was assigned to all of the isomers. Generally, it was a CAS # for the compound with "unstated stereochemistry." This caused some incompatibility with AMDIS as explained below.

AMDIS software differentiates among compounds using a "chemical identification number." The easiest and most consistent approach is to use each compound's CAS #. The default setting for AMDIS is to allow each CAS # to be used only once when analyzing a GC/MS data file. While this seems logical, it requires that each database entry have a different CAS #. It is possible to allow multiple hits per compound by checking the box in AMDIS found in the drop down menu under Analyze/Settings/Identif. However, this allows multiple peaks to be assigned the same compound name.

In the new RTL Pesticide Database (G1672AA), the Roman numeral designations remain and the first isomer in the series is given its genuine CAS #. Subsequent isomers in the series are given unique, but fictitious "CAS #s" generated by Agilent. The compound's real CAS # appears in braces after the compound name. For example, the cyfluthrin isomers are entered into the database as shown in Table 3.

Table 3. Method for Listing Compounds with Multiple Stereoisomers in the New G1672AA RTL Pesticide Database

RT	Compound name*		CAS #**
32.218	Cyfluthrin I		68359-37-5
32.359	Cyfluthrin II	{CAS # 68359-37-5}	999028-03-4
32.477	Cyfluthrin III	{CAS # 68359-37-5}	999029-03-7
32.536	Cyfluthrin IV	{CAS # 68359-37-5}	999030-03-4

* In a series, the earliest eluting isomer is identified with "I" and is assigned its legitimate CAS #. Subsequent isomers are assigned unique, but fictitious CAS #s (see footnote **). Their actual CAS # is put in braces behind the compound name.

** Cyfluthrin I has been given it's genuine CAS #. Cyfluthrin II-IV have been given unique numbers that can be distinguished from actual CAS numbers because they all have six digits before the first hyphen (9 total) and all begin with the series 999.

Figure 5 shows how permethrin was identified in a spinach sample using both databases with AMDIS configured to allow one hit per compound. Using the older 567-compound database (G1049A) only one permethrin isomer was identified because its CAS # could be used only once. With the new format used in the 926-compound RTL Pesticide Database (G1672AA), both isomers of permethrin were identified. Not surprisingly, the NIST library search found no hits with the same fictitious CAS # assigned to permethrin II. So, the software printed the best match on the following line. This compound, a cyclopropanecarboxylic acid derivative, is a permethrin isomer.

So long as the NIST library search is turned on in DRS, it will always print another line after reporting a compound with a fictitious CAS #. Note that these fictitious CAS #s always contain 9 digits and begin with 999.

A)

			Agilent			NIST	
RT	**Cas #**	**Compound name**	**ChemStation amount (ng)**	**AMDIS match**	**RT Diff (sec.)**	**reverse match**	**Hit number**
31.6158	52645531	Permethrin II		88	3.9	91	3

B)

			Agilent			NIST	
RT	**Cas #**	**Compound name**	**ChemStation amount (ng)**	**AMDIS match**	**RT Diff (sec.)**	**reverse match**	**Hit number**
31.4127	52645531	Permethrin I		78	2.6	81	3
31.6088	999046036	Permethrin II {CAS # 52645-53-1}		65	3.5		
31.6088	51877748	Cyclopropanecarboxylic acid, 3-(2,2-dichlorovinyl)-2,2-dimethyl-, (3-phenoxyphenyl)methyl ester, (1R-trans)-				95	1

Figure 5. A) A single isomer of permethrin was identified by DRS using the G1049A 567-compound database when AMDIS was not allowed to use multiple hits per compound.
B) Two permethrin isomers are identified by DRS with the G1672AA 926-compound database under the same circumstances.

Conclusions

The new G1672AA RTL Pesticide and Endocrine Disruptor library contains substantially more target analytes than its predecessor. With the addition of 359 new compounds, it is the most comprehensive library of its type available today. Many new pesticides, metabolites, and endocrine disruptors were added along with important PCBs, PBBs, PAHs, synthetic musk compounds, Sudan dyes, and organophosphorus fire retardants. The database contains all of the analytes specified for GC/MS analysis in the new Japanese "Positive List" regulations.

When combined with the complete DRS solution, one can screen GC/MS data files for all 926 compounds in about two minutes per sample. This is the fastest, most comprehensive, most accurate, and least tedious method for screening food and environmental samples for these compounds.

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Acknowledgments

The author wishes to thank Dr. G. Kempe of the Landesuntersuchungsanstalt Sachsen, Institut, Chemnitz, Germany for his help in acquiring much of the data for this library update. The author also thanks Dr. Mark Lee and Mr. Steve Siegel of the California Department of Food and Agriculture for providing the surface water extract data files.

Appendix A

Lists of Compounds in Databases

1,2,4-Trichlorobenzene
1,2-Dibromo-3-chloropropane
1,3,5-Tribromobenzene
1,3-Dichlorbenzene
17a-Ethynylestradiol
1-naphthalenol
2-(1-naphthyl)acetamide
2-(2-Butoxyethoxy)ethyl thiocyanate
2-(Octylthio)ethanol
2,3,4,5-Tertrachloronitrobenzene
2,3,4,5-Tetrachlorophenol
2,3,4,6-Tetrachlorophenol
2,3,5,6-Tetrachlorophenol
2,3,5,6-Tetrachloro-p-terphenyl
2,3,5-Trichlorophenol
2,3,5-Trimethacarb
2,3,6-Trichloroanisole
2,3,7,8-Tetrachlorodibenzofuran
2,3,7,8-Tetrachlorodibenzo-p-dioxin
2,4,5,6-Tetrachloro-m-xylene
2,4,5-T methyl ester
2,4,5-Trichloroaniline
2,4,5-Trichlorophenol
2,4,5-Trichloro-p-terphenyl
2,4,5-Trimethylaniline
2,4,6-Tribromoanisole
2,4,6-Tribromophenol
2,4,6-Trichloroanisole
2,4,6-Trichlorophenol
2,4-D methyl ester
2,4-D sec-butyl ester
2,4-DB methyl ester
2,4'-Dichlorobenzophenone (2,4'-Dicofol decomposition product)
2,4-Dichlorophenol
2,4-Dichlorophenyl benzenesulfonate
2,4-Dimethylaniline
2,4-Dimethylphenol
2,6-Dichlorobenzamide
2,6-Dichlorobenzonitrile
2,6-Dimethylaniline
2-[3-Chlorophenoxy]propionamide
2-Chlorophenol
2-Ethyl-1,3-hexanediol
2-ethyl-6-methylaniline
2-Hydroxyestradiol
2-Methyl-4,6-dinitrophenol
2-Methylphenol
2-Nitrophenol
2-Phenoxypropionic acid
3,4,5-Trimethacarb
3,4-Dichloroaniline
3,5-Dichloroaniline
3-Aminophenol
3-Chloro-4-fluoroaniline
3-Chloro-4-methoxyaniline
3-Chloroaniline
3-Hydroxycarbofuran
3-Indolylacetonitrile
3-Trifluormethylaniline
4,4'-Dichlorobenzophenone
4,4'-Oxydianiline
4,6-Dinitro-o-cresol (DNOC)
4-Aminodiphenyl
4-Bromoaniline
4-Chloro-2-methylaniline
4-Chloro-3-methylphenol
4-Chloroaniline
4-Chlorophenyl isocyanate
4-Isopropylaniline
4-Methylphenol
4-Nitrophenol
4-Nonylphenol
5,7-Dihydroxy-4'-methoxyisoflavone
9,10-Anthraquinone
Acenaphthene
Acenaphthylene
Acephate
Acequinocyl
acetamiprid
Acetochlor
Acifluorfen methyl ester
Aclonifen
Acrinathrin
Alachlor
Aldrin
Allidochlor
Ametryn
Amidithion
Aminocarb
Amitraz
Amitraz metabolite [Methanimidamide, N-(2,4-dimethylphenyl)-N'-methyl-]
Ancymidol
Anilazine
Aniline
Anilofos
Anthracene
Aramite I
Aramite II {CAS # 140-57-8}
Atraton
Atrazine
Atrazine-desethyl
Azaconazole
Azamethiphos
Azibenzolar-S-methyl
Azinphos-ethyl
Azinphos-methyl
Aziprotryn metabolite [2-Amino-4-isopropylamino-6-methylthio-1,3,5-triazine]
Aziprotryne
Azobenzene
Azoxybenzene
Azoxystrobin
Barban
Beflubutamid
Benalaxyl
Benazolin-ethyl
Bendiocarb
Benfluralin

Benfuracarb
Benfuresate
Benodanil
Benoxacor
Bentazone
Bentazone methyl derivative
Benthiocarb
Benzene, 1,3-*bis*(bromomethyl)-
Benzenesulfonamide
Benzidine
Benzo(a)anthracene
Benzo(a)pyrene
Benzo[b]fluoranthene
Benzo[g,h,i]perylene
Benzo[k]fluoranthene
Benzophenone
Benzoximate metabolite
Benzoylprop ethyl
Benzyl benzoate
b-Estradiol
BHC alpha isomer
BHC beta isomer
BHC delta isomer
BHC epsilon isomer
Bifenazate metabolite
(5-Phenyl-o-anisidine)
Bifenox
Bifenthrin
Binapacryl
Bioallethrin
Bioallethrin S-cyclopentenyl isomer
Bioresmethrin
Biphenyl
Bis(2,3,3,3-tetrachloropropyl) ether
Bis(2-butoxyethyl) phthalate
Bis(2-ethylhexyl)phthalate
Bisphenol A
Bitertanol I
Bitertanol II {CAS # 55179-31-2}
Boscalid (Nicobifen)
Bromacil
Bromfenvinphos-(E)
Bromfenvinphos-(Z)
Bromobutide
Bromocyclen
Bromophos
Bromophos-ethyl
Bromopropylate
Bromoxynil
Bromoxynil octanoic acid ester
Bromuconazole I
Bromuconazole II {CAS # 116255-48-2}
Bufencarb
Bupirimate
Buprofezin
Butachlor
Butafenacil
Butamifos
Butoxycarboxim
Butralin
Butyl benzyl phthalate
Butylate
Butylated hydroxyanisole
Cadusafos
Cafenstrole
Caffeine
Captafol
Captan
Carbaryl
Carbetamide
Carbofuran
Carbofuran-3-keto
Carbofuran-7-phenol
Carbophenothion
Carbosulfan
Carboxin
Carfentrazone-ethyl
Carpropamid
Carvone
Cashmeran
Cekafix
Celestolide
Chinomethionat
Chloramben methyl ester
Chloranocryl
Chlorbenside
Chlorbenside sulfone
Chlorbicyclen
Chlorbromuron
Chlorbufam
Chlordecone
Chlordene, *trans*-
Chlordimeform
Chlorethoxyfos
Chlorfenapyr
Chlorfenethol
Chlorfenprop-methyl
Chlorfenson
Chlorfenvinphos
Chlorfenvinphos, *cis*-
Chlorfenvinphos, *trans*-
Chlorflurecol-methyl ester
Chlormefos
Chlornitrofen
Chlorobenzilate
Chloroneb
Chloropropylate
Chlorothalonil
Chlorotoluron
Chlorpropham
Chlorpyrifos
Chlorpyrifos Methyl
Chlorthal-dimethyl
Chlorthiamid
Chlorthion
Chlorthiophos
Chlorthiophos sulfone
Chlorthiophos sulfoxide
Chlozolinate
Chrysene
Cinerin I
Cinerin II
Cinidon-ethyl
cis-Chlordane
Clodinafop-propargyl
Clomazone
Cloquintocet-mexyl
Coumaphos
Crimidine
Crotoxyphos
Crufomate
Cyanazine
Cyanofenphos
Cyanophos
Cyclafuramid
Cycloate
Cyclopentadecanone
Cycluron

Cyflufenamid
Cyfluthrin I
Cyfluthrin II {CAS # 68359-37-5}
Cyfluthrin III {CAS # 68359-37-5}
Cyfluthrin IV {CAS # 68359-37-5}
Cyhalofop-butyl
Cyhalothrin I (lambda)
Cyhalothrin (Gamma)
Cymiazole
Cymoxanil
Cypermethrin I
Cypermethrin II {CAS # 52315-07-8}
Cypermethrin III {CAS # 52315-07-8}
Cypermethrin IV {CAS # 52315-07-8}
Cyphenothrin *cis*-
Cyphenothrin *trans*- {CAS # 39515-40-7}
Cyprazine
Cyproconazole
Cyprodinil
Cyprofuram
Cyromazine
d-(*cis-trans*)-Phenothrin-I
d-(*cis-trans*)-Phenothrin-II {CAS # 260002-80-2}
Dazomet
DDMU [1-Chloro-2,2-*bis*(4'-chlorophenyl)]
Decachlorobiphenyl
Deltamethrin
Demephion
Demeton-S
Demeton-S-methylsulfon
Desbromo-bromobutide
Desmedipham
Desmetryn
Dialifos
Di-allate I
Di-allate II {CAS # 2303-16-4}
Diamyl phthalate
Diazinon
Diazinon-oxon
Dibenz[a,h]anthracene
Dicamba
Dicamba methyl ester
Dicapthon
Dichlofenthion
Dichlofluanid
Dichlofluanid metabolite (DMSA)
Dichlone
Dichlormid
Dichlorophen
Dichlorprop
Dichlorprop methyl ester
Dichlorvos
Diclobutrazol
Diclocymet I
Diclocymet II {CAS # 139920-32-4}
Diclofop methyl
Dicloran
Dicrotophos
Dicyclohexyl phthalate
Dicyclopentadiene
Dieldrin
Diethatyl ethyl
Diethofencarb
Diethyl dithiobis(thionoformate) (EXD)
Diethyl phthalate
Diethylene glycol
Diethylstilbestrol
Difenoconazol I
Difenoconazol II {CAS # 119446-68-3}
Difenoxuron
Diflufenican
Diisobutyl phthalate
Dimefox
Dimepiperate
Dimethachlor
Dimethametryn
Dimethenamid
Dimethipin
Dimethoate
Dimethomorph-(E)
Dimethomorph-(Z) {CAS # 110488-70-5}
Dimethylphthalate
Dimethylvinphos(z)
Dimetilan
Dimoxystrobin
Di-n-butylphthalate
Di-n-hexyl phthalate
Diniconazole
Dinitramine
Di-n-nonyl phthalate
Dinobuton
Dinocap I
Dinocap II {CAS # 39300-45-3}
Dinocap III {CAS # 39300-45-3}
Dinocap IV {CAS # 39300-45-3}
Di-n-octyl phthalate
Dinoseb
Dinoseb acetate
Dinoseb methyl ether
Dinoterb
Dinoterb acetate
Di-n-propyl phthalate
Diofenolan I
Diofenolan II {CAS # 63837-33-2}
Dioxabenzofos
Dioxacarb
Dioxathion
Diphacinone
Diphenamid
Diphenyl phthalate
Diphenylamine
Dipropetryn
Dipropyl isocinchomeronate
Disulfoton
Disulfoton sulfone
Ditalimfos
Dithiopyr
Diuron
Diuron Metabolite [3,4-Dichlorophenyl isocyanate]
Dodemorph I
Dodemorph II {CAS # 1593-77-7}
Drazoxolon
Edifenphos
Empenthrin I
Empenthrin II {CAS # 54406-48-3}
Empenthrin III {CAS # 54406-48-3}
Empenthrin IV {CAS # 54406-48-3}
Empenthrin V {CAS # 54406-48-3}
Endosulfan (alpha isomer)
Endosulfan (beta isomer)
Endosulfan ether
Endosulfan lactone
Endosulfan sulfate
Endrin
Endrin aldehyde
Endrin ketone

EPN
Epoxiconazole
EPTC
Erbon
Esfenvalerate
Esprocarb
Etaconazole
Ethalfluralin
Ethidimuron
Ethiofencarb
Ethiolate
Ethion
Ethofenprox
Ethofumesate
Ethofumesate, 2-Keto
Ethoprophos
Ethoxyfen-ethyl
Ethoxyquin
Ethylenethiourea
Etoxazole
Etridiazole
Etridiazole, deschloro- (5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole)
Etrimfos
Eugenol
Exaltolide [15-Pentadecanolide]
Famoxadon
Famphur
Fenamidone
Fenamiphos sulfoxide
Fenamiphos-sulfone
Fenarimol
Fenazaflor
Fenazaflor metabolite
Fenazaquin
Fenbuconazole
Fenchlorazole-ethyl
Fenchlorphos
Fenchlorphos-oxon
Fenclorim
Fenfuram
Fenhexamid
Fenitrothion
Fenitrothion-oxon
Fenobucarb
Fenoprop
Fenoprop methyl ester
Fenothiocarb
Fenoxanil
Fenoxaprop-ethyl
Fenoxycarb
Fenpiclonil
Fenpropathrin
Fenpropidin
Fenson
Fensulfothion
Fensulfothion-oxon
Fensulfothion-oxon -sulfone
fensulfothion-sulfone
Fenthion
Fenthion sulfoxide
Fenthion-sulfone
Fenuron
Fenvalerate I
Fenvalerate II {CAS # 51630-58-1}
Fepropimorph
Fipronil
Fipronil, desulfinyl-
Fipronil-sulfide
Fipronil-sulfone
Flamprop-isopropyl
Flamprop-methyl
Fluacrypyrim
Fluazifop-p-butyl
Fluazinam
Fluazolate
Flubenzimine
Fluchloralin
Flucythrinate I
Flucythrinate II {CAS # 70124-77-5}
Fludioxonil
Flufenacet
Flumetralin
Flumiclorac-pentyl
Flumioxazin
Fluometuron
Fluoranthene
Fluorene
Fluorodifen
Fluoroglycofen-ethyl
Fluoroimide
Fluotrimazole
Fluoxastrobin *cis*-
Fluquinconazole
Flurenol-butyl ester
Flurenol-methylester
Fluridone
Flurochloridone I
Flurochloridone II {CAS # 61213-25-0}
Flurochloridone, deschloro-
Fluroxypyr-1-methylheptyl ester
Flurprimidol
Flurtamone
Flusilazole
Fluthiacet-methyl
Flutolanil
Flutriafol
Fluvalinate-tau-I
Fluvalinate-tau-II {CAS # 102851-06-9}
Folpet
Fonofos
Formothion
Fosthiazate I
Fosthiazate II {CAS # 98886-44-3}
Fuberidazole
Furalaxyl
Furathiocarb
Furilazole
Furmecyclox
Halfenprox
Haloxyfop-methyl
Heptachlor
Heptachlor epoxide isomer A
Heptachlor exo-epoxide isomer B
Heptenophos
Hexabromobenzene
Hexachlorobenzene
Hexachlorophene
Hexaconazole
Hexazinone
Hexestrol
Hydroprene
Imazalil
Imazamethabenz-methyl I
Imazamethabenz-methyl II {CAS # 81405-85-8}
Imibenconazole
Imibenconazole-desbenzyl

Indeno[1,2,3-cd]pyrene

Indoxacarb and Dioxacarb decomposition product [Phenol, 2-(1,3-dioxolan-2-yl)-]

Ioxynil

Ioxynil octanoate

Ipconazole

Iprobenfos

Iprodione

Iprovalicarb I

Iprovalicarb II {CAS # 140923-25-7}

Irgarol

Isazophos

Isobenzan

Isobornyl thiocyanoacetate

Isocarbamide

Isocarbophos

Isodrin

Isofenphos

Isofenphos-oxon

Isomethiozin

Isoprocarb

Isopropalin

Isoprothiolane

Isoproturon

Isoxaben

Isoxadifen-ethyl

Isoxaflutole

Isoxathion

Jasmolin I

Jasmolin II

Jodfenphos

Kinoprene

Kresoxim-methyl

Lactofen

Lenacil

Leptophos

Leptophos oxon

Lindane

Linuron

Malathion

Malathion-o-analog

MCPA methyl ester

MCPA-butoxyethyl ester

MCPB methyl ester

m-Cresol

Mecarbam

Mecoprop methyl ester

Mefenacet

Mefenpyr-diethyl

Mefluidide

Menazon

Mepanipyrim

Mephosfolan

Mepronil

Metalaxyl

Metamitron

Metasystox thiol

Metazachlor

Metconazole I

Metconazole II {CAS # 125116-23-6}

Methabenzthiazuron [decomposition product]

Methacrifos

Methamidophos

Methfuroxam

Methidathion

Methiocarb

Methiocarb sulfone

Methiocarb sulfoxide

Methomyl

Methoprene I

Methoprene II {CAS # 40596-69-8}

Methoprotryne

Methoxychlor

Methoxychlor olefin

Methyl (2-naphthoxy)acetate

Methyl paraoxon

Methyl parathion

Methyl-1-naphthalene acetate

Methyldymron

Metobromuron

Metolachlor

Metolcarb

Metominostrobin (E)

Metominostrobin (Z) {CAS # 133408-50-1}

Metrafenone

Metribuzin

Mevinphos

Mirex

Molinate

Monalide

Monocrotophos

Monolinuron

Musk amberette

Musk Ketone

Musk Moskene

Musk Tibetene (Moschustibeten)

Musk xylene

Myclobutanil

N,N-Diethyl-m-toluamide

N-1-Naphthylacetamide

Naled

Naphthalene

Naphthalic anhydride

Naproanilide

Napropamide

Nicotine

Nitralin

Nitrapyrin

Nitrofen

Nitrothal-isopropyl

N-Methyl-N-1-naphthyl acetamide

Nonachlor, *cis*-

Nonachlor, *trans*-

Norflurazon

Norflurazon, desmethyl-

Nuarimol

o,p'-DDD

o,p'-DDE

o,p'-DDT

Octachlorostyrene

o-Dianisidine

o-Dichlorobenzene

Ofurace

Omethoate

o-Phenylphenol

Orbencarb

ortho-Aminoazotoluene

Oryzalin

Oxabetrinil

Oxadiazon

Oxadixyl

Oxamyl

Oxycarboxin

Oxychlordane

Oxydemeton-methyl

Oxyfluorfen

p,p'-DDD
p,p'-DDE
p,p'-DDM [*bis*(4-chlorophenyl)methane]
p,p'-DDT
p,p'-Dibromobenzophenone
p,p'-Dicofol
Paclobutrazol
Paraoxon
Parathion
PBB 52 Tetrabrombiphenyl
PBB 101
PBB 15
PBB 169 Hexabrombiphenyl
PCB 101
PCB 105
PCB 110
PCB 118
PCB 126
PCB 127
PCB 131
PCB 136
PCB 138
PCB 153
PCB 169
PCB 170
PCB 180
PCB 30
PCB 31
PCB 49
PCB 77
PCB 81
p-Dichlorobenzene
Pebulate
Penconazole
Pendimethalin
Pentachloroaniline
Pentachloroanisole
Pentachlorobenzene
Pentachloronitrobenzene
Pentachlorophenol
Pentanochlor
Permethrin I
Permethrin II {CAS # 52645-53-1}
Perthane
Phantolide
Phenamiphos
Phenanthrene
Phenanthrene-d10
Phenkapton
Phenol
Phenothiazine
Phenothrin I
Phenothrin II
Phenoxyacetic acid
Phenthoate
Phorate
Phorate sulfone
Phorate sulfoxide
Phorate-oxon
Phosalone
Phosfolan
Phosmet
Phosphamidon I
Phosphamidon II {CAS # 13171-21-6}
Phthalide
Phthalimide
Picloram methyl ester
Picolinafen
Picoxystrobin
Pindone
Piperalin
Piperonyl butoxide
Piperophos
Pirimicarb
Pirimiphos-ethyl
Pirimiphos-methyl
Plifenat
p-Nitrotoluene
Potasan
Prallethrin, *cis*-
Prallethrin, *trans*- {CAS # 23031-36-9}
Pretilachlor
Probenazole
Prochloraz
Procymidone
Prodiamine
Profenofos
Profenofos metabolite (4-Bromo-2-chlorophenol)
Profluralin
Prohydrojasmon I
Prohydrojasmon II {CAS # 158474-72-7}
Promecarb
Promecarb artifact [5-isopropyl-3-methylphenol]
Prometon
Prometryn
Propachlor
Propamocarb
Propanil
Propaphos
Propargite
Propargite metabolite [Cyclohexanol, 2-(4-tert-butylphenoxy)]
Propazine
Propetamphos
Propham
Propiconazole-I
Propiconazole-II {CAS # 60207-90-1}
Propisochlor
Propoxur
Propyzamide
Prosulfocarb
Prothioconazole-desthio
Prothiofos
Prothoate
Pyracarbolid
Pyraclofos
Pyraflufen-ethyl
Pyrazon
Pyrazophos
Pyrazoxyfen
Pyrene
Pyrethrin I
Pyrethrin II
Pyributicarb
Pyridaben
Pyridaphenthion
Pyridate
Pyridinitril
Pyrifenox I
Pyrifenox II {CAS # 88283-41-4}
Pyriftalid
Pyrimethanil
Pyrimidifen
Pyriminobac-methyl (E)
Pyriminobac-methyl (Z) {CAS # 136191-64-5}

Pyriproxyfen
Pyroquilon
Quinalphos
Quinoclamine
Quinoxyfen
Quintozene metabolite (pentachlorophenyl methyl sulfide)
Quizalofop-ethyl
Rabenzazole
Resmethrin
Resmethrine I
Resmethrine II {CAS # 10453-86-8}
Rotenone
S,S,S-Tributylphosphorotrithioate
Schradan
Sebuthylazine
Sebuthylazine-desethyl
Secbumeton
Silafluofen
Silthiopham
Simazine
Simeconazole
Simetryn
Spirodiclofen
Spiromesifen
Spiroxamine I
Spiroxamine II {CAS # 118134-30-8}
Spiroxamine metabolite (4-tert-butylcyclo-hexanone)
Sudan I
Sudan II
Sudan Red
Sulfallate
Sulfanilamide
Sulfentrazone
Sulfotep
Sulfur (S8)
Sulprofos
Swep
Tamoxifen
TCMTB
Tebuconazole
Tebufenpyrad
Tebupirimifos
Tebutam
Tebuthiuron
Tecnazene
Tefluthrin, *cis*-
Temephos
Terbacil
Terbucarb
Terbufos
Terbufos-oxon-sulfone
Terbufos-sulfone
Terbumeton
Terbuthylazine
Terbuthylazine-desethyl
Terbutryne
Tetrachlorvinphos
Tetraconazole
Tetradifon
Tetraethylpyrophosphate (TEPP)
Tetrahydrophthalimide, *cis*-1,2,3,6-
Tetramethrin I
Tetramethrin II {CAS # 7696-12-0}
Tetrapropyl thiodiphosphate
Tetrasul
Thenylchlor
Theobromine
Thiabendazole
Thiazopyr
Thifluzamide
Thiofanox
Thiometon
Thionazin
Thymol
Tiocarbazil I
Tiocarbazil II {CAS # 36756-79-3}
Tolclofos-methyl
Tolfenpyrad
Tolylfluanid
Tolylfluanid metabolite (DMST)
Tolyltriazole [1H-Benzotriazole, 4-methyl-]
Tolyltriazole [1H-Benzotriazole, 5-methyl-]
Tonalide
Toxaphene Parlar 26
Toxaphene Parlar 50
Toxaphene Parlar 62
trans-Chlordane
Transfluthrin
Traseolide
Triadimefon
Triadimenol
Tri-allate
Triamiphos
Triapenthenol
Triazamate
Triazophos
Tributyl phosphate
Tributyl phosphorotrithioite
Trichlamide
Trichlorfon
Trichloronate
Triclopyr methyl ester
Triclosan
Triclosan-methyl
Tricresylphosphate, *meta*-
Tricresylphosphate, *ortho*-
Tricresylphosphate, *para*
Tricyclazole
Tridemorph, 4-tridecyl-
Tridiphane
Trietazine
Triethylphosphate
Trifenmorph
Trifloxystrobin
Triflumizole
Trifluralin
Triphenyl phosphate
Tris(2-butoxyethyl) phosphate
Tris(2-chloroethyl) phosphate
Tris(2-ethylhexyl) posphate
Triticonazole
Tryclopyrbutoxyethyl
Tycor (SMY 1500)
Uniconizole-P
Vamidothion
Vernolate
Vinclozolin
XMC (3,4-Dimethylphenyl N-methylcarbama
XMC (3,5-Dimethylphenyl N-methylcarbama
Zoxamide
Zoxamide decomposition product

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Printed in the USA
April 18, 2006
5989-5076EN

Agilent Technologies

Identifying Pesticides with Full Scan, SIM, µECD, and FPD from a Single Injection

Application

Food Safety, Environmental

Authors

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Abstract

In this application note, a gas chromatography/mass spectrometry (GC/MS) system capable of providing up to four signals from a single injection is described. When a three-way micro-fluidic splitter is added to the end of the column, two additional signals from GC detectors can be acquired together with the MS data from a single injection. This multi-signal configuration provides: full-scan data for library searching, selective ion monitoring (SIM) data for trace analysis, micro-electron capture detector and flame photometric detector data for excellent selectivity and sensitivity from complex matrices. A combination of element selective detectors, SIM/Scan, and deconvolution reporting software makes a very powerful pesticide analysis system. Examples for trace-level compound quantitation/confirmation or for screening are discussed.

Introduction

Many laboratories in the world are analyzing pesticide residue levels in both foods and the environment to protect human health. The process usually involves homogenizing the sample, extracting the pesticides, and analyzing the target compounds with a Gas Chromatograph (GC) or a Liquid Chromatograph (LC) depending on the nature of the compounds. For GC amenable compounds, the traditional detectors are NPD (Nitrogen Phosphorus Detector), µECD (micro-Electron Capture Detector), and FPD (Flame Photometric Detector) for their excellent sensitivity and selectivity. However, even with dual-column confirmation analysis, these GC detectors cannot be used to verify the identity of the compounds with high confidence.

Full scan mass spectral data and library searching are typically used for final compound verification. However, full-scan analysis has a worse (higher) detection limit (DL) compared to selective detectors on a GC. To improve the DL, the technique selective ion monitoring (SIM) is often used. With SIM, the MS monitors only a few characteristic ions for each target compound within the retention time (RT) range that the target elutes from the column. By monitoring only a few specific ions, the signal-to-noise ratio (S/N) improves significantly. The ions monitored are time programmed in groups corresponding to the RTs of the targets. SIM analyses with closely eluting targets require precise alignment of chromatographic RTs with the time programming of SIM groups. The Retention Time Locking (RTL) technique can be applied to eliminate the need to adjust SIM group time-windows after column maintenance or replacement.

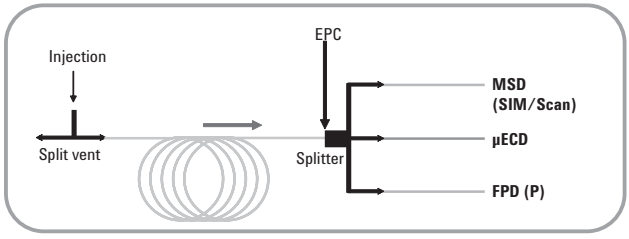
In this application note, a GC/MS system capable of providing up to four signals from a single injection is described. The benefits of the multi-signal detection include:

- Confirmatory information – Full-scan data for library search capability

- Maximum sensitivity – SIM data enables trace analysis
- Excellent selectivity – µECD and FPD detect trace-level hetero-compounds from complex matrices

Experimental

A recent technical note describes "Synchronous SIM/Scan", which takes advantages of the Performance Electronics in the 5975 inert MSD to get both SIM and full-scan signals in a single run without sacrificing performance [1]. The SIM method can be easily developed automatically using the ChemStation's AutoSIM tool [2]. By simply selecting a checkbox in the method, the SIM and full-scan data can be acquired together. The trade-off is giving up some cycles per second but gaining an additional signal (full-scan data or SIM data) for the whole analysis. With properly chosen acquisition parameters, for example, increasing the scan speed, the decrease of cycles per second is usually not significant and does not affect peak quantitation or the quality of results (for example, S/N).



At the end of the column, effluent flow is split three ways according to the length and diameter of the capillary tubing (restrictor) used.

Figure 1. A schematic of the multi-signal configuration. Note: the EPC flow adds to the column flow into the splitter.

Besides the SIM/Scan data, the ChemStation software can simultaneously acquire up to two additional GC detector signals, for example, FPD (in phosphorus- or sulfur- mode) and NPD (nitrogen-phosphorus detector) signals or both P- and S- signals from a dual-wavelength FPD (DFPD). See Figure 1.

Figure 1 is a schematic for multi-signal detection. At the end of the column, a three-way micro-fluidic splitter was used to split the column effluent to different detectors [3]. For this study, an FPD and a µECD were installed. Notice on the figure that an Auxiliary Electronic Pneumatics Control (Aux EPC) gas channel was connected to the splitter to maintain the pressure at the end of the column so that the split ratios/flows are kept constant throughout a run. Figure 2 shows a close-up view of the micro-fluidic splitter installed in the GC oven.

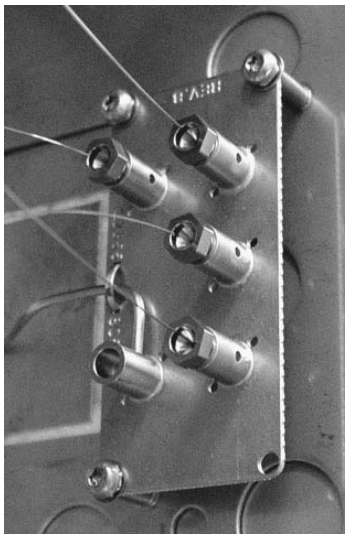


Figure 2. A close-up view of the micro-fluidic three-way splitter in the 6890 GC oven.

The size of the micro-fluidic plate is 1.25 inches (3.2 cm) wide and 2.5 inches (6.4 cm) tall. The device was designed to eliminate the common problems of large thermal mass, excess dead volume, and leaky connection due to oven temperature cycling etc. The splitter's flow paths and connection points are laid out and etched onto a thin, stainless steel plate using photolithography and chem-milling technologies. The plate is diffusion bonded, mounted with column connectors, and surface deactivated, resulting in an integrated and compact micro-fluidic splitter. Metal ferrules are used at the connectors that are leak-free after temperature cycling and will not absorb solvents or sample matrix, improving sensitivity for trace analysis applications.

Deactivated capillary tubing between the splitter and each detector was used as a flow restrictor. Aux EPC pressure and the restrictor dimensions were determined using a spreadsheet-like calculator program to achieve the proper split ratio among all detectors. The three-way splitter can easily turn into a two-way splitter when a connector is capped.

Other advantages of a splitter include back-flushing [3] and quick-swapping. The Aux EPC flow can be run-time programmed to a higher pressure, while at the same time the inlet pressure is lowered to near ambient. This causes the column flow to reverse direction, back-flushing the less volatile materials out of the split vent of the inlet. The Aux EPC on the splitter also allows column changing and inlet maintenance without cooling and venting the MSD. The splitter's flow paths and connection points were designed in such a way

that when the column fitting is removed, the helium gas from the Aux EPC purges the fitting, preventing air from entering the splitter/MSD. See Table 1 for hardware details and settings.

Table 1. Gas Chromatograph, Mass Spectrometer, and Three-Way Splitter Operating Parameters

GC	Agilent Technologies 6890		
Inlet	EPC Split/Splitless		
Mode	Splitless, 1.0 µL injected (7683 ALS)		
Inlet temp	280 °C		
Pressure	~27 psi (chlorpyrifos methyl RT locked to 16.596 min)		
Purge flow	50.0 mL/min		
Purge time	0.75 min		
Total flow	55.3 mL/min		
Gas saver	Off		
Gas type	Helium		
Inlet liner	Siltek Cyclosplitter, 4-mm id, Restek p/n 20706-214.1		
Oven			
Oven ramp	°C/min	Final (°C)	Hold (min)
Initial		70	2.00
Ramp 1	25	150	0.00
Ramp 2	3	200	0.00
Ramp 3	8	280	15
Total run time		46.87 min (last standard elutes around 35 min)	
Equilibration time		0.5 min	
Oven max temp		325 °C	
Column		Agilent Technologies HP 5-ms, p/n 19091S-433	
Length		30.0 m	
Diameter		0.25 mm	
Film thickness		0.25 µm	
Mode		Constant pressure	
Nominal initial flow		2.5 mL/min	
Outlet		Unspecified	
Outlet pressure		3.8 psi (Aux EPC pressure to splitter)	
Front detector (FPD)			
Phosphorus mode		**Sulfur mode**	
Hydrogen flow:	75.0 mL/min	Hydrogen flow:	50.0 mL/min
Oxidizer flow:	100.0 mL/min	Oxidizer flow:	60.0 mL/min
Temperature:	250 °C		
Oxidizer gas type:	Air		
Mode:	Constant makeup flow		
Makeup flow:	60.0 mL/min		
Makeup gas type:	Nitrogen		
Lit offset:	2.00		
Data rate:	5 Hz		

Table 1. Gas Chromatograph, Mass Spectrometer, and Three-Way Splitter Operating Parameters (Continued)

Back detector (μECD)	
Temperature:	300 °C
Mode:	Constant makeup flow
Makeup flow:	60.0 mL/min
Makeup gas type:	Nitrogen
Date rate:	5 Hz
Thermal AUX 2	
Use:	MSD Transfer line heater
Initial temp:	280 °C
Pressure AUX 5	
Gas type:	Helium
Initial pressure:	3.80 psi
Initial time:	0.00 min (this value will follow oven ramp)
MSD	Agilent Technologies 5975 inert MSD
Tune file	Atune.U
Mode	Scan
Solvent delay	3.00 min
EM voltage	Atune voltage
Low mass	45 amu
High mass	555 amu
Threshold	100
Sampling	2
A/D Samples	4
Scans/s	2.89
Quad temp	150 °C
Source temp	230 °C
Three-way splitter	Agilent 6890N Option 890, when installed on the GC during factory assembly
Split ratio	10:10:1 MSD:FPD:μECD
MSD restrictor	1.444 m × 0.18-mm id Deactivated fused silica tubing
FPD restrictor	0.532 m × 0.18-mm id Deactivated fused silica tubing
μECD restrictor	0.507 m × 0.10-mm id Deactivated fused silica tubing
Flow to MSD (at 280 °C)	1.53 mL/min
Flow to FPD (at 280 °C)	1.53 mL/min
Flow to μECD (at 280 °C)	0.153 mL/min
Makeup flow (at 280 °C)	1.38 mL/min

Software Used in this Application Note

GC/MSD ChemStation	G1701DA
Deconvolution Reporting Software (DRS)	G1716AA
NIST Library	G1033A
AMDIS (included for free with the NIST library CD)	

Results and Discussion

Figure 3 shows four signals that were simultaneously acquired from a single injection of a pesticide mixture. Due to the high sensitivity of the µECD, the split ratios for the three detectors was set to MSD:FPD:µECD = 10:10:1. This split ratio distributes the sample of a 1-µL splitless injection of a 1-ppm (1000 pg/µL) sample to the different detectors as labeled in Figure 3.

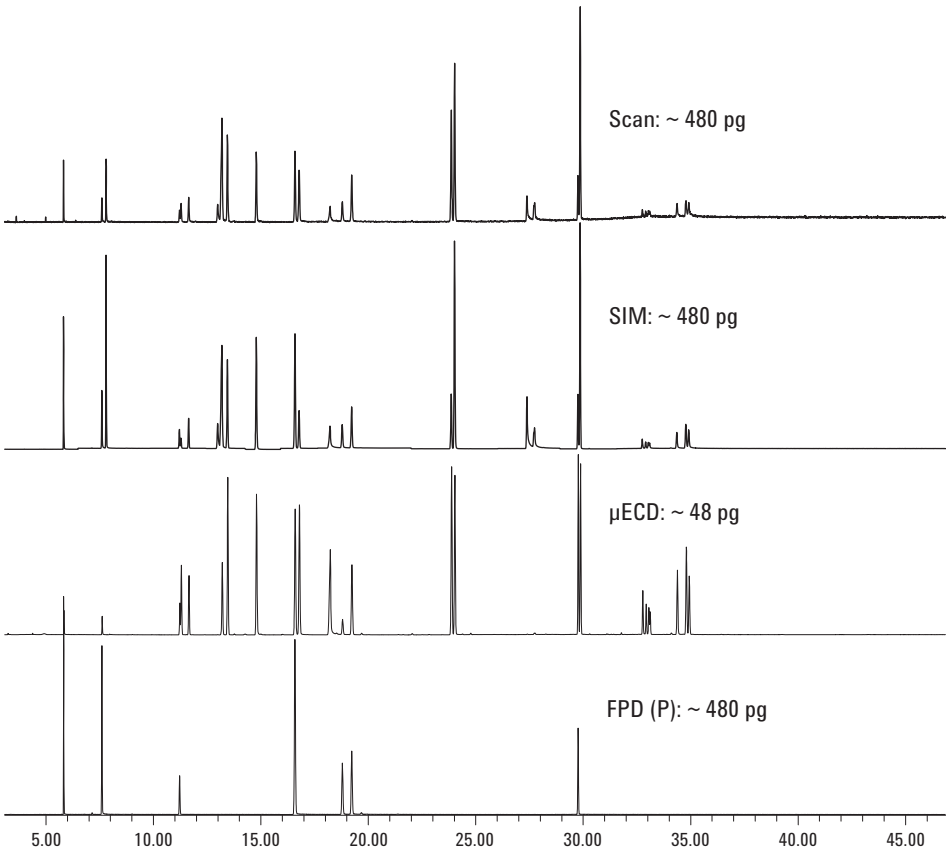


Figure 3. Signals acquired simultaneously from a 1-µL splitless injection of 1-ppm standard. The split ratios were MSD:FPD:µECD = 10:10:1.

Figure 4 shows the signals when the pesticide standard was diluted 100-fold in a produce matrix. The total ion chromatogram (TIC) from full scan was not shown due to the lack of sensitivity. The FPD(P) and µECD were able to detect all the pesticides spiked in this extract. For trace-level target compound analysis, the SIM signal can be used for quantitation and the GC signals used for further confirmation.

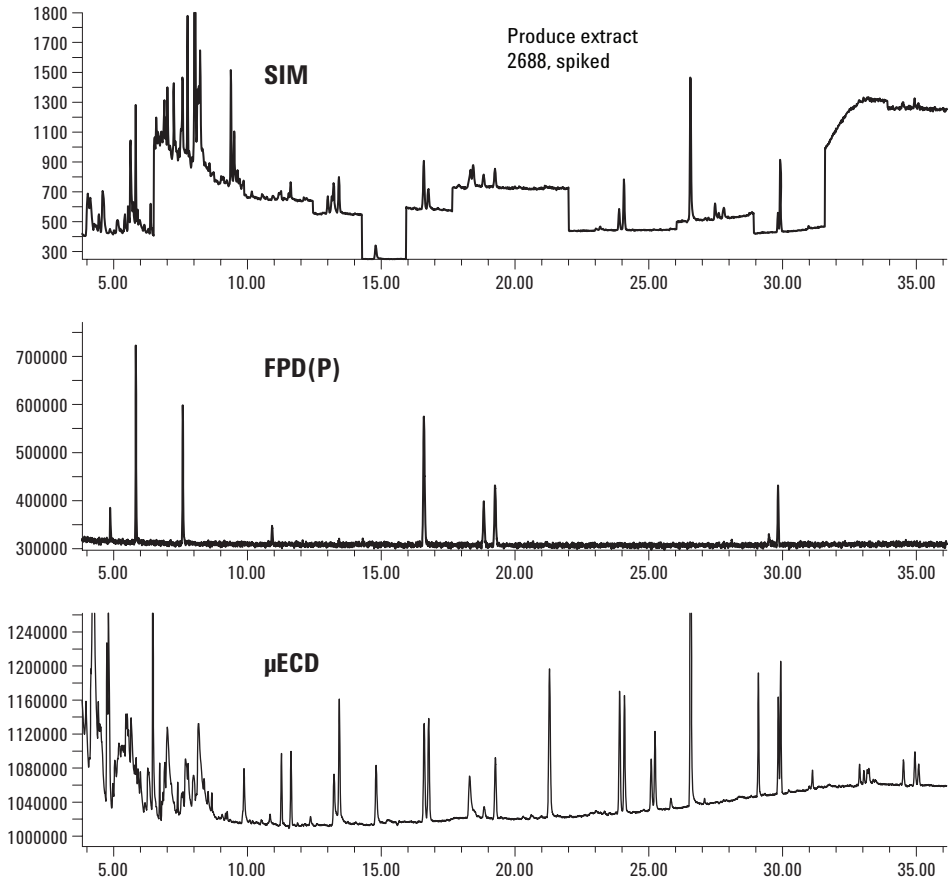


Figure 4. Data of a produce extract spiked at 10 ppb. FPD and µECD were able to detect the respective standards spiked into the extract.

Another application for this multi-signal system is for screening. In screening, no target list is available for the analysis; therefore, SIM acquisition or MS/MS is not possible. Figure 5 shows three signals (no SIM) from a produce extract.

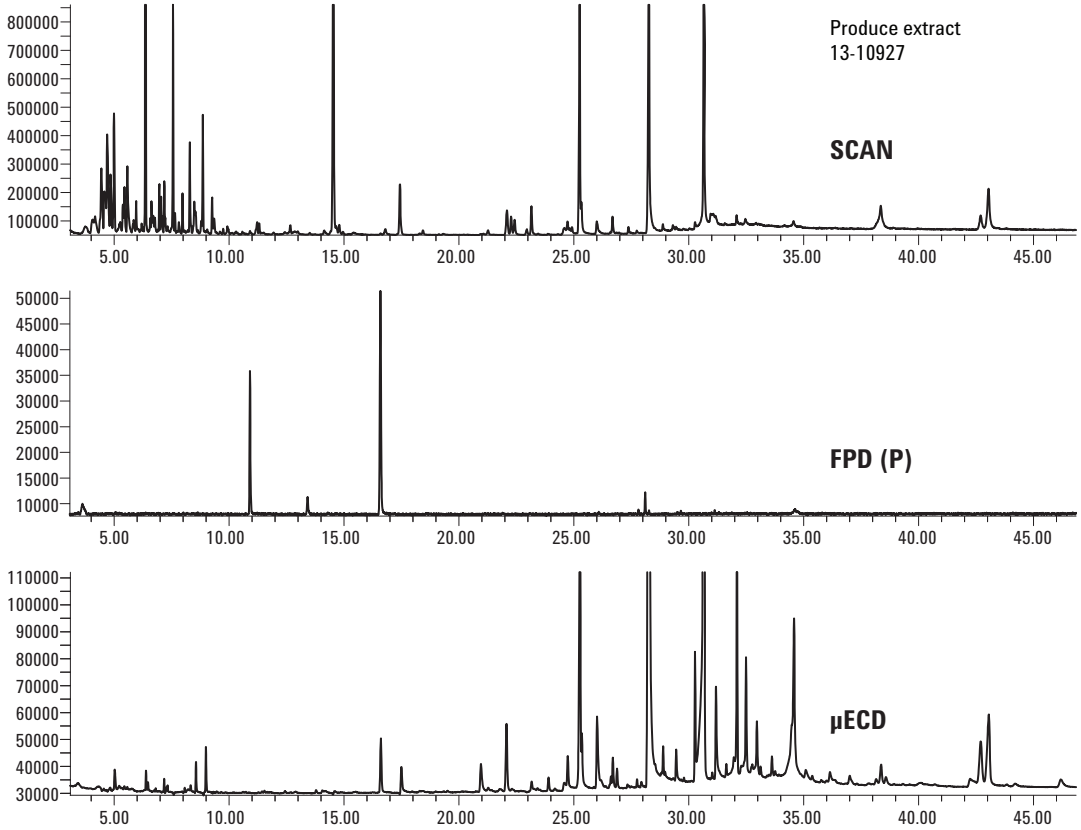
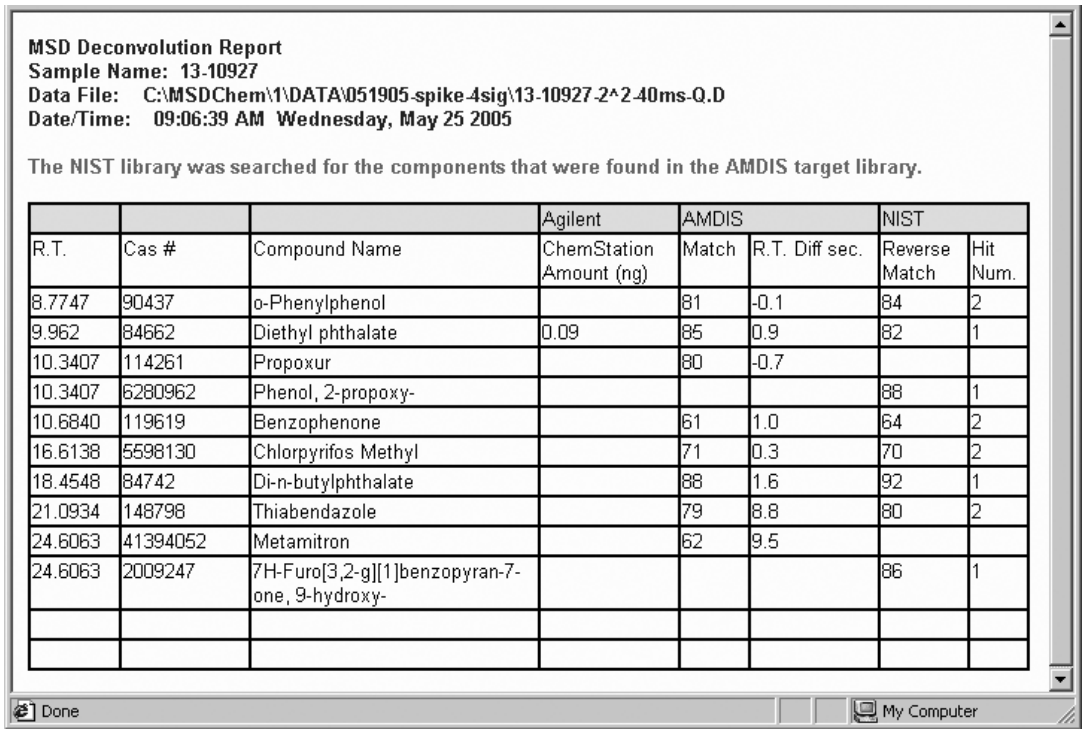


Figure 5. Full-scan, FPD(P), and µECD data for extract 13-10927.

The Deconvolution Reporting Software (DRS) [3, 4] found several pesticides in the TIC as shown in Figure 6.



MSD Deconvolution Report
Sample Name: 13-10927
Data File: C:\MSDChem\1\DATA\051905-spike-4sig\13-10927-2^2-40ms-Q.D
Date/Time: 09:06:39 AM Wednesday, May 25 2005

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
8.7747	90437	o-Phenylphenol		81	-0.1	84	2
9.962	84662	Diethyl phthalate	0.09	85	0.9	82	1
10.3407	114261	Propoxur		80	-0.7		
10.3407	6280962	Phenol, 2-propoxy-				88	1
10.6840	119619	Benzophenone		61	1.0	64	2
16.6138	5598130	Chlorpyrifos Methyl		71	0.3	70	2
18.4548	84742	Di-n-butylphthalate		88	1.6	92	1
21.0934	148798	Thiabendazole		79	8.8	80	2
24.6063	41394052	Metamitron		62	9.5		
24.6063	2009247	7H-Furo[3,2-g][1]benzopyran-7-one, 9-hydroxy-				86	1

Done My Computer

Figure 6. Report for extract 13-10927 generated from DRS.

The possible pesticides in the sample were benzophenone, chlorpyrifos methyl, and thiabendazole. Propoxur and metamitron were not confirmed by both AMDIS and NIST; therefore, they were most likely false positives.

Due to the complexity of the sample matrix and other interferences, it is sometimes difficult to get a high library match factor from peaks in the TIC, even after background subtraction. Therefore, element selective detectors would be very useful in providing the supporting information for compound confirmation. The multi-signal system was retention time locked, therefore, from the RT and the aligned peaks from the FPD(P) and the µECD responses, chlorpyrifos methyl ($C_7H_7Cl_3NO_3PS$) was confirmed.

It usually takes less than 3 minutes to turn off the FPD photomultiplier, swap the P-filter with the S-filter, and turn the photomultiplier back on. After the swap, adjust the detector gas flows to optimize the response in either P- or S- mode. A new injection of the same extract was made in FPD(S) mode. The FPD(S) result is shown with previously acquired signals in Figure 7. Two major peaks were seen on the FPD(S) chromatogram. From the peak RTs, they supported the presence of chlorpyrifos methyl and thiabendazole ($C_{10}H_7N_3S$) respectively. Note that the full-scan TIC barely showed a peak for either compound, which made it impossible for traditional data analysis to identify both compounds. The FPD(S) mode is very selective, but it is not as sensitive as the FPD(P) mode. Although the µECD is very sensitive, it is not as selective as the FPD. A combination of GC detectors, SIM/Scan, and DRS makes a very powerful pesticide analysis system.

Conclusion

The Synchronous SIM/Scan provides users with library searchable full-scan spectra as well as trace level SIM data in a single analysis. When a three-way micro-fluidic splitter is added to the end of the column, two additional signals from element selective detectors can be acquired together with the MS data from a single injection. This configuration makes it very attractive for the analysis of trace-level pesticide residues in foods or environmental samples.

This multi-signal configuration provides: full-scan data for library searching, SIM data for trace analysis, µECD and FPD data for excellent selectivity and sensitivity from complex matrices. In this application note, examples of µECD signal and FPD signal (P- or S- mode) were acquired together with the SIM/Scan data from a single injection for trace-level compound quantitation/confirmation, or for screening.

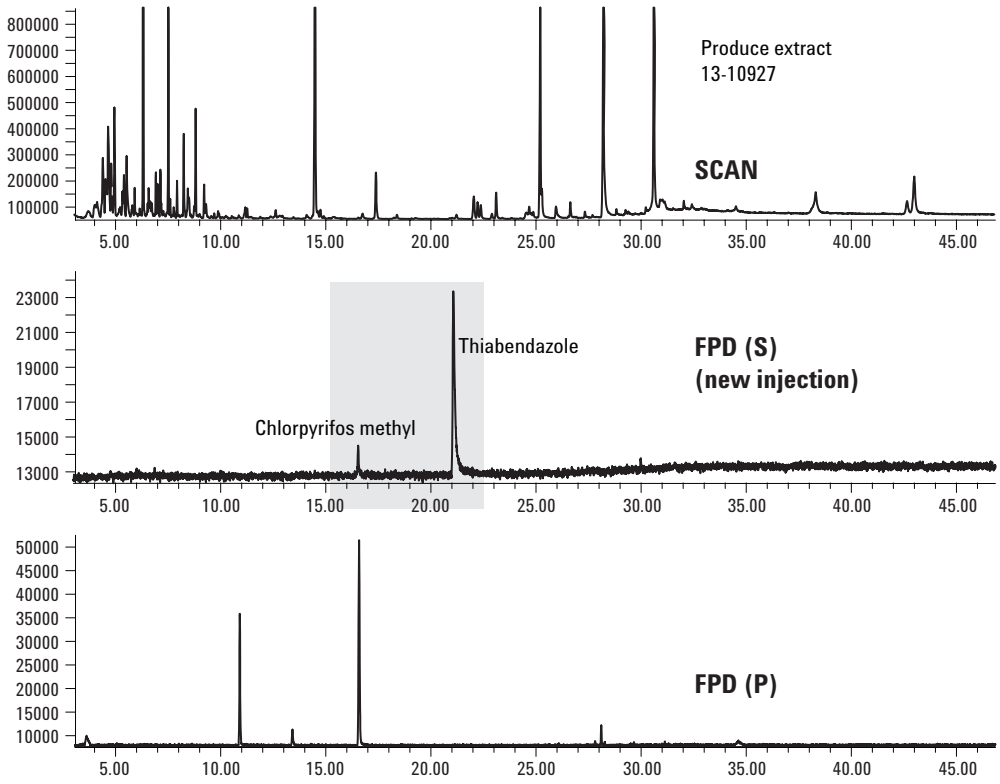


Figure 7. Full-scan, FPD(S), and FPD(P) data for extract 13-10927.

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Printed in the USA
July 6, 2005
5989-3299EN

Agilent Technologies

Comprehensive Pesticide Screening by GC/MSD using Deconvolution Reporting Software

Application

Food Safety

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Introduction

According to The Pesticide Manual, more than 700 pesticides are currently approved for use around the world [1]. About 600 more were used in the past, but are either banned or no longer marketed. In spite of their discontinuance, some of these still persist in the environment where they may bioaccumulate in the flora and fauna. Many pesticides or their degradation products can be found at trace levels in food and beverages; in soil, water, and air; in aquatic and terrestrial flora and fauna; and in human blood, adipose tissue, and breast milk. The World Health Organization has classified pesticides into five groups based upon their acute toxicity to humans [2]. The categories range from "Acutely Hazardous" to those that are "Unlikely to Present Acute Hazard in Normal Use." Certain pesticides are classified as persistent organic pollutants (POPs), carcinogens, teratogens, or endocrine disrupters. It is now common to analyze for pesticides in food and environmental samples to track their distribution in the environment and to ensure a safe food supply.

Current analytical methods target only a subset of the possible compounds. Whether for food or environmental samples, analyses are often complicated by the presence of co-extracted natural products. Food or tissue extracts can be exceedingly complex matrices that require several stages of sample cleanup prior to analysis [3]. Even then, it can be difficult to detect trace levels of contaminants in the presence of the remaining matrix.

For efficiency, multiresidue methods (MRMs) must be used to analyze for most pesticides. Traditionally, these methods have relied upon gas chromatography (GC) with a constellation of element-selective detectors to locate pesticides in the midst of a variable matrix [4, 5, 6]. GC with mass spectral detection (GC/MS) has been widely used for confirmation of hits. Liquid chromatography (LC) has been used for those compounds that are not amenable to GC [2]. Today, more and more pesticide laboratories are relying upon LC with mass spectral detection (LC/MS) and GC/MS as their primary analytical tools [7, 8]. Still, most MRMs are target compound methods that look for a small subset of the possible pesticides. Any compound not on the target list is likely to be missed by these MRMs.

Using the techniques of retention time locking (RTL) [9, 10, 11] and spectral deconvolution [12], a method has been developed to screen for 567 pesticides and suspected endocrine disrupters in a single GC/MS analysis. Spectral deconvolution

helps to identify pesticides even when they are buried under co-eluting matrix compounds. RTL helps to eliminate false positives and gives greater confidence in the results. Users can easily add compounds to the method if they wish.

Experimental

Table 1 lists the instrumentation, software, and analytical parameters used by Agilent for pesticide analysis. Depending upon the desired injection volume, a PTV inlet or split/splitless inlet can be used.

Samples

Vegetable extracts were obtained from Dr. Mark Lee and Stephen Siegel at The California Department of Food and Agriculture (CDFA; Sacramento, CA USA) and from Dr. J.G.J. Mol at TNO Nutrition and Food Research (Zeist, The Netherlands). Seventeen data files from the GC/MS analysis of surface water samples were also contributed by CDFA and were processed in this laboratory using the Deconvolution Reporting Software (DRS). GC/MS data files (locked to the Agilent Pesticide Method) for 17 crop extracts were supplied by NRM Laboratories, Berkshire, UK.

Table 1. Instrumentation and Conditions of Analysis

Gas chromatograph	**Agilent 6890N**
Automatic sampler	Agilent 7683
Inlet	Agilent PTV operated in the solvent vent mode
Column	Agilent 30 m × 0.25 mm × 0.25 µm HP-5MS (p/n 19091S-433)
Carrier gas	Helium in the constant pressure mode
RTL	Chlorpyrifos-methyl locked to 16.596 min (nominal column head pressure = 17.1 psi)
Oven temperature program	70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C /min to 200 °C (0 min), 8 °C /min to 280 °C (10–15 min)
PTV inlet parameters	Temp program: 40 °C (0.25 min), 1600 °C/min to 250 °C (2 min); Vent time: 0.2 min; Vent flow: 200 mL/min; Vent pressure: 0.0 psi; Purge flow: 60.0 mL/min; Purge time: 2.00 min
Injection volume	15 µL (using a 50-µL syringe)
Mass Selective Detector (MSD)	**Agilent 5973 inert**
Scan range	50–550 amu
Source, quad, transfer line temperatures	230, 150, and 280 °C, respectively
Solvent delay	4.00 min
Multiplier voltage	Autotune voltage
Software	
GC/MSD ChemStation	Agilent p/n G1701DA (Version D01.00 sp1)
Deconvolution Reporting Software (DRS)	Agilent p/n G1716AA
Library searching software	NIST MS Search (version 2.0) (included with NIST '02 mass spectral library, Agilent p/n G1033A)
Deconvolution software	Automated Mass Spectral Deconvolution and Identification Software (AMDIS) (included with NIST '02 mass spectral library, Agilent p/n G1033A)
MS Libraries	NIST '02 mass spectral library (Agilent p/n G1033A); Agilent RTL Pesticide Library (p/n G1049A)

Results and Discussion

RTL and RTL Databases

RTL is a technique developed by Agilent that allows users to match analyte retention times (RTs) on any Agilent 6890 GC, in any laboratory in the world, so long as the same nominal GC method and capillary column are used [13]. Using RTL, Agilent has developed several retention-time-locked databases for GC and GC/MS that include the locked retention time, compound name, CAS number, molecular formula, molecular weight, and mass spectrum (GC/MS databases only) for each entry [14]. The Agilent RTL Pesticide Library contains this information for almost all GC-amenable pesticides, as well as several endocrine disrupters - 567 compounds in all. For use with the DRS discussed below, this library was converted into the NIST format [15]. Separate Automated Mass Spectral Deconvolution and Identification Software (AMDIS) libraries for the RTs and compound information were created from the original RTL Pesticide Library. Users can easily augment these libraries with newer pesticides or other compounds of interest [15].

Basics of Deconvolution

In GC/MS, deconvolution is a mathematical technique that "separates" overlapping mass spectra into "cleaned" spectra of the individual components. Figure 1 is a simplified illustration of this process. Here, the total ion chromatogram (TIC) and apex spectrum are shown. As is often the case, the peak is composed of multiple overlapping components and the apex spectrum is actually a composite of these constituents. A mass spectral library search would give a poor match, at best, and certainly would not identify all of the individual components that make up the composite "spectrum."

The deconvolution process finds ions whose individual abundances rise and fall together within the spectrum. In this case, it first corrects for the spectral skew that is inherent in quadrupole mass spectra and determines a more accurate apex RT of each chromatographic peak. As illustrated in Figure 1, deconvolution produces "clean" spectra for each overlapping component. These individual spectra can be library searched with a high expectation for a good match.

The AMDIS that is incorporated into the Agilent DRS is supplied by the National Institute of Science and Technology (NIST) [12].

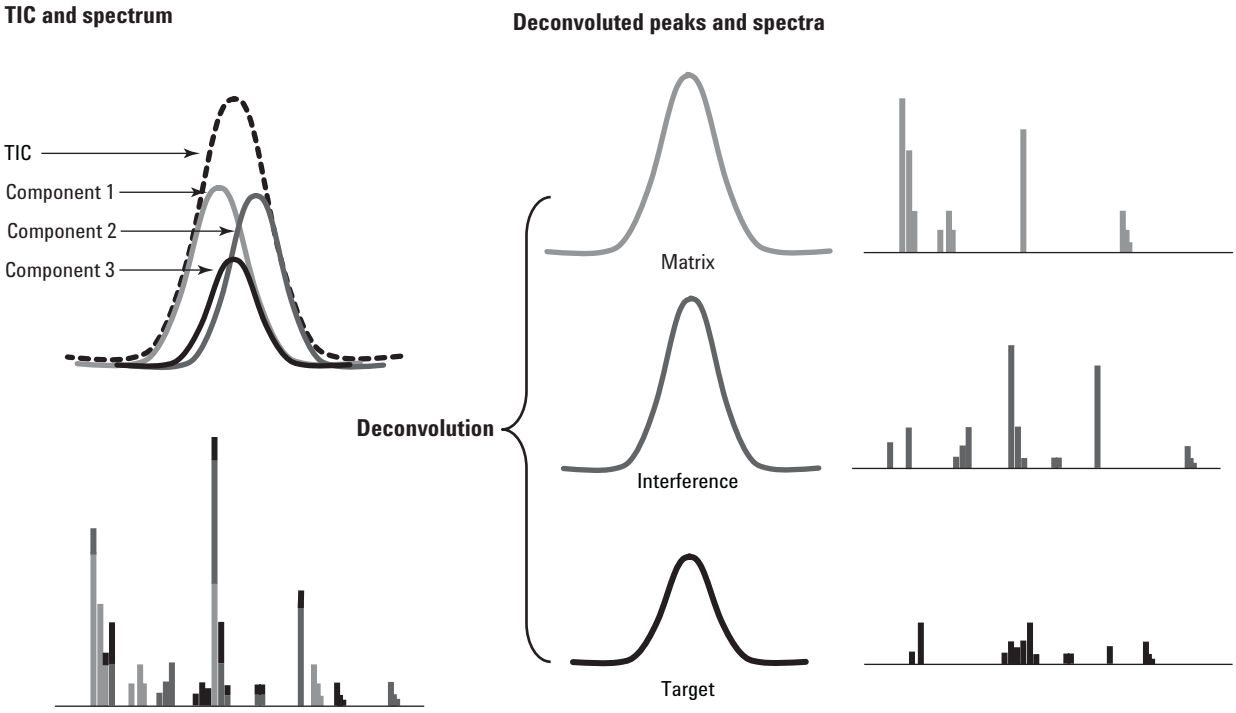

Figure 1. An illustration of mass spectral deconvolution process.

DRS

Agilent's DRS results from the combination of three different GC/MS software packages: 1) the Agilent GC/MS ChemStation, 2) the NIST Mass Spectral Search Program with the NIST '02 MS Library, and 3) the AMDIS software, also from NIST. Included in the DRS, are mass spectral and locked RT libraries for 567 pesticides and suspected endocrine disrupters.

Three separate, but complimentary, data analysis steps are combined into the DRS. First, the GC/MS ChemStation software performs a normal quantitative analysis for target pesticides using a target ion and up to three qualifiers. An amount is reported for all calibrated compounds that are detected. For other compounds in the database, an estimate of their concentration can be reported based upon an average pesticide response factor (RF) that is supplied with the DRS software. The DRS then sends the data file to AMDIS, which deconvolutes the spectra and searches the Agilent RTL Pesticide Library (in AMDIS format) using the deconvoluted full spectra. A filter can be set in AMDIS, which requires the analyte's RT to fall within a user-specified time window. Because RTL is used to reproduce the RTL database RTs with high precision, this window can be quite small - typically 20 seconds or less. Finally, the deconvoluted spectra for all of the targets found by AMDIS are searched against the 147,000-compound NIST mass spectral library for confirmation; for this step, there is no RT requirement.

Once the appropriate method is loaded, the DRS report can be generated with a single mouse click as shown in Figure 2. The software can run automatically after each analysis or at a later time on a single file or a batch of files.

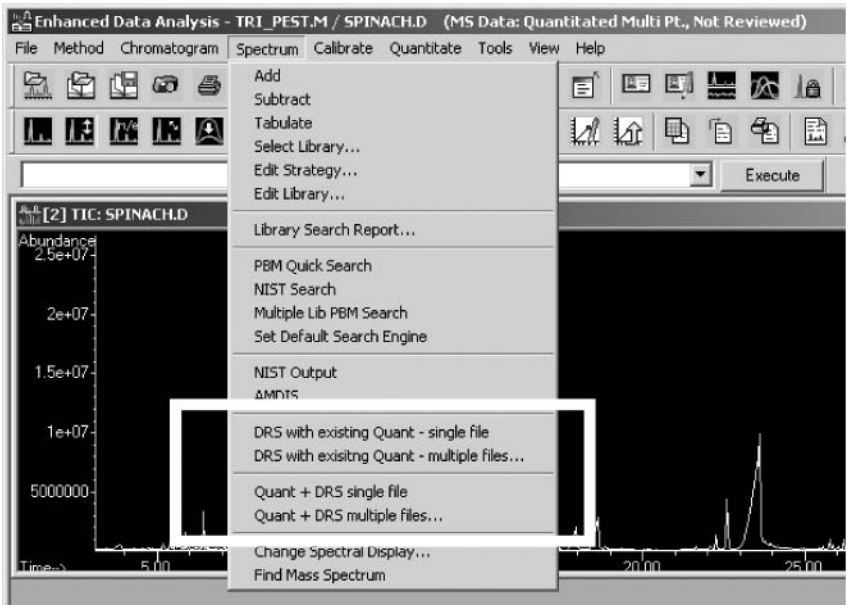

Figure 2. ChemStation pull down menu showing options for running the DRS on single or multiple files.

Pesticides in an Herbal Mix

Figure 3 shows a TIC from the extract of an herbal mix. Figure 4 shows the MSD Deconvolution Report for this sample, which is produced in html format so it can easily be emailed or copied into a spreadsheet. This sample was chosen because herbs are among the most difficult vegetable products to analyze. Their extracts contain a large number of natural products that interfere with pesticide analysis.

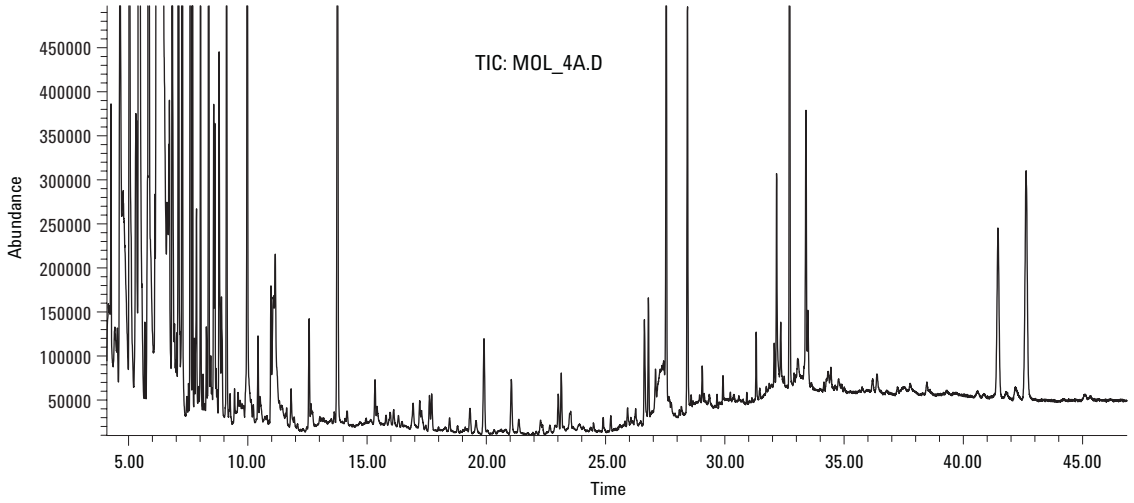

Figure 3. TIC of an herbal mix.

Document - Microsoft Internet Explorer provided by Agilent Technologies, Inc.

File Edit View Favorites Tools Help

Address C:\MSDChem\1\DATA\Hans Mol Data Feb 04 samples\Mar03_X4\MOL_4A.D\MOL_4A.htm

MSD Deconvolution Report
Sample Name: Herbal Mix Control
Data File: C:\MSDChem\1\DATA\Hans Mol Data Feb 04 samples\Mar03_X4\MOL_4A.D
Date/Time: 02:03:11 PM Tuesday, Apr 27 2004

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
13.038	1610180	Prometon		84	2.5	71	1
18.468	84742	Di-n-butylphthalate	1.7	90	2.5	94	1
23.654	38727558	Diethatyl ethyl		69	3.2	73	1
24.079	72559	p,p'-DDE		64	3.3	55	1
27.436	51235042	Hexazinone		61	3.3	80	1
29.681	117817	Bis(2-ethylhexyl)phthalate	0.62	92	2.7	88	3
29.770	21609905	Leptophos		87	3.0	71	1
29.864	2385855	Mirex	0.06	63	2.4	66	2
34.344	51630581	Fenvalerate I		70	5.3	83	2
34.779	102851069	Fluvalinate-tau-I		63	4.6		
34.779	69409945	Fluvalinate				71	1
13.766		Phenanthrene-d10	10				

Done My Computer

Figure 4. MSD Deconvolution Report generated for the herbal mix extract shown in Figure 3.

The DRS report in Figure 4 lists the RT, CAS number, and compound name for each hit. Phenanthrene-d_{10}, listed at the bottom of the report, is the internal standard (ISTD) used by the ChemStation to estimate the quantity of each compound that it found. Since an average pesticide response factor was used for all 567 target compounds, the amounts listed in column 4 are only estimates. Experience has shown that most estimates reported using an average pesticide response factor fall within a factor of 10 of their actual values. True quantitation requires calibration with pesticide standards in the normal way, but this is not practical for all of the pesticides in the database. A laboratory would normally generate calibration curves for their target set of pesticides and use the average RF for the remaining compounds in the database. In this way, when a new compound is detected, the lab can immediately get a rough estimate of its concentration and decide if it should be added to the calibration list.

Column 5 in the report shows the match factor obtained through AMDIS deconvolution and RTL Pesticide Library searching using the deconvoluted full spectra. In this case, several more targets were identified by AMDIS than were found by the ChemStation software (for example, Prometon and p,p'-DDE), which is typical for complex samples. When locked RTs are available, it is a significant advantage to set a RT requirement in the AMDIS software. In this case, hits that did not fall within ±10 seconds of the database RT were eliminated. Column 6 shows the RT difference (in seconds) between the compound's library RT and its actual value in the chromatogram.

Figure 4 shows that the software identified two phthalates (suspected endocrine disrupters) in addition to the pesticides. Phthalates are ubiquitous in the environment and are extremely difficult to remove from the background. In this case, no attempt was made to determine if the phthalates were actually extracted from the sample or were introduced in the laboratory.

The last two columns in the DRS report show the results from searching all of the AMDIS hits against the NIST 147,000-compound mass spectral library. When the NIST library search finds a compound in the top 100 matches (a user-settable value) that agrees with the AMDIS results, its match factor is listed in column seven. The hit number is shown in the last column, with "1" being the best match (highest match factor) in the NIST database. Occasionally, the NIST library search does not find the AMDIS hit among the top 100 spectral matches. In this case, the next line in the report shows the best library match for that spectrum. This is evident for fluvalinate-tau-I (Figure 4), which eluted at 34.779 min. The next line shows the best NIST library match for that spectrum - fluvalinate. In this case, no compound with the same CAS number as fluvalinate-tau-I is contained in the NIST mass spectral library. In fact, fluvalinate-tau-I is the D isomer, while fluvalinate is the DL isomer mixture.

Blind Comparison Between DRS and Traditional Data Review

Many comparisons have shown that the DRS is much better than conventional methods at identifying target compounds in complex samples, such as food and environmental extracts. Two such studies are described here. In the first case, 17 unspiked crop samples were analyzed by NRM Laboratories in Berkshire, UK using Agilent's RT-locked pesticide method. The data files, but not their list of pesticide hits, were sent to Agilent for analysis using the new DRS. Table 2 shows a comparison of the results from the two laboratories. Using manual data review, NRM identified 28 pesticides in the 17 samples, four of which were below their lowest calibration level. Using the same data files, the DRS identified 33 pesticides.

Agilent's automated method did not identify azoxystrobin in the spring onion sample because it is not included in the RTL pesticide library. While it can be found in the NIST library, it has a molecular ion at 403 amu and method used at NRM only scanned to 400 amu. The DRS method confirmed all four pesticides that were below the NRM calibration range and found five more (terbacil, pyrimethanil, methiocarb, pyridaben, and propamocarb) that were not included in their method.

The agreement between the manual and automated methods was excellent. However, the DRS looks for many more pesticides and was able to find several that were missed by the manual method. In addition, manual data review took a chemist about 7 hours for the 17 samples while the DRS finished the task in 50 minutes of unattended computer time.

Table 2. A Comparison of the Pesticides Found in 17 Unspiked Crop Samples Using Conventional Data Review and Agilent's DRS. Pesticides that Were Found by Only One Method Are Underlined

Sample	Agilent DRS results*	NRM Manual Analysis**
Coriander	Propyzamide Chlorthal-dimethyl p,p'-DDE	Propyzamide Chlorthal-dimethyl p,p'-DDE
Rosemary	Terbacil Pirimicarb Chlorthal-dimethyl	Not found*** Pirimicarb Chlorthal-dimethyl
Spring Onion	Propyzamide Pyrimethanil Pirimicarb Metalaxyl Iprodione Not in DRS library†	Propyzamide Not found*** Pirimicarb Metalaxyl Iprodione Azoxystrobin
Chives	Methiocarb Iprodione	Not found*** Iprodione
Cherry Tomato	Procymidone Pyridaben	Procymidone Not found***
Courgette	Propamocarb	Not found***
Aubergine	Procymidone Buprofezin Endosulfan sulfate Iprodione	Procymidone Buprofezin Endosulfan sulfate Iprodione
Flat Leaf Parsley	Chlorthal-dimethyl	Chlorthal-dimethyl
Lambs Lettuce	Iprodione	Iprodione†††
Cos Lettuce	Dimethoate Metalaxyl Procymidone Terbuconazole Omethoate††	Dimethoate Metalaxyl Procymidone Terbuconazole††† Omethoate
Fine Endive	Procymidone lamda-Cyhalothrin	Procymidone lamda-Cyhalothrin
Red Potato	Chloropropham Pirimicarb	Chloropropham Pirimicarb†††
Fine Endive	Pirimicarb	Pirimicarb†††

* Pesticides found by re-analyzing NRM datafiles using Agilent's DRS software.

** Pesticides found by NRM using target compound analysis and manual verification.

*** This compound was not in the NRM target compound list.

† This compound is not included in the Agilent RTL Pesticide Library or the DRS software.

†† Found by the Agilent ChemStation but not found by AMDIS or NIST library searching after deconvolution. After careful review of this hit, omethoate was judged not to be in the sample.

††† Compound was detected but was below the calibration range.

Analysis of Surface Water Samples: In another study, the CDFA analyzed 17 surface water extracts for pesticides. TICs for two typical samples are shown in Figure 5. The CDFA used RTL and RTL database searching but without the benefit of spectral deconvolution. The same data files were then analyzed using the DRS for comparison.

Table 3 shows the results from the CDFA manual analysis of the 17 samples compared to results using the DRS. The CDFA found 38 pesticide hits in the 17 samples, some of which were for the same pesticide in multiple samples. It took a skilled analyst about 8 hours to review the results, eliminate false positives, and verify all of the hits. The DRS found 37 of the compounds seen by the CDFA and identified one CDFA hit as a false positive. In addition, 34 more pesticides were found for a total of 71 hits in the 17 samples. The process was fully automated and took about 20 minutes of unattended computer time to process all of the data files.

Table 3. A Comparison of Results from the Analysis of 17 Surface Water Samples by GC/MS. The CDFA Used RTL and RTL Database Searching, but No Deconvolution. Agilent's DRS Was Used to Analyze the Same Data Files

	CDFA	DRS
Number of pesticide hits	37	Same 37 + 34 additional
Number of false positives	1	0
Time required for analysis	~ 8 hours	20 minutes

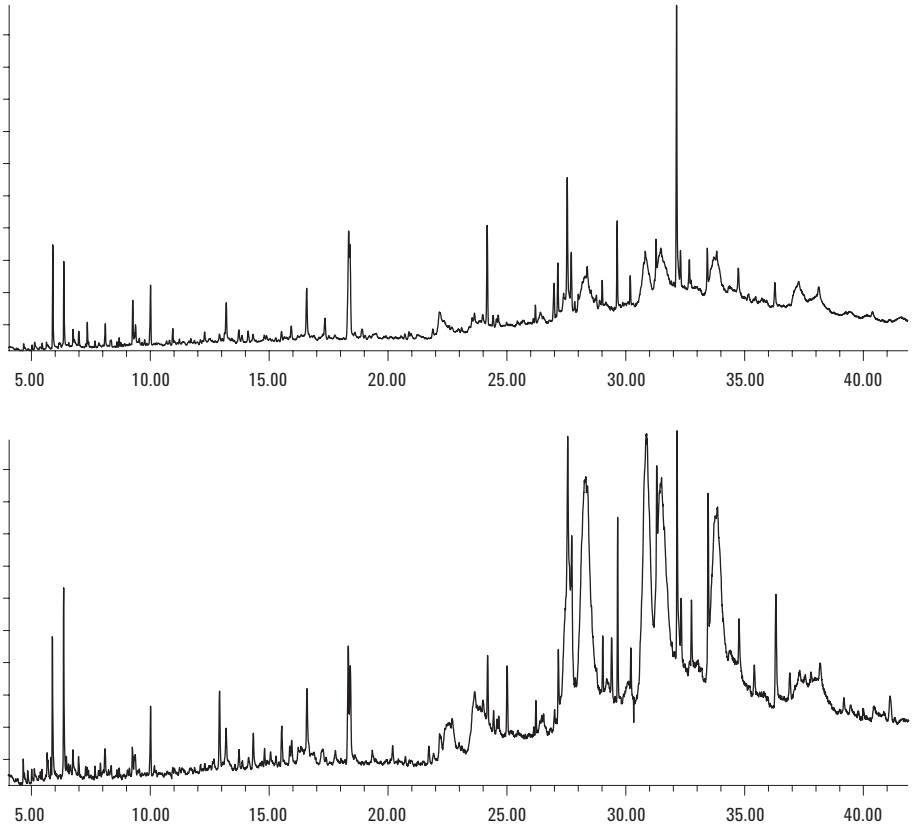

Figure 5. TICs of typical surface water extracts provided by the CDFA.

Conclusions

Agilent's new DRS solution for pesticide analysis offers laboratories a number of real benefits.

- **Ease of use**: This software solution is very simple to use and takes no more skill than is needed to operate the 6890N/5973 inert GC/MS system. There is no need for the user to learn about the intricacies of deconvolution or to master a new software package.
- **Automation**: The deconvolution report can be generated automatically after each run or a batch of samples can be processed all at once.
- **Time savings**: Data review is reduced from hours to minutes.
- **Quality**: It produces results with the fewest false positives and false negatives.
- **Reproducibility**: Results are not dependent upon the skill or experience of the operator.
- **Accuracy**: Comparisons such as those discussed in this application note show that the DRS finds pesticides with greater accuracy than manual methods of data analysis. It is particularly useful for relatively complex samples where co-eluting matrix components might obscure traces of target pesticides.
- **Comprehensive**: This method screens for almost all GC-amenable pesticides as well as several suspected endocrine disrupters in a single GC/MS run. With 567 compounds in the method, it is the most comprehensive pesticide-screening tool available. Users can add more compounds to the method as needed.
- **Produces quantitative, semi-quantitative, and qualitative results**: All calibrated compounds can be quantified. The concentrations of any other compounds can be estimated using an average pesticide response factor provided with the software.

Use of the DRS is not limited to pesticide analysis. Other target compound mass spectral libraries can be converted into the AMDIS format and used with this software. For example, one could use existing libraries for forensic drugs, flavors and fragrances, organic pollutants, etc. Users can even generate their own libraries and use them with the DRS. While not required, it is a big advantage to have an RTL library with locked RTs for each entry, as this will give the fewest false positives.

Acknowledgements

The authors wish to thank Dr. Mark Lee and Stephen Siegel of the California Department of Food and Agriculture, Dr. J.G.J. Mol of TNO Research, The Netherlands, and the management and staff at NRM Laboratories, UK, for their contribution of samples and data.

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Printed in the USA
May 19, 2004
5989-1157EN

Agilent Technologies

Validated Method for the Determination of Phenyl Urea and Triazine Herbicides in Potable and Groundwater by LC/MS Using Selective Ion Monitoring

Application

Environmental

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Abstract

This application note describes a validated liquid chromatography/mass spectrometry method for phenyl urea and triazine herbicides in potable and groundwater using an atmospheric pressure electrospray ionisation source in both positive and negative ion mode. The performance requirements set by the Drinking Water Inspectorate for standard deviation, bias, and total error are all met for each of the 16 herbicides studied.

Introduction

This application note describes the analysis of a single analytical suite composed of five phenyl urea herbicides including eight triazine herbicides, carbetamide (a carbamate herbicide), chloridazon (a pyridazinone herbicide), and metamitron (a triazinone herbicide). Phenyl urea herbicides, such as isoproturon, are widely used for weed control in crops. The triazine herbicides, such as atrazine, are extensively used general weed control agents. Both phenyl urea and triazine herbicides are found in environmental samples and are detected in drinking water.

The Prescribed Concentration or Value (PCV) for an individual pesticide in drinking water in the UK, as defined by the Water Supply (Water Quality) Regulations, is set at 0.1 µg/L. Ideally, the method of analysis should be capable of detecting 10% to 20% of the PCV, that is 0.01 to 0.02 µg/L.

Standard methods for the determination of these herbicides in water matrices involve either liquid-liquid extraction or, more recently, solid phase extraction (SPE) followed by high performance liquid chromatography (HPLC) with ultra violet (UV)/diode array detection for both classes of herbicide, but more typically gas chromatograph/nitrogen phosphorus detector (GC/NPD) or gas chromatography/mass spectrometry (GC/MS) detection for the triazines. The phenyl urea herbicides are not suited to GC analysis as they are thermally labile. This application note details the extraction method (SPE) and analysis using liquid chromatography/mass spectrometry (LC/MS) as a combined suite, using a relatively small volume of sample.

Experimental

All analyses were performed using the Agilent 1100 series LC/MS quadrupole coupled to an Agilent 1100 series LC system consisting of a binary pump, autosampler, thermostated column compartment, and vacuum degasser. The system also had a diode array detector in-line before the mass spectrometer, as a trouble shooting tool. The quadrupole mass spectrometer was operated with an atmospheric pressure electrospray ionisation (API-ES) source in positive and negative ion modes.

LC Conditions

Column:	Zorbax Eclipse XDB-C8		
	50 mm long x 2.1 mm id, 3.5 µm particles,		
40 °C			
Flow rate;	0.5 mL/min		
Injection volume:	25 µL		
Mobile phase:	A = 0.001% formic acid in water		
	B = Methanol		
		A	B
Gradient program:	Initial	90%	10%
	2.0 min	90%	10%
	15.0 min	30%	70%
	15.1 min	90%	10%
	22.0 min	90%	10%

MS Conditions

Ionisation mode:	Positive/Negative API-ES
Drying gas flow:	13.0 L/min
Nebulizer pressure:	40 psig
Drying gas temperature:	350 °C
V_{cap} voltage:	3000 V (positive), 2500 V (negative)

The selected ion monitoring (SIM) ions and fragmentor voltages listed in the SIM table parameters of Table 1 were all optimised using Flow Injection Analysis (FIA). Ten mg/L standard solutions of each herbicide were injected using scan mode 150 – 400 amu and the fragmentor voltage was ramped from 70 to 150 V in steps of 5 V.

Sample Preparation

SPE was performed using automated equipment and Baker SDB1 200 mg, 3 mL cartridges. The cartridges were conditioned with 5 mL of ethyl acetate, followed by 5 mL methanol, followed by 2 mL HPLC grade water. Fifty mL of sample was diluted to 200 mL using de-ionised water. One hundred mL of the diluted solution was pumped through the conditioned cartridge at 10 mL/min. Cartridges were dried for 25 minutes by forcing air through, followed by elution using ethyl acetate 1 × 1.5 mL and 1 × 1 mL. The final extracts were evaporated to dryness using a heated block set at 45 °C

Table 1. SIM Table parameters, positive ion mode

Compound #	Compound	Time	Group	SIM ions, Quantitation, Qualification (q)	Fragmentor voltage
1	Metamitron	2.50	1	203.0, 204.0q	70
2	Chloridazon			222.0, 224.0q	100
3	Monuron	7.50	2	199.0, 201.0q	115
5	Simazine			202.0, 204.0q	70
4	Carbetamide			237.0, 238.0q	95
6	Cyanazine			241.0, 243.0q	130
9	Isoproturon	10.25	3	207.0, 208.0q	140
7	Chlortoluron/ Isoproturon-d6			213.0, 215.0q	130
8	Atrazine			216.0, 218.0q	135
12	Propazine	12.40	4	230.0, 232q	130
13	Terbuthylazine				
15	Trietazine				
14	Prometryn			242.0, 243.0q	130
16	Terbutryn				
SIM Table parameters, negative ion mode					
10	Diuron	2.50	1	231.0, 233.0q	130
11	Linuron			247.0, 249.0q	115

q Qualifier ion

and a gentle stream of air. The residue was redissolved in 250 µL 90:10 HPLC grade water:methanol. The final make-up solution contains an internal standard at a concentration of 0.1 µg/L. The internal standard used is isoproturon-d6. Isoproturon-d6 is also present in the calibration standards at the above concentration for all levels of the calibration.

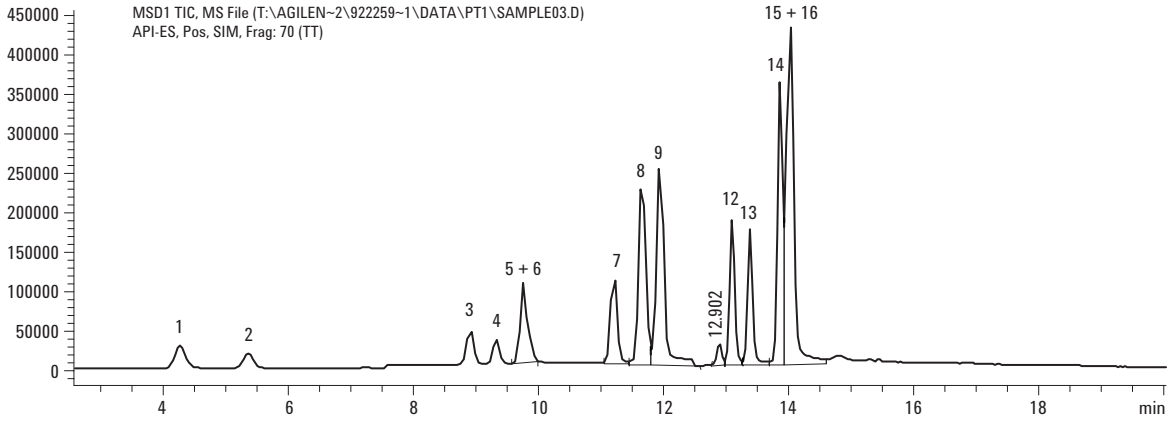

Figure 1. Chromatogram for the low level standard in positive ion mode.

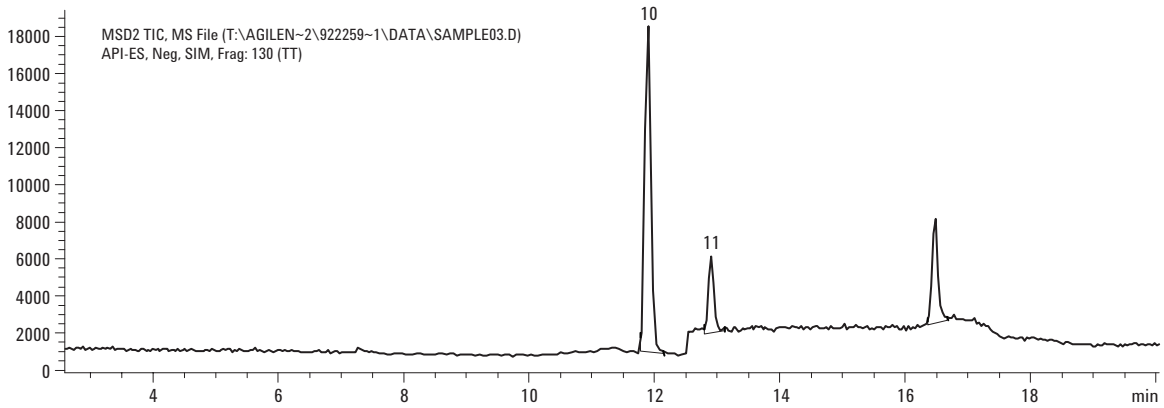

Figure 2. Chromatogram for the low level standard in negative ion mode.

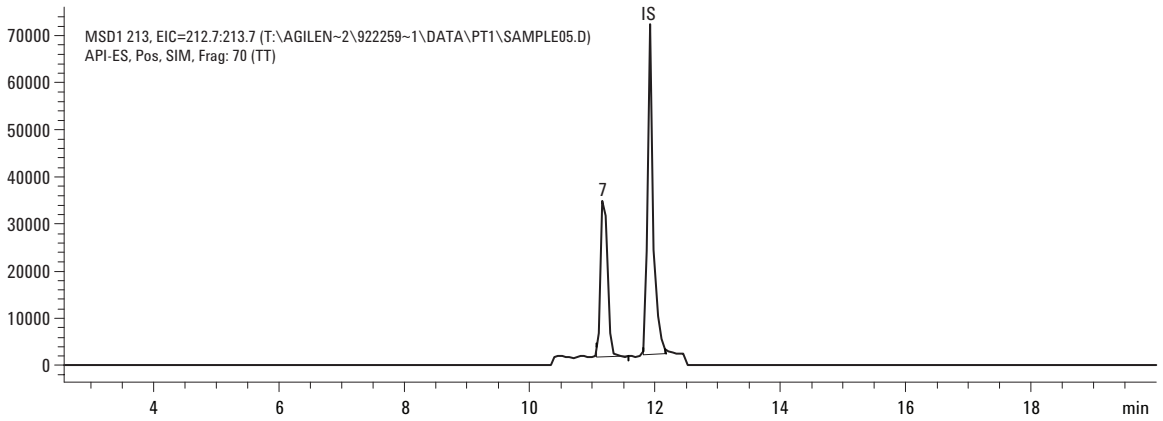

Figure 3. Extracted ion chromatogram from a spiked tap water sample containing chlortoluron at 0.10 µg/L. The isoproturon-d6 internal standard (IS) is also shown.

Results

A typical chromatogram for the low level standard (each compound at 0.1 µg/L) appears in Figures 1 and 2 in both positive and negative ion modes respectively. Figure 3 shows an extracted ion chromatogram from a spiked tap water sample containing chlortoluron (0.10 µg/L) and the isoproturon-d6 internal standard.

Validation of the method was done on 11 batches of samples. Standards were spiked at three levels: 0.01, 0.10, and 0.40 µg/L. The borehole raw water was spiked at two levels: 0.01 and 0.10 µg/L. The potable tap water (which was from a surface water source) was also spiked at two levels: 0.01 and 0.10 µg/L . All samples were analysed in duplicate in each batch in a random order. See Table 2.

The limit of detection (LOD) for each herbicide was calculated from the within-batch standard deviation of the standard spiked at 0.01 µg/L. Recovery for both the groundwater and potable water samples was calculated from the 0.10 µg/L spike after the subtraction of 0.01 µg/L, hence the recovery value is based on 0.09 µg/L.

Calibration curves were produced using three calibration levels at 0.1, 0.3, and 0.5 µg/L. The calibration curves were all produced using a quadratic fit and forced through the origin and referenced against an internal standard. Typical correlation values are 0.9997 or better for all the herbicides in the suite.

Discussion

All 16 compounds could be analysed in positive ionisation mode, but diuron and linuron were analized in negative ionisation mode because some interferences were observed around the 0.01 µg/L level. No interferences were observed for these compounds in negative ionisation mode and good limits of detection were obtained.

The recovery values obtained for all compounds in both water matrices tested are all in agreement. It appears that there is no suppression of ionisation, which has not been the case for other methods [1] where suppression is noted by a reduction in the recovery of individual compounds in some matrices.

Complete separation of all 16 compounds was not achieved by this method. Compounds 5 and 6 coeluted as well as compounds 15 and 16. However, all compounds were still quantitatively analysed as they differ in molecular weight. For instance, since compound 5 (simazine) has a molecular weight of 201 amu ($[M + H]^+ = 202$ *m/z*) and compound 6 (cyanazine) has a molecular weight of 240 amu ($[M + H]^+ = 241$ *m/z*), they were distinguished by selected ion mass spectroscopy. The same is applicable for compounds 15 and 16. Three compounds 12, 13, and 15 are isomers and all have a molecular weight of 229, but all three compounds show good chromatographic separation.

Table 2. Results

	Groundwater sample		Potable water sample		
Compound	**Recovery %**	**RSD %**	**Recovery %**	**RSD %**	**LOD µg/L**
Atrazine	83.4	5.1	83.3	5.6	0.00146
Carbetamide	92.0	7.5	88.5	7.8	0.00544
Chloridazon	94.8	5.8	94.0	4.6	0.00293
Chlortoluron	89.5	3.9	89.5	4.6	0.00219
Cyanazine	95.1	4.3	96.3	4.7	0.00352
Diuron	92.7	4.6	94.3	4.5	0.00348
Isoproturon	93.0	2.6	93.2	3.3	0.00209
Linuron	89.4	4.9	91.0	4.6	0.00330
Metamitron	102.4	2.8	102.8	3.1	0.00257
Monuron	96.6	4.6	97.3	4.8	0.00221
Prometryn	81.8	4.6	82.6	4.4	0.00208
Propazine	85.8	5.8	85.6	6.2	0.00218
Simazine	91.0	4.7	91.1	4.8	0.00179
Terbuthylazine	78.1	8.3	80.2	6.8	0.00397
Terbutryn	81.9	4.4	83.6	4.6	0.00268
Trietazine	79.1	4.7	78.7	4.8	0.00179

Figure 4 shows an extracted ion chromatogram, at 230 *m/z* ($[M + H]^+$) for compounds 12, 13, and 15. Two other compounds, 14 and 16, are also isomers with a molecular weight of 241 amu ($[M + H]^+$ = 242 *m/z*). Compounds 14 and 16 are also separated by the chromatography.

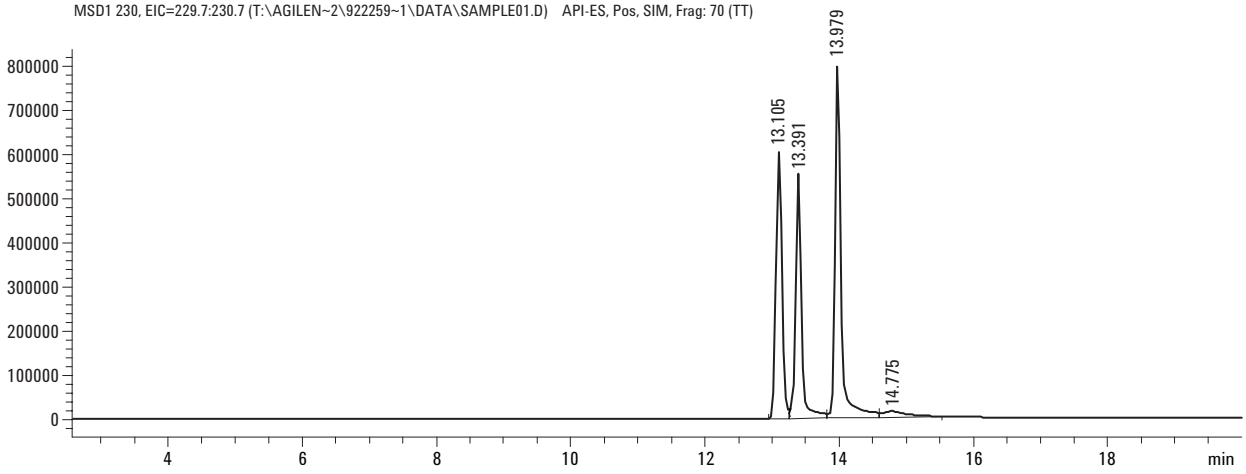

Figure 4. Extracted positive ion chromatogram, 230 *m/z*, for compounds 12, 13, and 15.

Conclusion

The data shows that the method presented is capable of quantitative analysis for the 16 herbicides in a single analytical suite. The performance requirements set by the Drinking Water Inspectorate (DWI) for standard deviation, bias, and total error are all met for each individual herbicide. Although spiked recovery targets of 90%–110% are not achieved in all cases, this can be compensated for by the application of recovery factors calculated from the performance data.

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Printed in the USA
April 2, 2003
5988-8595EN

Agilent Technologies

Identification of Steroids in Water by Ion Trap LC/MS/MS

Application

Environmental

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Abstract

The analytical boundaries for the rapid and sensitive identification and analysis of eight representative steroids were investigated using several LC/MS options including ion trap LC/MS/MS. Analysis was favored by direct large volume injections of aqueous sample and the appropriate choice of MS methodology.

Introduction

Synthetic steroids, potent hormones that can impact ecosystems at very low levels, are capable of causing significant and undesirable mutations in fauna. They can enter the environment from many real and potential sources and eventually enter into water drainage systems to lakes and seas. There is, therefore, a need to develop efficient and sensitive methods to monitor these compounds.

This paper summarizes an investigation to determine the analytical boundaries for the identification and analysis of these compounds in water using ion trap MS/MS methodology.

Experimental

Eight steroids, representing three different substituent classes, and diethylstilbestrol, were investigated and are shown in Figure 1. They were dissolved in water or selected solvents, injected into a liquid chromatograph for separation and characterized using several mass spectroscopic modes.

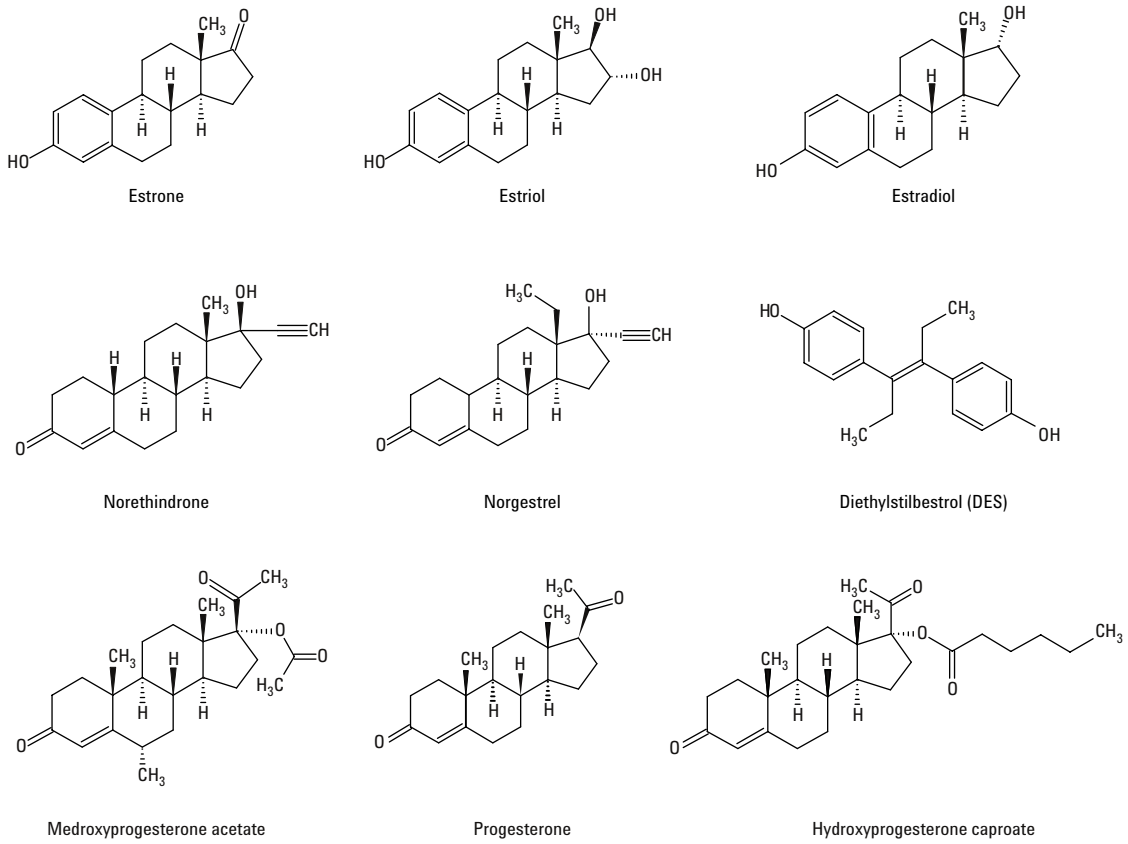

Figure 1. The compounds investigated.

Sample Preparations

The experimental steroids used here were USP reference standards, and diethylstilbestrol was obtained from Aldrich. Experimental standards and their mixtures were prepared in isopropyl alcohol (IPA).

Instrument

Agilent 1100 Series LC/MSD Trap VL

LC Conditions

Column:	Zorbax Eclipse XDB-C18, 5 cm long × 2.1 mm id, 3.5 µm particles
Mobile phase A:	0.25 mM ammonium acetate in water
Mobile phase B:	Acetonitrile (ACN)

Gradient:

Time, min	% ACN
0	20
0.2	35
8	90
9	90
9.7	20

Flow:	0.25 mL/min
Run time:	9.8 min
Post time:	8 min
Sample size injected:	1 to 100 µL, to deliver each at 5 to 50 ng on column

Binary pump with UV/VIS diode array detector (DAD)

Ion Trap Mass Spectrometry

Vaporizer:	475 °C average
Nebulizer:	30 psi
Dry gas:	8 L/min, 300 °C
V_{cap}:	-3.0 KV for APCI positive ion mode
V_{cap}:	+1.5 KV for APCI negative ion mode
V_{cap}:	+2.0 KV for APPI negative ion mode

Chromatography

A liquid chromatogram of the test compounds is shown in Figure 2. Both name and a peak number identify the steroid peaks. These same peak numbers will be used to identify the peaks in other figures.

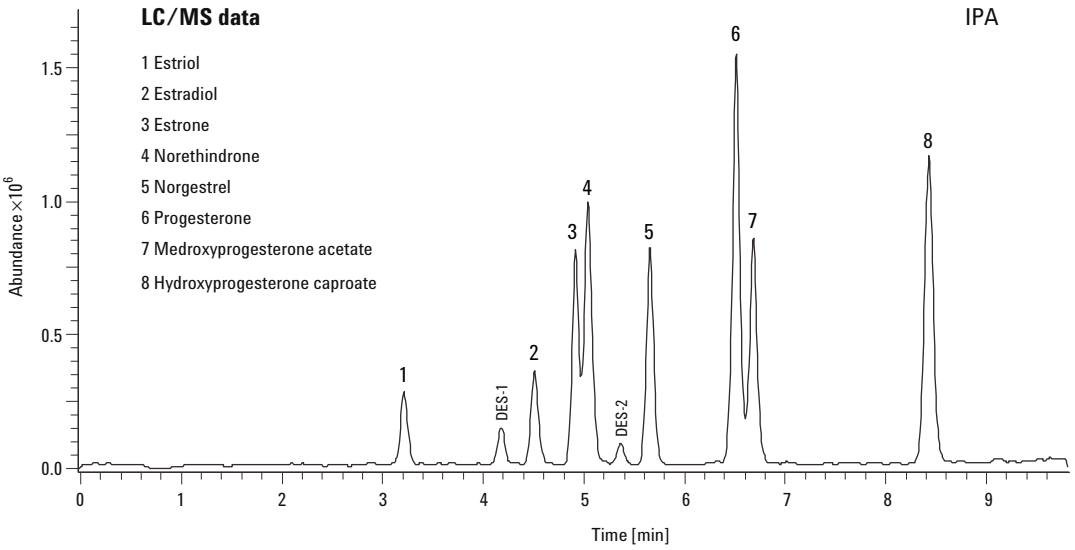

Figure 2. Liquid chromatogram of target compounds, detected by ion trap MS.

Injection Volume

The effect of strong solvent injection, using IPA as an example, on the chromatography is shown in Figure 3. It is obvious that there is substantial loss of resolution as the injection volume increases.

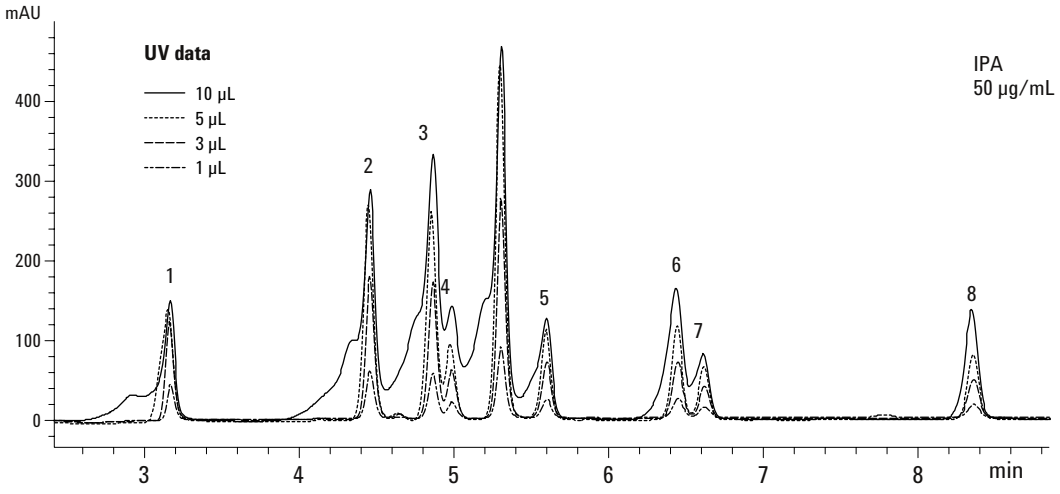

Figure 3. DAD chromatograms using injection volumes of 1, 3, 5, and 10 µL on column, in IPA.

Solvent Choice

The effect of solvent choice on the sharpness of chromatographic peaks is shown in Figure 4. Here the reciprocal of peak width is plotted against injection volume for both water and IPA solutions. As much as 100 µL of an aqueous sample can be injected and still obtain the same peak width as a 1 µL IPA solution of the same concentration, which in this case is 5.0 µg/mL.

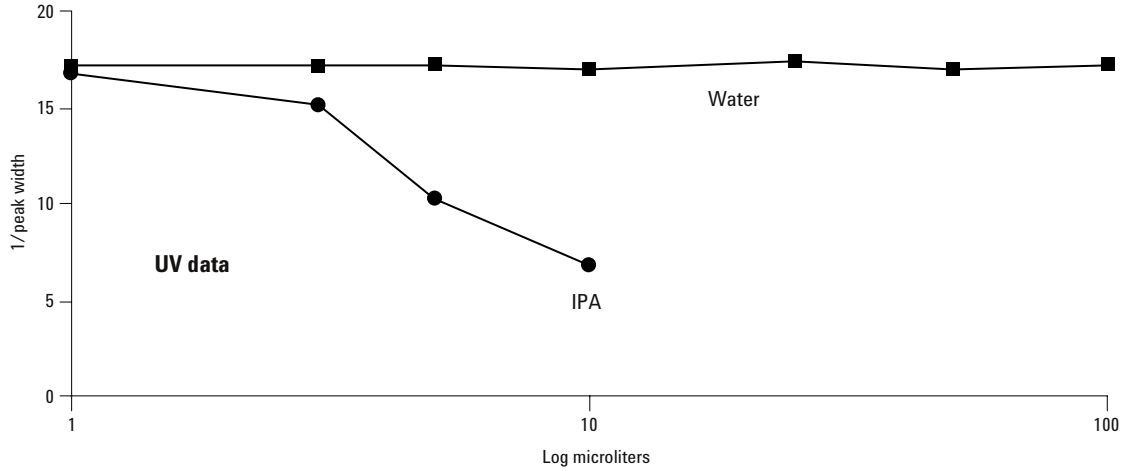

Figure 4. The effect of solvent choice on the maximum allowable injection volume.

Figure 5 compares the DAD chromatograms obtained from these solutions. Note that the water solution exhibits 10 times the signal intensity using a tenth of the concentration.

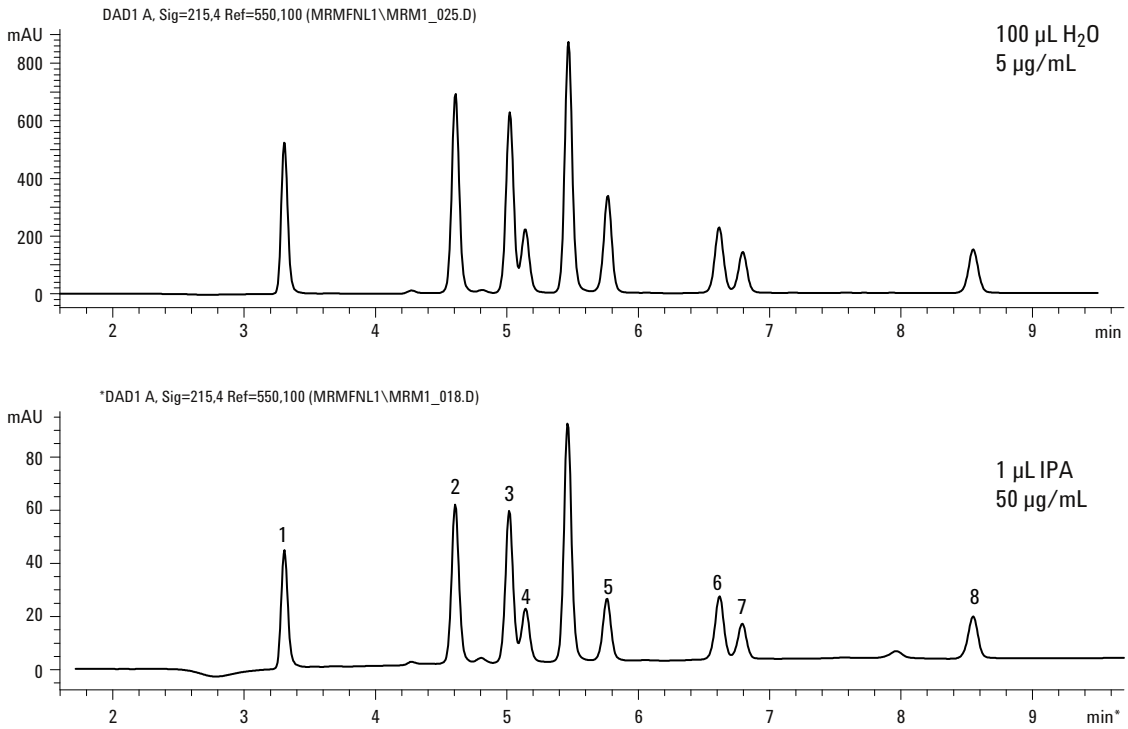

Figure 5. Comparison of DAD chromatograms produced from water and IPA solutions of the target compounds.

Vaporizer Temperature

Signal intensity is a function of both target compound and vaporizer temperature. The relationship for four steroids is shown in Figure 6. The signal intensity for estriol is greatest when the vaporizer temperature is about 450 °C, while for the others the signal intensity keeps increasing up to the experimental limit. When analyzing for all compounds in this set the vaporizer temperature must be a compromise, hence the 475 °C setting.

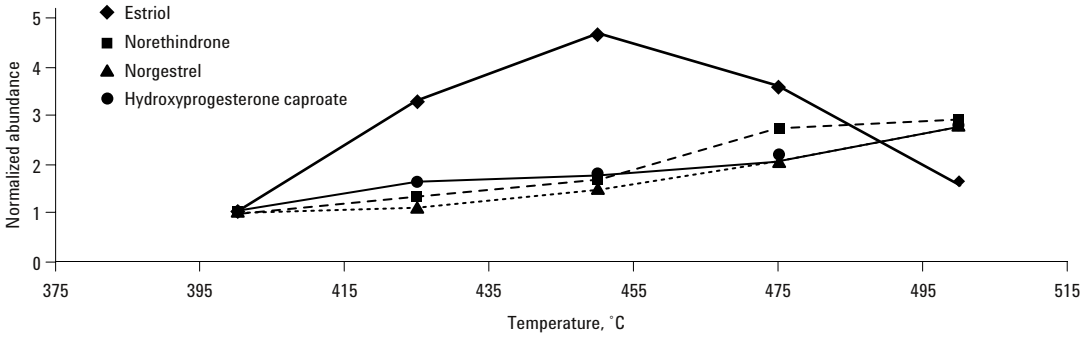

Figure 6. Signal intensity vs. vaporizer temperature relationship for estriol, norethindrone, norgestrel, and hydroxyprogesterone caproate.

Atmospheric Pressure Ionization and Mass Spectrometry

Several mass spectrometric options were evaluated and described. The major ions observed in these experiments are identified in Table 1. In the negative ion experiments the indicated solvents were added post column. We note that certain steroids were not detected by all modes. We also note that estriol and estradiol lose water during APCI positive ionization.

Table 1. Ions Observed Using Different Atmospheric Pressure Ionization Modes

			APCI positive ion			APCI negative ion, CH_2Cl_2			APPI negative ion, acetone		
			Expected	Measured		Expected	Measured		Expected	Measured	
No.	Steroid	MW	*m/z*	*m/z*	Ion	*m/z*	*m/z*	Ion	*m/z*	*m/z*	Ion
1	Estriol	288	289	271	$[M+H-H2O]^+$	287	287	$[M-H^+]^-$	287	287	$[M-H^+]^-$
2	Estradiol	272	273	255	$[M+H-H2O]^+$	271	271	$[M-H^+]^-$	271	271	$[M-H^+]^-$
3	Estrone	270	271	271	$[M+H]^+$	269	269	$[M-H^+]^-$	269	269	$[M-H^+]^-$
4	Norethindrone	298	299	299	$[M+H]^+$	297	Not found		297	Not found	
5	Norgestrel	312	313	313	$[M+H]^+$	311	Not found		311	Not found	
6	Progesterone	314	315	315	$[M+H]^+$	313	313	$[M-H^+]^-$	313	Not found	
7	Medroxyprogesterone acetate	386	387	387	$[M+H]^+$	385	385	$[M-H^+]^-$	385	Not found	
8	Hydroxyprogesterone caproate	428	429	429	$[M+H]^+$	427	427	$[M-H^+]^-$	427	427	$[M-H^+]^-$

The mass spectra for the APCI positive ions are stacked and shown in Figure 7. Arrows indicate the peaks chosen for additional dissociation to produce the second generation mass spectra shown in Figure 8.

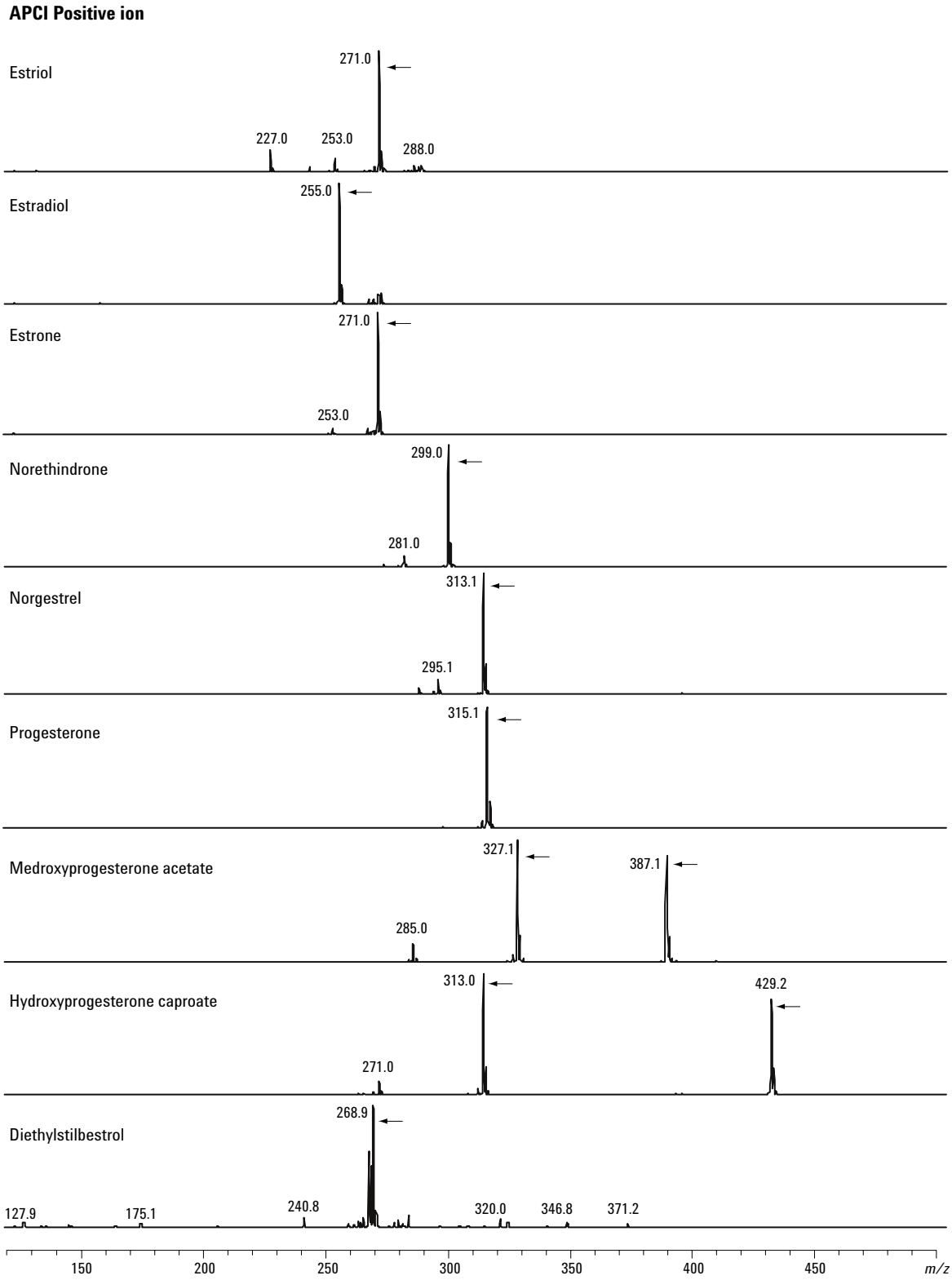

Figure 7. Stacked APCI positive ion mass spectra for the target compounds.

Auto MS/MS

One major feature of the Agilent LC/MSD trap is its ability to automatically sense, from an initial scan of a chromatographic peak, the major masses worthy of MS^2. The compound mixture was processed this way and the resulting second generation mass spectra are shown in Figure 8. Here only one MS^2 spectrum is shown for each target compound, although many could have been automatically selected had the precursor ion exceeded threshold. Arrows in Figure 7 indicate the precursor ions chosen for further dissociation in the trap. Two precursor ions exceeding threshold were allowed for MS^2 in these Auto MS/MS experiments.

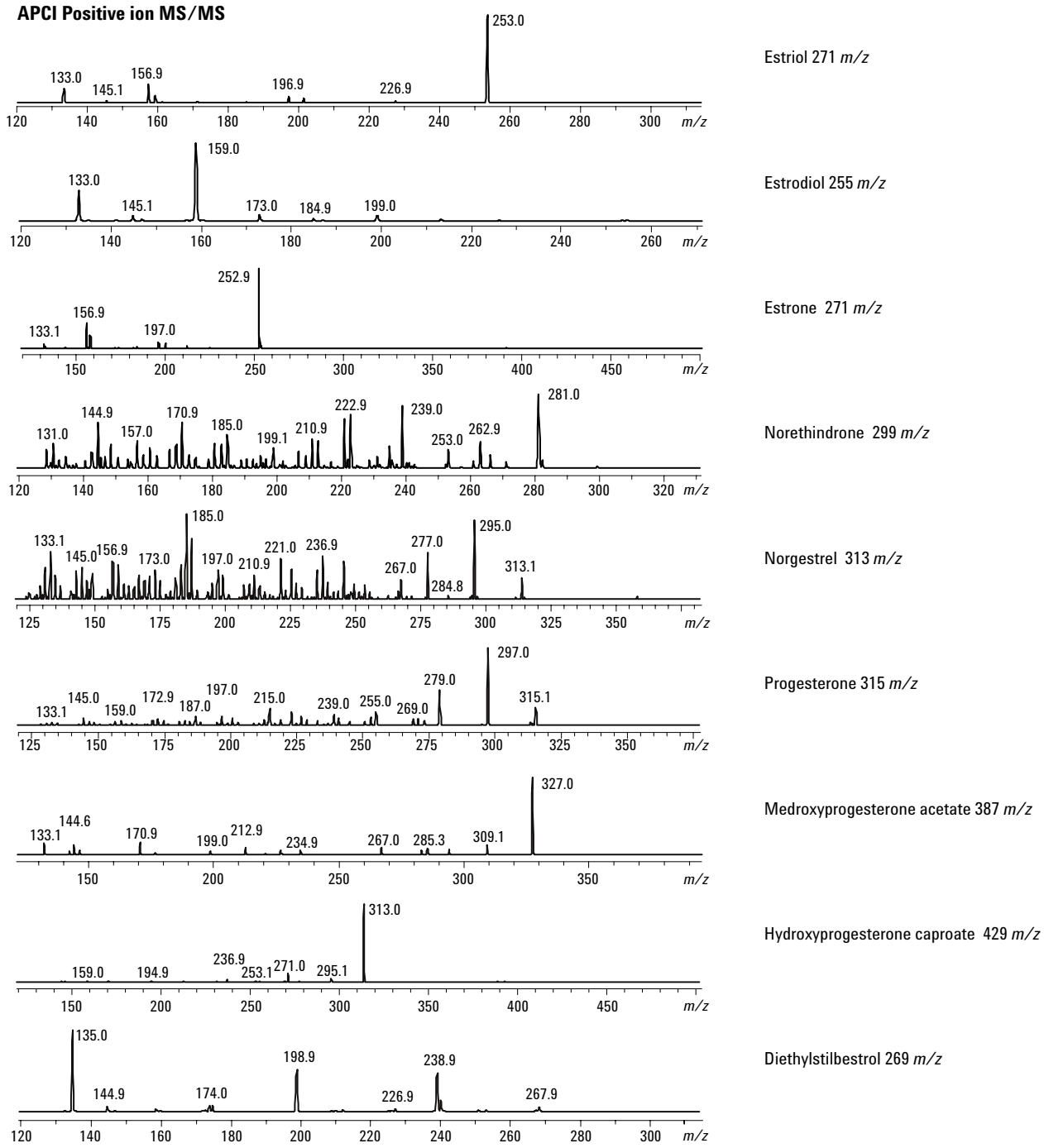

Figure 8. Stacked second generation (MS^2) mass spectra for one of each target compound's precursor ions.

Chromatograms were generated using both first (MS) and second-generation (MS^2) data for each chromatographic peak at compound concentrations of 5 and 50 ng on column. These are compared in Figure 9. The separate chromatograms at each concentration represent different order MS data as indicated in the figure key. The MS/MS chromatograms represent sums of product ions from each parent. The ability to use multiple precursor ions is an analytical advantage when there are overlapping chromatographic peaks exhibiting similar primary dissociation ions.

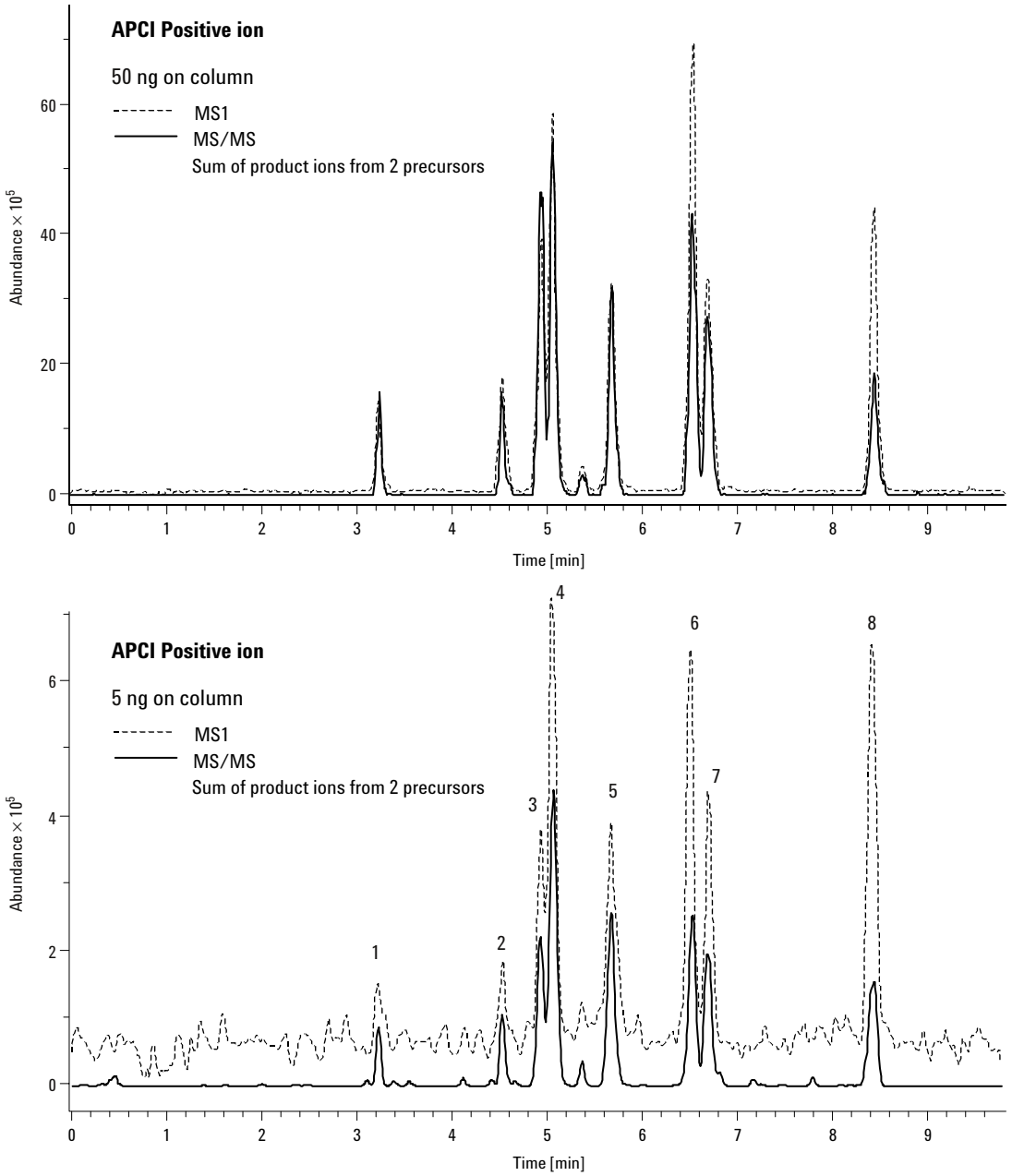

Figure 9. MS and MS/MS chromatograms at 5 and 50 ng on column for the target compounds.

Multiple Reaction Monitoring (MRM)

MRM is an analytical option, which directly produces the desired second-generation mass spectrum. This is useful when the compound's primary mass spectrum is already known and further analysis will only use second generation data from pre-selected primary ions.

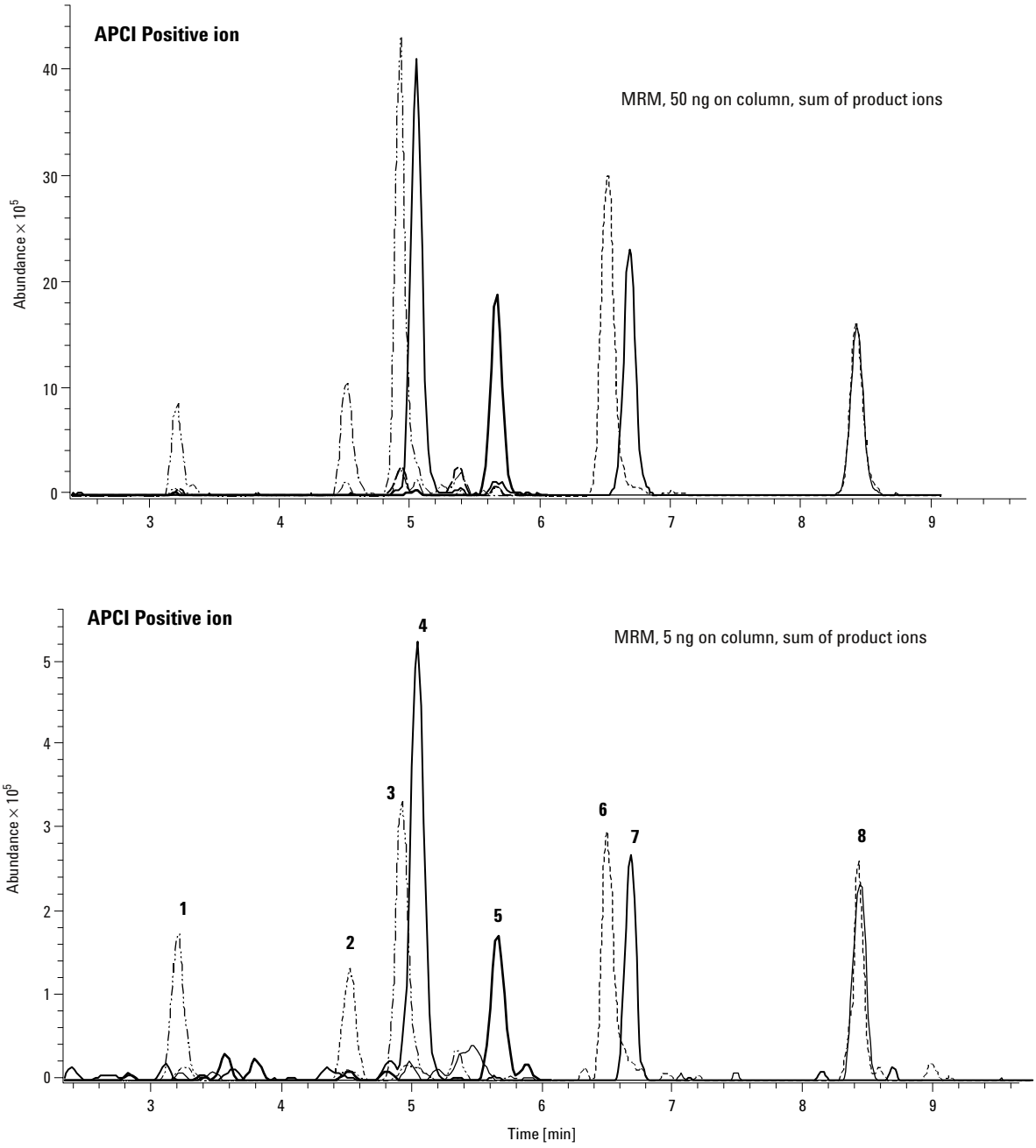

Figure 10. MRM second generation chromatograms of target compounds at two concentration levels using two precursor ions per compound.

Another useful analytical option is the ability to add signal intensities derived from multiple secondary ions from the same chromatographic peak to further enhance sensitivity. This is shown in Figure 11. Note the dramatic signal intensity increase for peaks 4, 5, and 6 when compared to neighboring peaks 3 and 7. This is most advantageous for situations where the secondary dissociation produces many low intensity peaks and complex MS/MS spectra. (For example, MS/MS spectra of northindrone, norgestrel, and progesterone in Figure 8.)

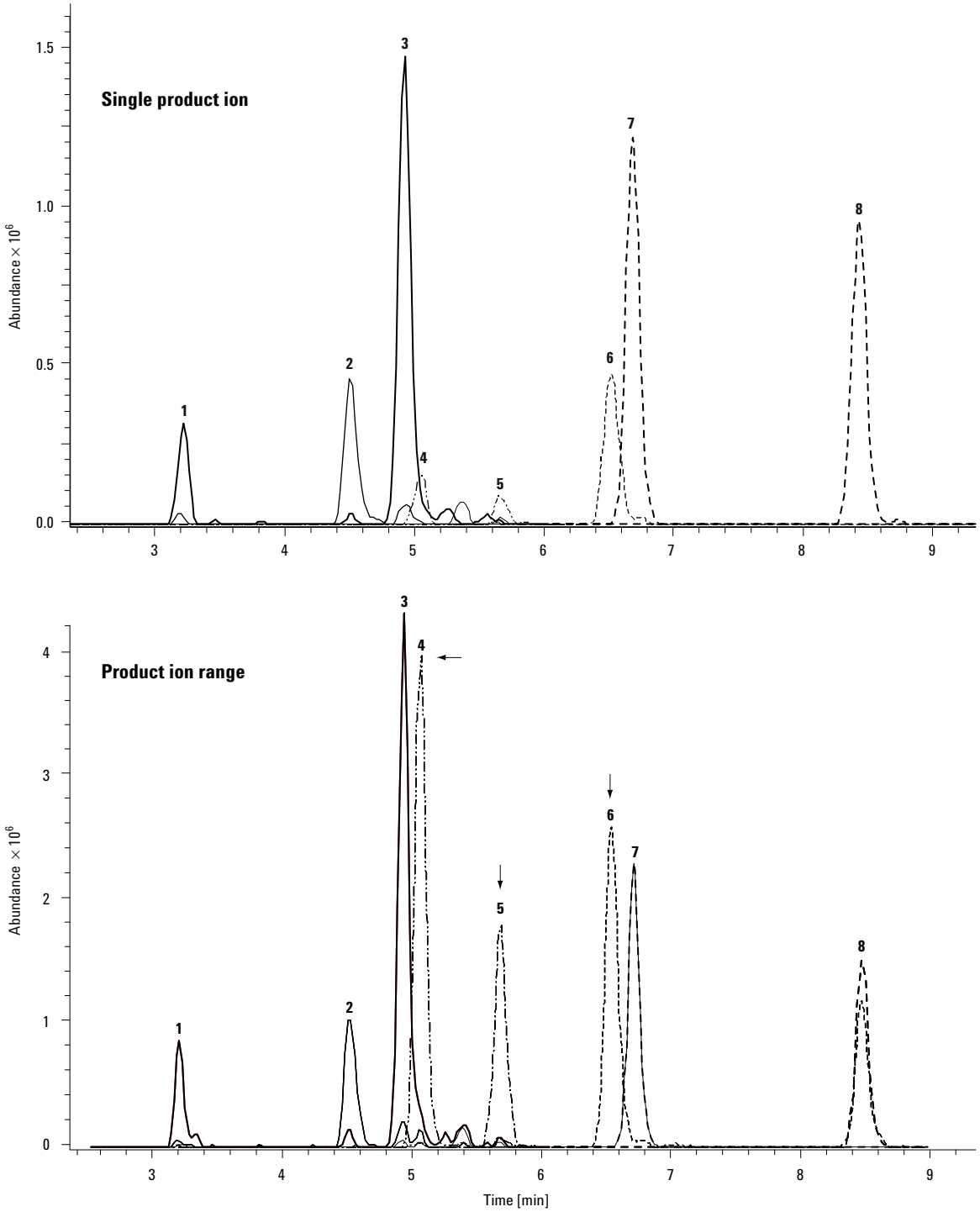

Figure 11. Comparison of MRM signal intensities using single and multiple product ion contributions.

Observations and Conclusions

- Chromatographic properties favor direct large volume aqueous sample injection. Rapid analysis is possible.
- Estriol, Estradiol and DES are best detected using negative ion APCI with 450–475 °C vaporizer temperature; all others are best detected in positive ion mode and at maximum temperature.
- APPI is very selective for some compounds under these conditions.
- Ionization in positive ion mode is usually simple. Some compounds lose water in the CID[1] region.
- Positive ion APCI is best for analysis of weak ionic strength mobile phases.
- Auto MS/MS is possible in scan mode to 5 ng of compound on column. TRAP SL optics is expected to give significantly better sensitivity and speed [1].
- MRM allows product ion scans to better than 5 ng on column. TRAP SL optics is expected to give significantly better sensitivity and speed [1].
- Monitoring the sum of product ions is best for compounds with complex product ion spectra.

[1]Collision induced dissociation

References

1. Supporting Agilent documents viewable via www.agilent.com
   a. Publication 5988-2870EN, June 1, 2001
   b. Publication 5988-3619EN, January 17, 2002
   c. Publication 5988-3620EN, October 31, 2001

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Printed in the USA
August 7, 2002
5988-6926EN

Agilent Technologies

Rapid Screening Method for the Analysis of Paraquat and Diquat by LC/MSD Using Selective Ion Monitoring and Large Volume Injection

Application

Environmental

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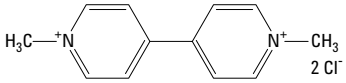
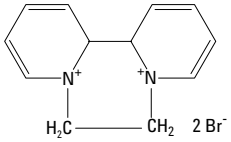


Paraquat



Diquat

Abstract

A rapid and sensitive method for the analysis of paraquat and diquat was developed using LC/MS, electrospray ionisation (ESI), positive ion mode, selective ion monitoring (SIM) large volume injections, and minimal sample preparation. No trace enrichment was required to obtain limits of detection (LOD) below 1 µg/L, which is well below the World Health Organisation (WHO) advisory value of 10 µg/L.

Introduction

Paraquat and diquat, bipyridilium salts, are used in agriculture as non-selective contact herbicides. They are also commonly used in commercial weed killer formulations for domestic weed control.

Standard analytical methods for these herbicides in water matrices involve solid phase extraction as the enrichment technique and analysis by liquid chromatography using diode array detection (DAD) [1]. The LOD for paraquat and diquat is reported to be less than 0.4 µg/L.

This application note gives details for the screening of these herbicides using liquid chromatography/ mass spectrometry (LC/MS) and a direct aqueous injection of the sample, thereby eliminating the trace enrichment step.

The WHO has set an advisory value of 10 µg/L for paraquat in drinking water. In this application, a LOD of well below 1 µg/L is achieved for paraquat and diquat, as well as for amitrole and chlormequat.

The method presented uses an ion-pairing reagent to help separate the compounds and to prepare

the analyte as an ion. Typical ion-pairing reagents such as hexanesulphonic acid are non-volatile and cause the ion-pair complex to become non-volatile. The ion-pair reagent used in this application is tridecafluoroheptanoic acid (TDFHA), which has been reported as suitable for the analysis of basic pesticides and sufficiently volatile for the electrospray interface [2].

Experimental

All analyses were performed using Agilent 1100 series LC/MSD quadrupole coupled to an Agilent 1100 series LC system consisting of a quaternary pump, autosampler, thermostated column compartment, vacuum degasser and diode array detector. The quadrupole mass spectrometer was operated with an atmospheric pressure electrospray ionisation (API-ES) source in positive ion mode.

HPLC conditions

Column:	Zorbax Extend C18, 150 mm long × 2.1 mm id, 3.5 µm particles, 60 °C
Flow rate:	0.4 mL/min
Injection volume:	250 µL
Mobile phase:	Isocratic elution A: 5 mM TDFHA in water (75%) B: Acetonitrile (ACN) (25%)

MS conditions

Ionisation mode:	API-ES, Positive
Drying gas flow:	13.0 L/min
Nebulizer gas pressure:	30 psig
Drying gas temperature:	350 °C
V_{cap} voltage:	3500 V

The SIM ions and fragmentor voltages listed in the SIM table parameters were all optimised using Flow Injection Analysis (FIA). Ten mg/L standard solutions of each herbicide were prepared in 5 mM TDFHA and injected using scan mode 50 to 1000 amu. The fragmentor voltage was ramped from 50 to 200 V in 10 V steps. The fragmentor voltage generating the maximum response for each SIM ion was selected.

Table 1. SIM Table Parameters

Compound	Time	Group	SIM ion	Frag volt	Gain	Res
Amitrole	0	1	85.1	120	1.0	Low
Chlormequat			122.0	130		
Diquat			183.0	140		
Paraquat			185.0	140		

Mass spectra for diquat and paraquat appear in Figures 1 and 2 respectively.

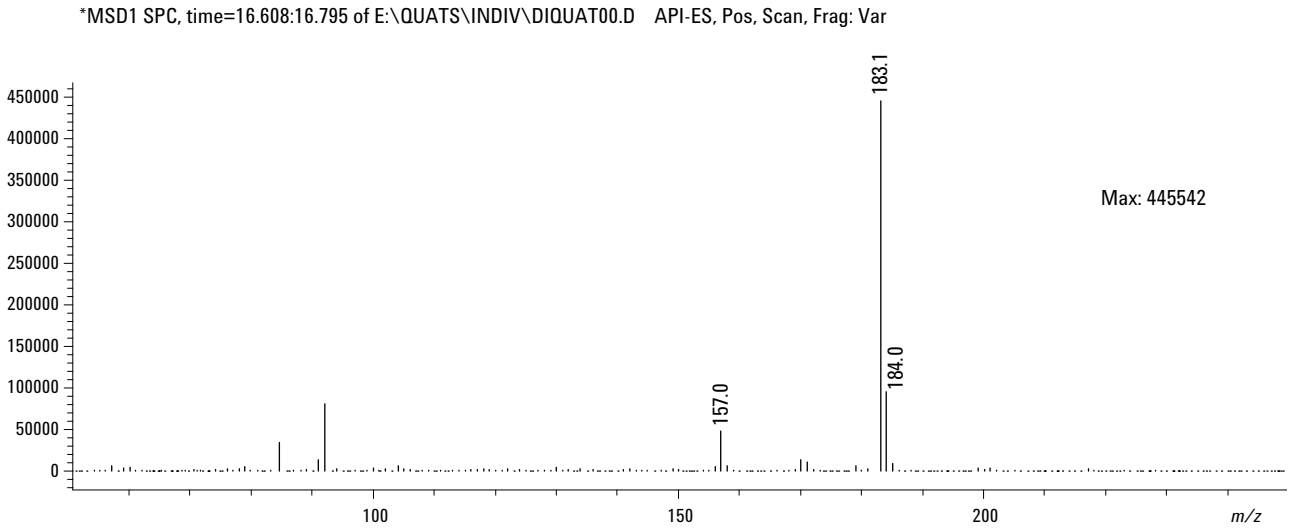


Figure 1. Mass spectrum of diquat.

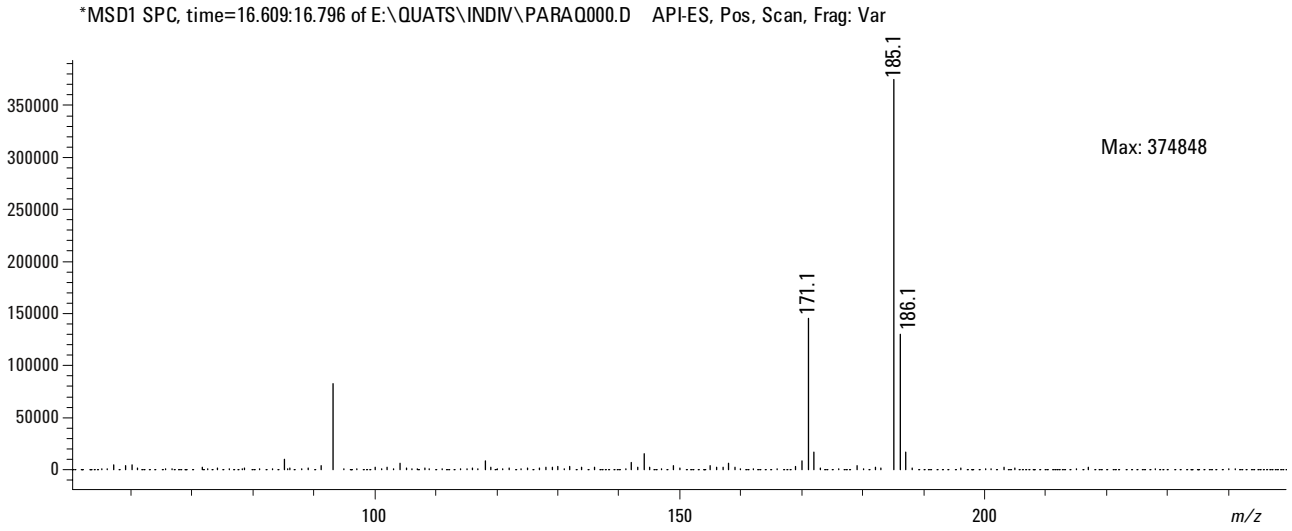


Figure 2. Mass spectrum of paraquat.

Sample Preparation

The only preparation required is that both working calibration standards and samples contain TDFHA at a concentration of 5 mM.

Figure 3 shows a typical chromatogram for a 10 µg/L calibration standard.

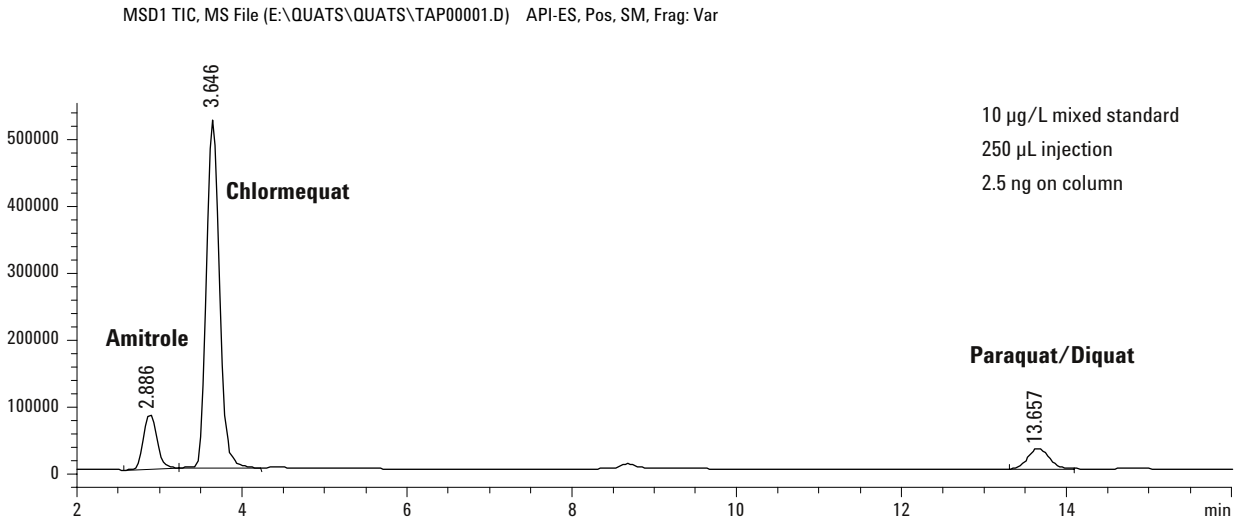


Figure 3. Total ion chromatogram of mixed standard, 10 µg/L, 250 µL injection, 2.5 ng on column.

Figures 4 and 5 show extracted ion chromatograms for diquat and paraquat, respectively, spiked into borehole water at a concentration of 1 µg/L.

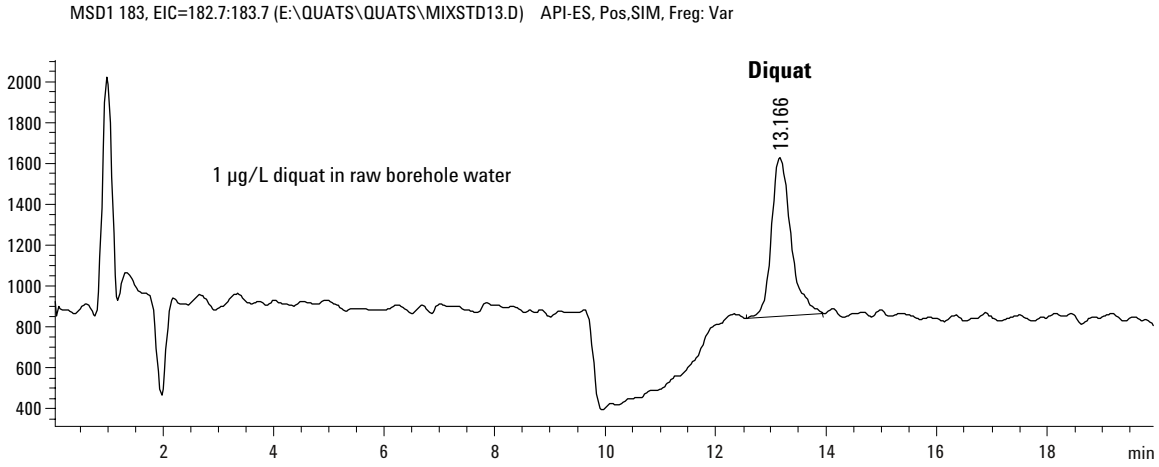


Figure 4. Extracted ion chromatogram of 1 µg/L diquat in raw borehole water.

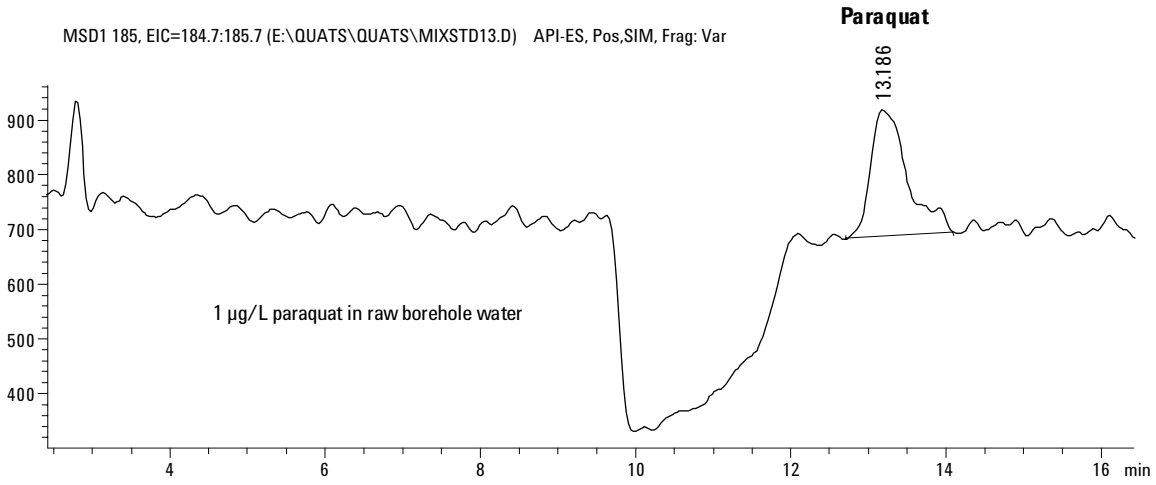


Figure 5. Extracted ion chromatogram of 1 µg/L paraquat in raw borehole water.

Conclusion

The data shows that the method presented is capable of screening for the four herbicides with a run time of 20 minutes per sample. No trace enrichment is required to obtain limits of detection below 1 µg/L with minimal sample preparation.

References

1. Analysis of Paraquat and Diquat by HPLC. Agilent publication number 5966-1875E, 1997.
2. Impact of Ion-pair Reagents on LC/MS Analysis. Agilent publication number 5968-8659E, 2000.

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Printed in the USA
August 7, 2002
5988-7220EN

Agilent Technologies

Analyzing Phenyl Ureas and Carbamate Pesticides Using ESI-, APPI-, and APCI-LC/MSD

Application

Environmental and Food

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Abstract

Carbamates (a class of highly effective insecticides) and phenyl ureas (a class of herbicides) were successfully analyzed by liquid chromatography/mass spectrometry using electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo ionization (APPI) sources. APPI and APCI exhibited lower background signals and fewer background peaks than ESI. Phenyl ureas, in general, exhibited better signal-to-noise ratios (S/N) from APCI or APPI than from ESI. However, it was just the opposite for carbamates. The S/N was better from ESI than from APCI or APPI. A small amount of post-column acetone was needed in the APPI source as a photoionizable dopant for charge transfer to the analyte.

Introduction

Carbamates, a class of highly effective insecticides, are widely used worldwide to protect crops from pests. Phenyl ureas, a class of herbicides, are highly potent chemicals for agricultural weed control. All are potential endocrine disrupters found in ground- and surface-water samples.

Gas chromatography/mass spectrometry (GC/MS) systems have traditionally been used for analyzing pesticides in environmental samples. Due to the thermally labile nature of these compounds, liquid chromatography has been the method of choice for the separation of these pesticides. Many of the pesticides within the same class exhibit similar ultraviolet (UV) spectra. With the development of atmospheric pressure ionization (API) techniques, liquid chromatography/mass spectrometry (LC/MS) systems have become the preferred method for the analysis of ground- and surface-water contaminants. The LC/MS provides better sensitivity and specificity than typical diode array detectors (DAD), even if the analytes are not fully resolved from neighboring eluants.

Three different API sources were used in this study for comparison: electrospray ionization (ESI), chemical ionization (APCI) [1], and photo ionization (APPI) [2]. In ESI, the ionization process happens before the solvent evaporation process. It is, therefore, a more universal ionization for polar compounds. In APCI and APPI, the analyte is not ionized until after solvent evaporation. APCI involves a charge transfer (proton or electron) between ionized reagent gas and the analyte. APPI requires that the analytes and/or a dopant be photoionized by absorbing photons from the 10.6 eV krypton light. The dopant then transfers the charge to the analyte, which could be thought of as photon-induced chemical ionization.

Experimental

A mixture of carbamates and urea pesticides at 10 ppm (ng/µL) in acetonitrile was purchased from AccuStandard (New Haven, CT). A series of dilutions in acetonitrile were made for the linearity studies. The compounds and their structures are shown in Figures 1 and 2, respectively. All experiments were performed on two G1946D LC/MSD systems equipped with ESI on one and APCI/APPI on the other. The LC/MS conditions are listed in Table 1.

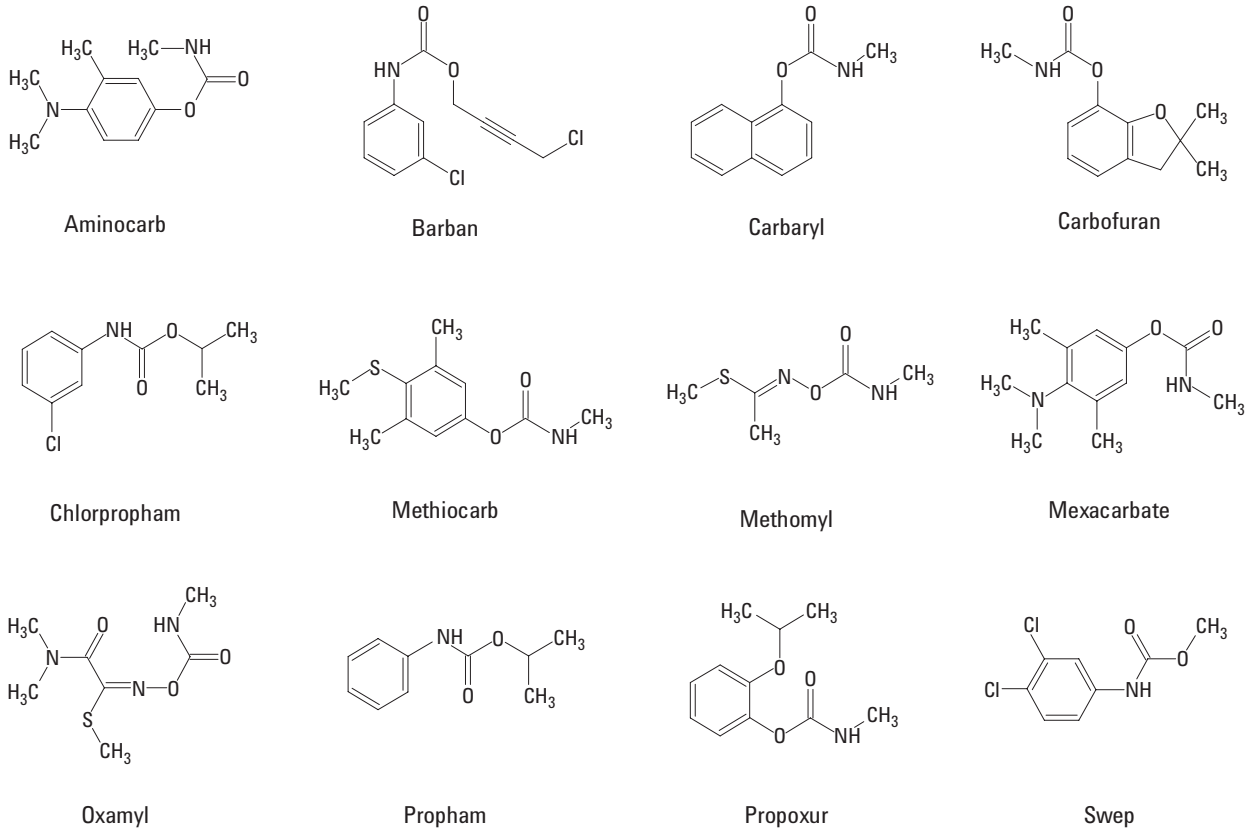


Figure 1. The 12 carbamates used in this study.

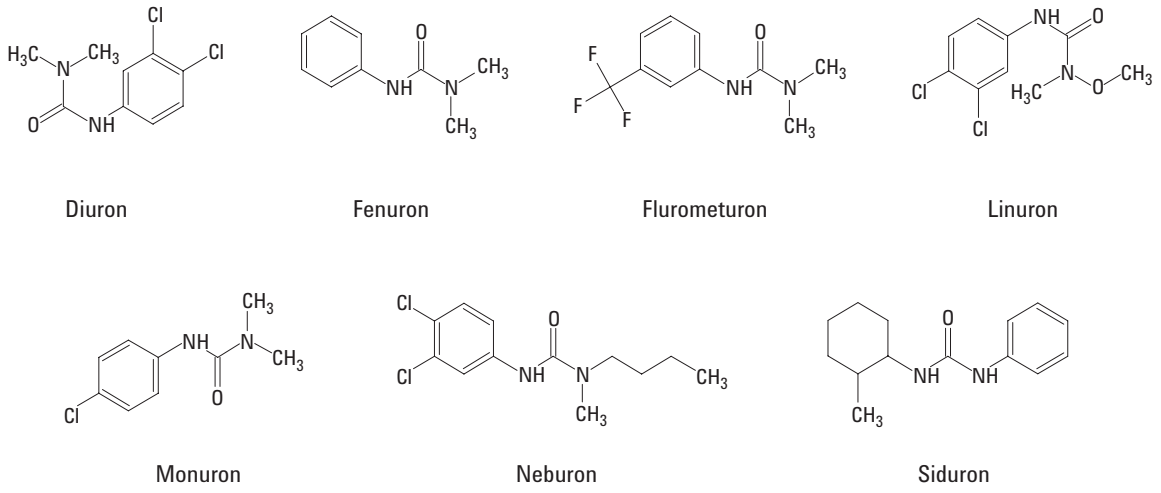


Figure 2. The seven phenyl ureas used in this study.

Table 1. LC/MS Conditions

	ESI	APCI	APPI
Column	Zorbax Eclipse XDB-C8, 4.6 x 50 mm, 3.5 µm (p/n 935967-906)		
Column temperature	30 °C	30 °C	30 °C
Column flow rate	1 mL/min	1 mL/min	1 mL/min
Solvent A	H_2O, 0.1% acetic acid or as specified	H_2O	H_2O
Solvent B	Acetonitrile, 0.1% acetic acid or as specified	Acetonitrile	Methanol
Post column	n/a	n/a	40 µL/min acetone added as dopant
Solvent gradient	B: 10% at 0 min, 30% at 4 min, 80% at 10 min, 80% at 16 min	B: 30% at 0 min, 40% at 4 min, 70% at 13 min, 80% at 16 min or as specified in the figure	
Injection volume	2 µL	2 µL	2 µL
Drying gas flow	12 L/min	11 L/min	11 L/min
Drying gas temperature	350 °C	275 °C	275 °C
Fragmentor voltage	60 V	110 V	110V
Vcap	3500 V	4500 V	4500V
Nebulizer pressure	60 psi	35 psi	35 psi
Vaporizer	n/a	225 °C	225 °C
Step size	0.1	0.1	0.1
Peak width (min)	0.15	0.15	0.15
Time filter	Off	Off	Off
Scan (*m/z*)	150–800	115–500	115–500
Polarity	Positive	Positive	Positive

Results and Discussion

Figure 3 shows the effect of adding a post-column dopant (acetone) in APPI to significantly improve the responses of the analytes. Acetone at a flow rate of 40 µL/min was introduced to the column effluent before entering the nebulizer. A gain of 1000× in peak height was seen for some of the analytes. The difference in peak-height gain among the analytes may be due to their structural difference and the charge transfer efficiency with the dopant.

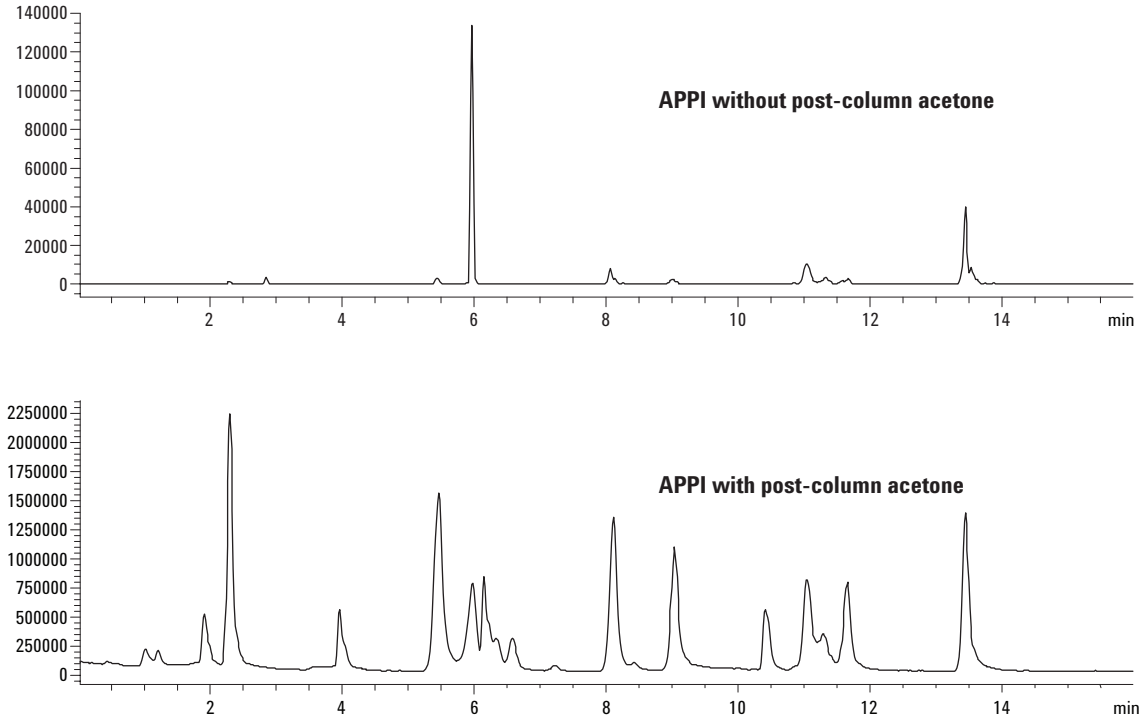


Figure 3. TICs show the effect of adding post-column acetone to enhance analyte signal in APPI. Methanol was used as solvent B for both TICs.

The LC mobile phase in APPI can interfere with ionization if the solvent has more affinity for the proton than the analyte. Figure 4 shows that acetonitrile can be a problem for baseline stability and it is always best to also try methanol in APPI.

Typical background spectra between 15 and 15.5 minutes from the three sources appear in Figure 5. Because ESI is a more universal ionization source for polar compounds and ionic modifiers are used in ESI, the baseline has a lot more peaks and the noise is usually 5 to 15 times higher than APCI and APPI.

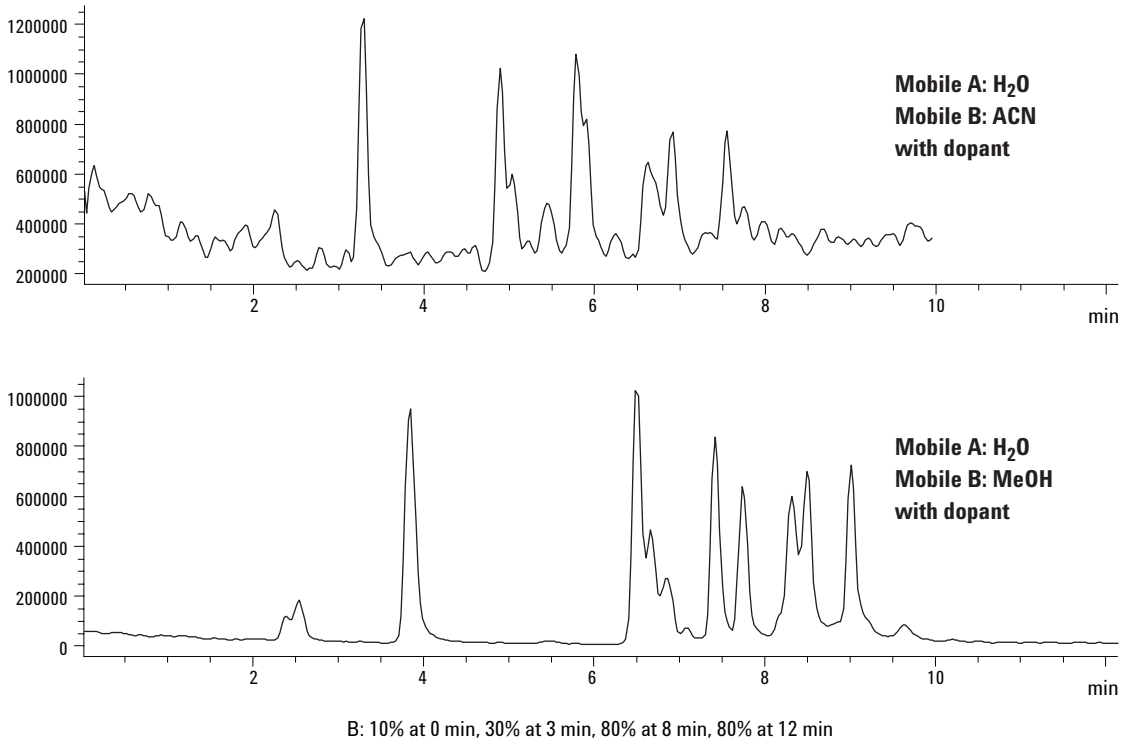


Figure 4. TICs show that methanol is a better mobile phase than acetonitrile in APPI. Acetone was used as dopant.

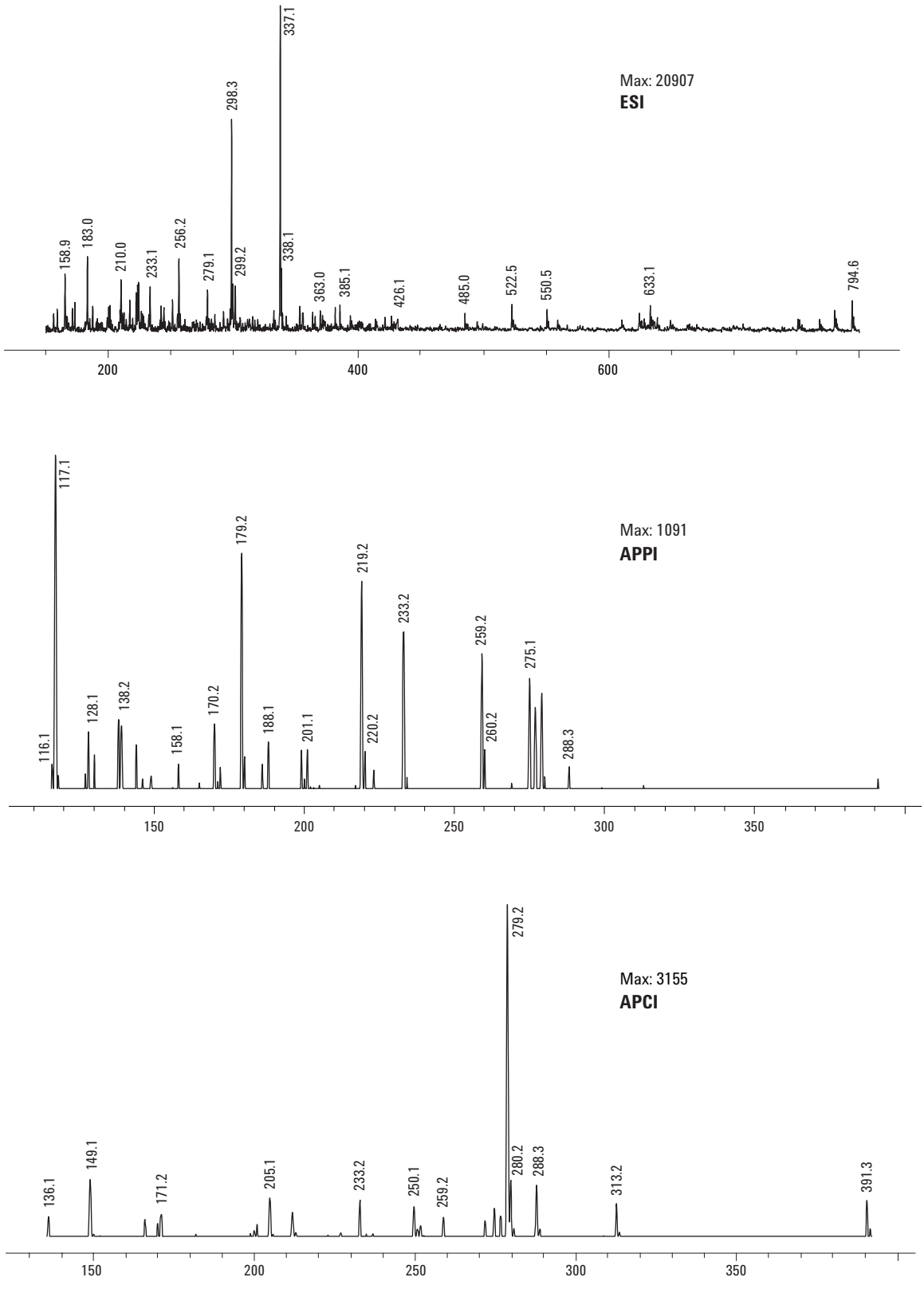
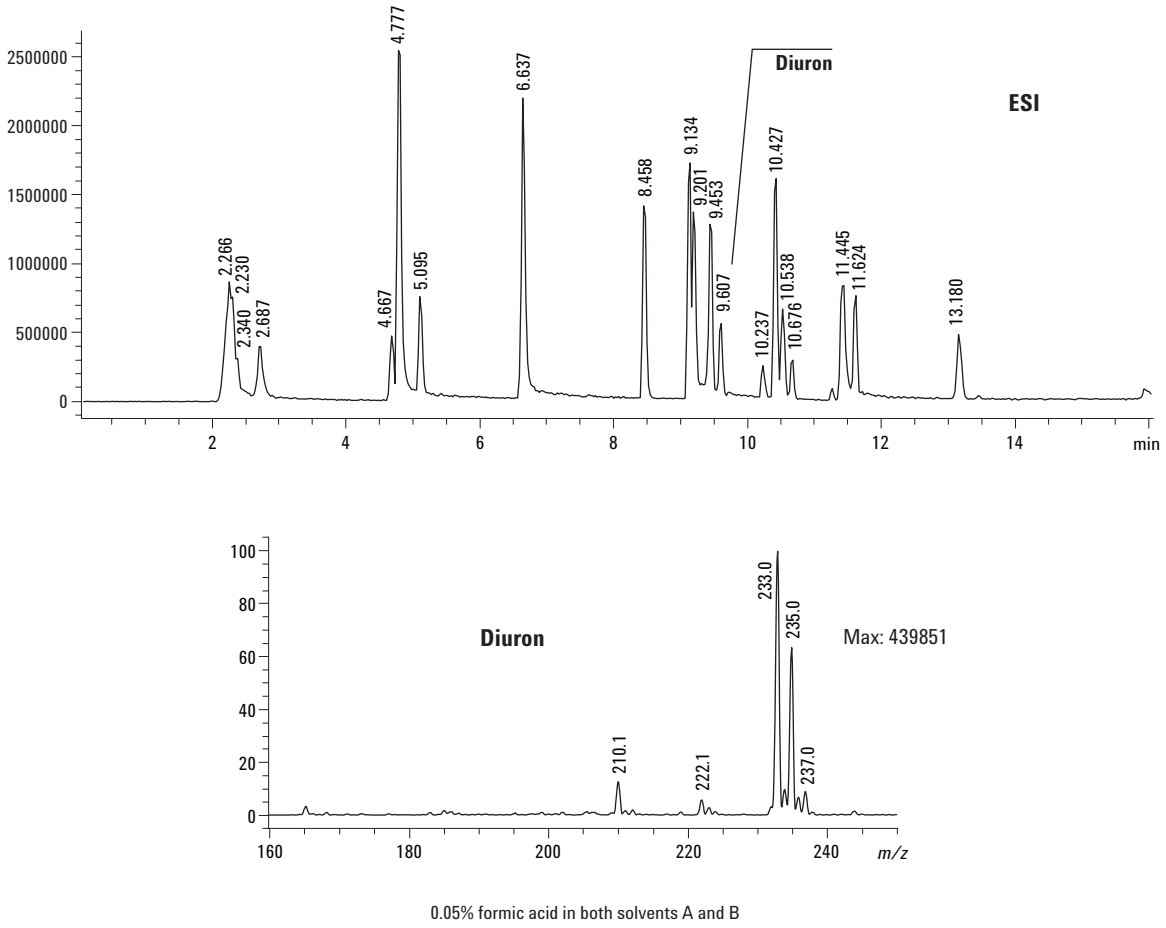


Figure 5. Baseline noise of the three sources are compared. ESI exhibits higher noise and more peaks in the baseline.

The spectra of 20 ng Diuron on column from the three sources (ESI, APCI, and APPI) appear in Figures 6, 7, and 8. The peak at mass 233 is the $[M+H]^+$ peak. Peaks at masses 233, 235, and 237 match the isotope peak-intensity pattern for two chlorine atoms, which is additional information for compound confirmation. Although ESI gives better response compared to the other two sources, it has lower signal-to-noise ratio (S/N).



0.05% formic acid in both solvents A and B

Figure 6. The TIC of all target compounds from ESI. The bottom half is the ESI spectrum of 20 ng Diuron on column.

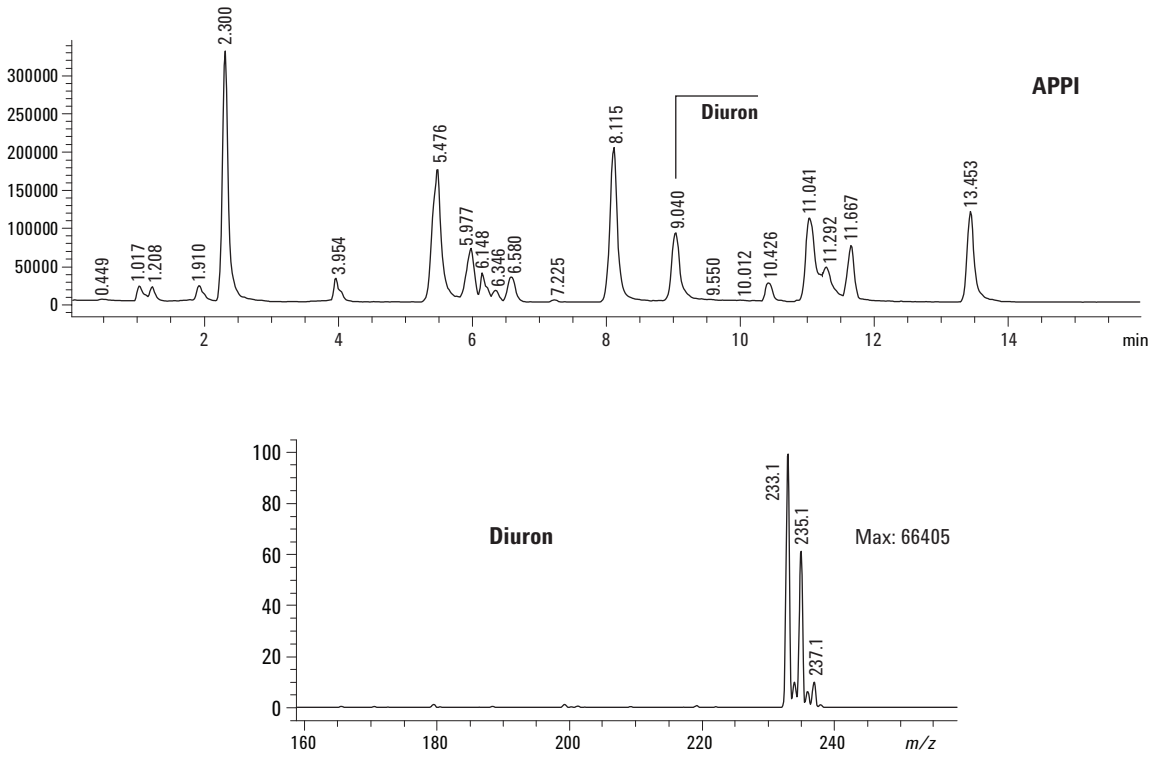


Figure 7. The TIC of all target compounds from APPI. The bottom half is the APPI spectrum of 20 ng Diuron on column.

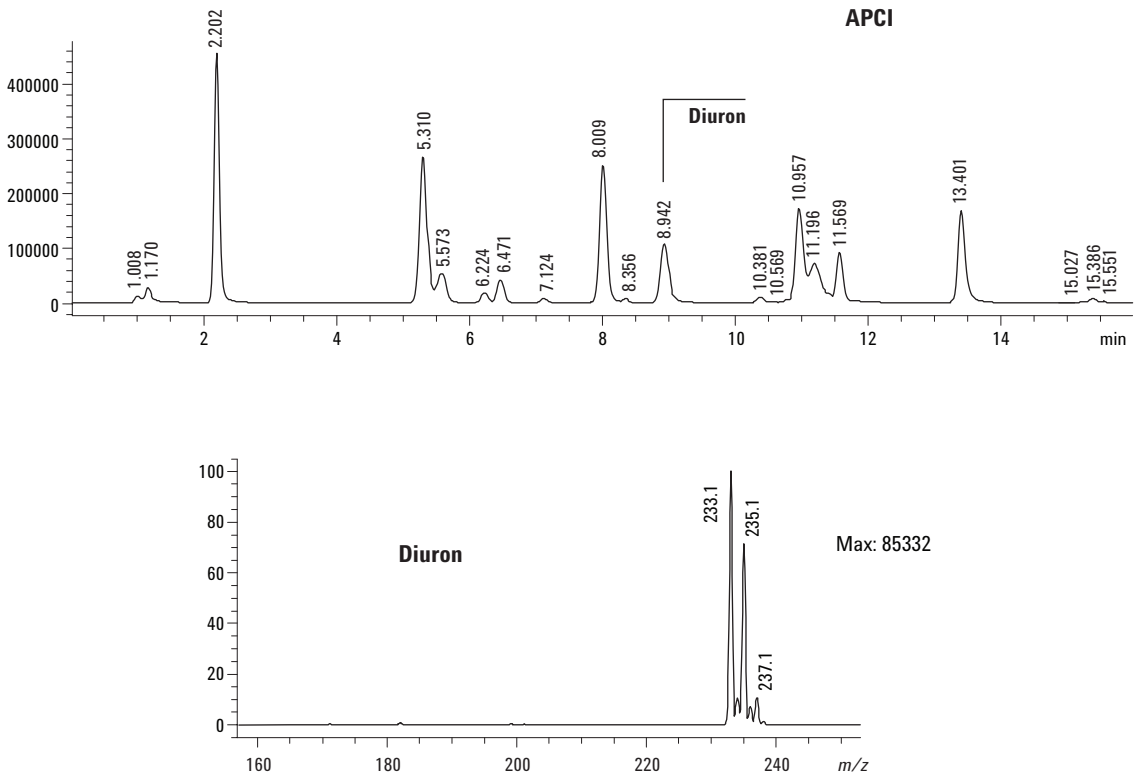


Figure 8. The TIC of all target compounds from APCI. The bottom half is the APCI spectrum of 20 ng Diuron on column.

Figure 9 shows the spectra of Siduron (protonated ion at *m/z* 233) from all three sources. Protonated dimer (*m/z* 465) and sodiated dimer (*m/z* 487) of Siduron are fairly significant peaks in the ESI spectrum. However, only negligible amounts of protonated dimers (no sodiated dimers) were observed in the APPI and APCI spectra. This is mainly due to the different sequence of desolvation and ionization steps. In ESI, ionization happens before desolvation. In APPI and APCI, ionization comes after desolvation.

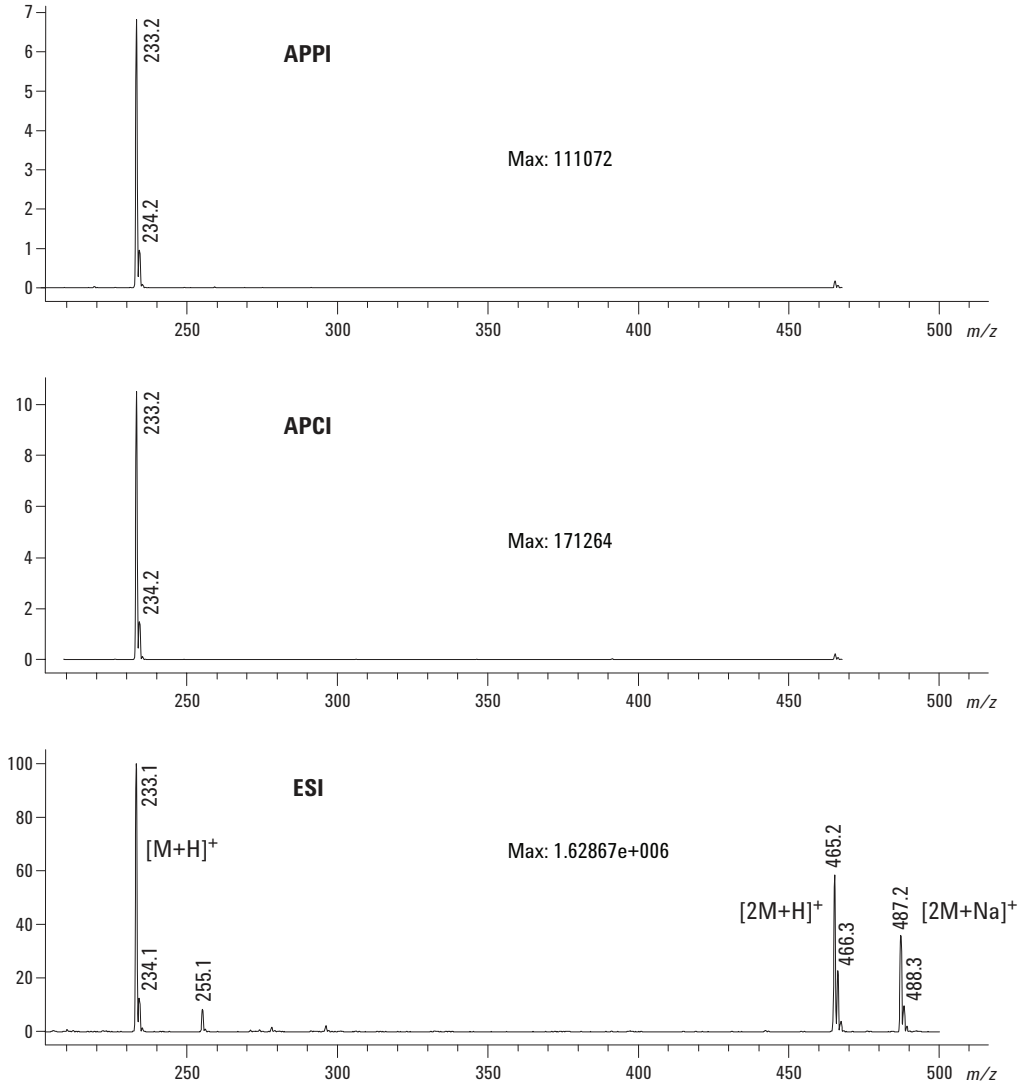


Figure 9. Spectra of Siduron from APPI, APCI, and ESI. Significant dimer peaks are observed in ESI.

Figure 10 compares the S/N of some of the target compounds analyzed by the three sources. The figure shows that carbamates, in general, give better S/N from ESI than from APCI or APPI. However, it is just the opposite for phenyl ureas, for example, the S/N is better from APCI or APPI than from ESI. It is also worth noting that APPI shows consistently higher S/N than APCI for this study.

A calibration based on the Methomyl $[M+H]^+$ (*m/z* 163) is linear over the concentration range of 20–2000 pg on column. Figure 11 shows the ESI calibration curve with the linear correlation coefficient of 0.9996. Four of the Methomyl peaks that were used for the linearity calibration appear in Figure 12.

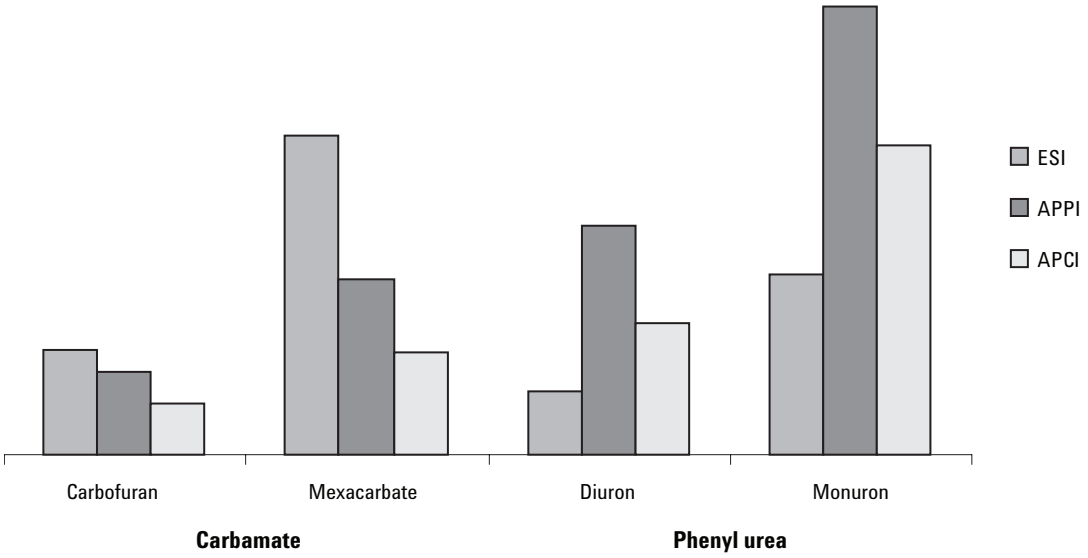


Figure 10. The S/N of four analytes from the three ionization modes.

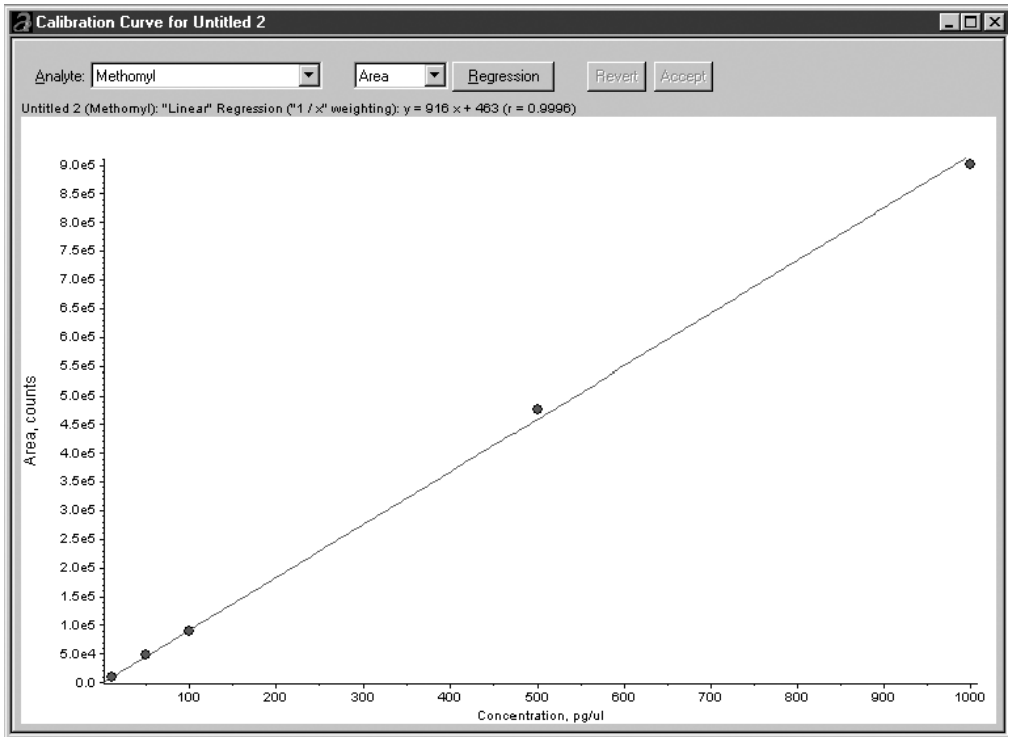


Figure 11. The calibration curve of Methomyl, by ESI in SIM mode (mass 163), shows good linearity over the concentration range of 20–2000 pg on column (2-µL injection).[1]

[1]The calibration curve was generated using the PE Sciex Analyst™ software.

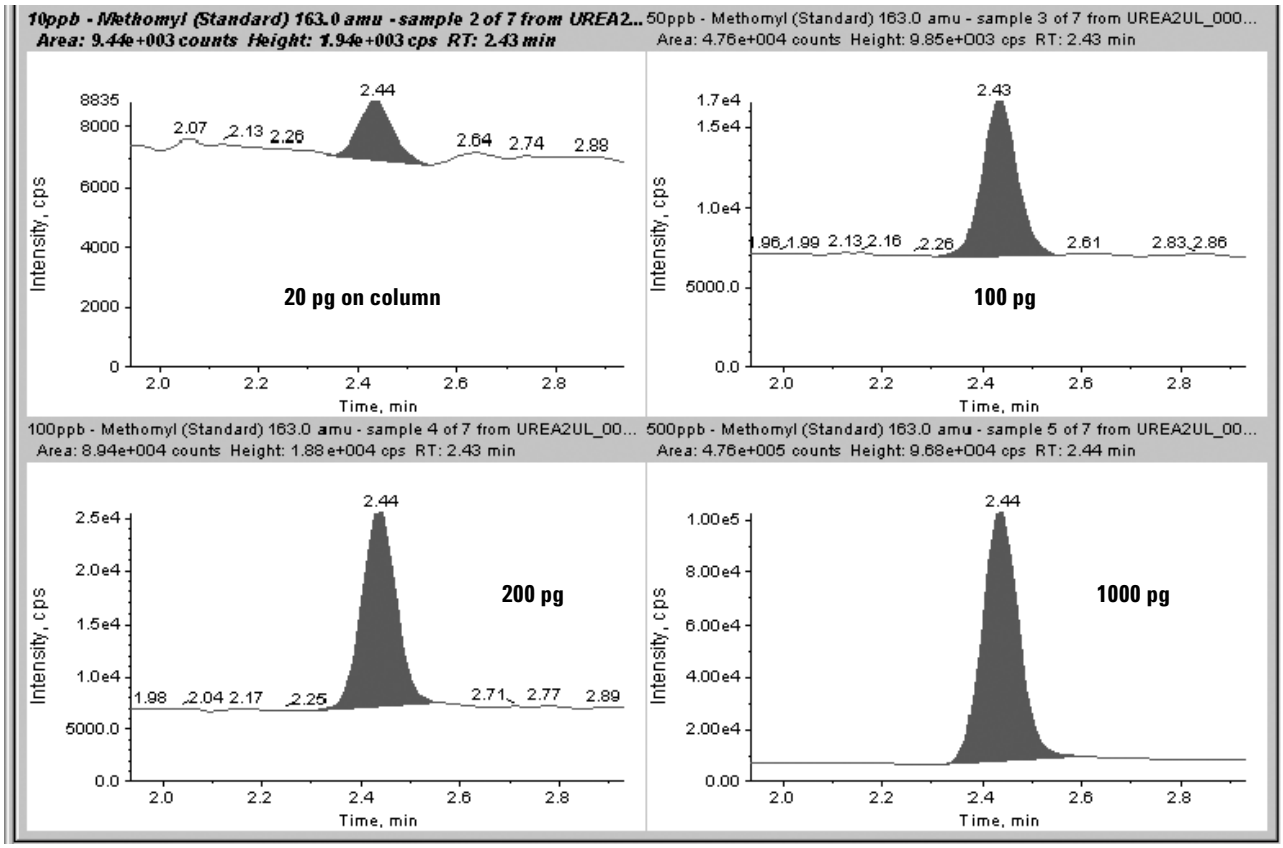


Figure 12. The Analyst™ software shows four of the Methomyl peaks (163 amu) that were used for generating the calibration curve in Figure 11.

Three different modifiers (formic acid, ammonium acetate, and acetic acid) were used in the ESI mode to compare their effectiveness. The results in Figure 13 show that, in general, the peak intensities are comparable among three modifiers. However, the elution order of the peak Mexacarbate (as well as Aminocarb) was different. Therefore, a single selected-ion-monitoring (SIM) method should not be used for different modifiers.

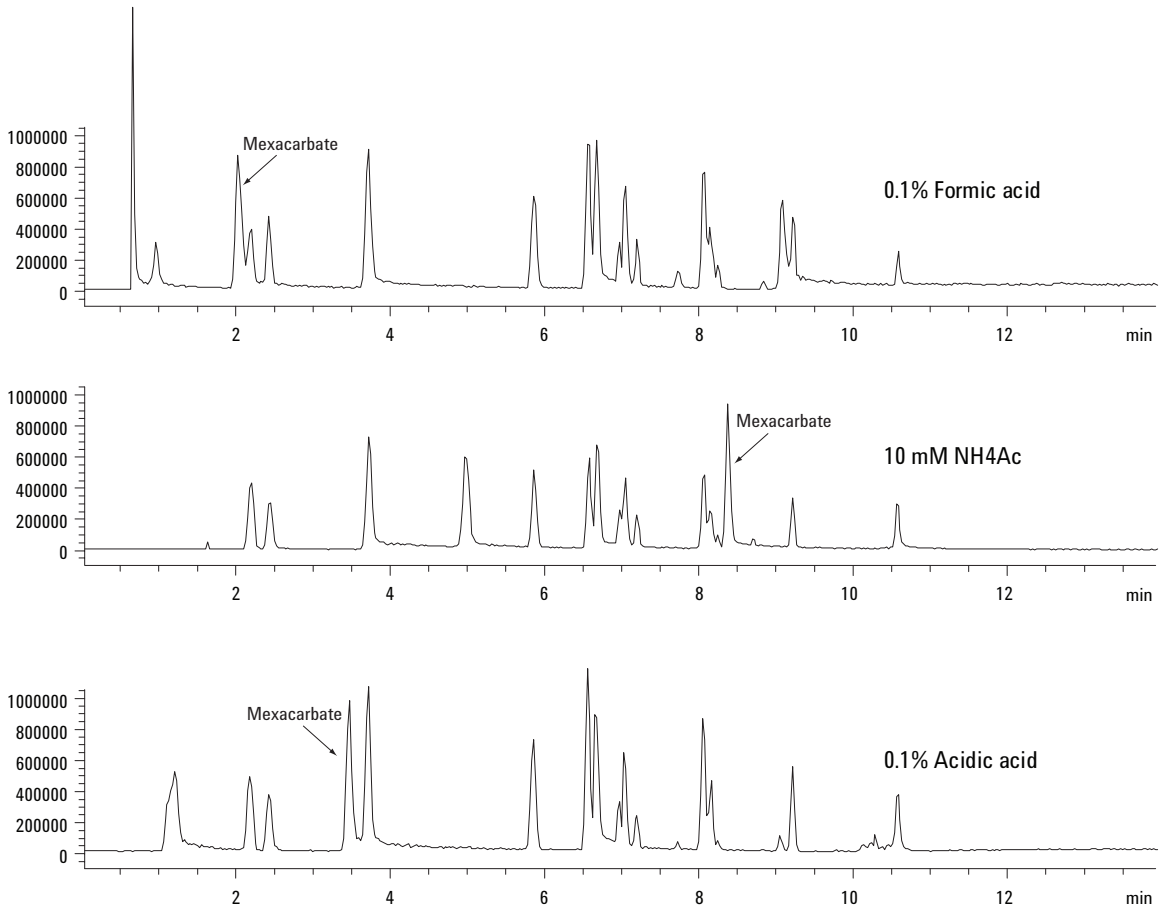


Figure 13. Different modifiers in ESI resulted in comparable peak intensities but changed the peak elution order.

Conclusions

Carbamates and phenyl ureas were successfully analyzed using ESI, APCI, and APPI sources. APPI and APCI have lower background signals and fewer background peaks than ESI. The results show that carbamates, in general, give better S/N from ESI than from APCI or APPI. However, it is just the opposite for phenyl ureas, for example, the S/N is better from APCI or APPI than from ESI. A small amount of post-column acetone was needed in the APPI source as a dopant. The dopant is photoionizable and is used to transfer charge to the analyte.

The typical ESI quantitation limit for these compounds is 20 pg on column using SIM. Some carbamates and phenyl ureas (for example, Propoxur, Carbofuran, and Siduron) exhibited protonated and sodiated dimers in the full scan ESI spectra.

References

1. "Basics of LC/MS," Agilent Technologies Primer, Publication 5988-2045EN, February 15, 2001.
2. "PhotoMate Atmospheric Pressure Photoionization Source from Syagen Technology," Agilent Technologies Brochure, Publication 5988-3130EN, May 25, 2001.

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Printed in the USA
May 24, 2002
5988-6635EN

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Analysis of the Active Compound in an Agricultural Fungicide Formulation by Liquid Chromatography

Application

Agricultural, Speciality Chemical, Environmental, Ag Chem

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USA

Abstract

Liquid chromatography was shown to be an excellent tool for the routine analysis of the active ingredient in an agricultural fungicide formulation. No extensive sample preparation or cleanup was required to remove the active ingredient from the rest of the sample matrix. The active ingredient was easily separated and detected using conventional reverse-phase conditions with a UV/VIS detector.

Introduction

Agricultural chemical formulations usually contain an active ingredient and several inert components, such as surfactants, that are designed to enhance the efficacy of the product. Gas chromatographic analysis of these formulations cannot be performed due to the polarity or thermal instability of the active ingredient as well as the high molecular weight and polarity of the surfactants. Therefore, liquid chromatography offers the best solution for the routine analysis of the active ingredients in an agricultural formulation.

This work was done on a commercially available fungicide formulation. The active ingredient in this product is 6.5 % (wt) of N,N-[1,4-piperazinediylbis (2,2,2-trichloroethylidene)] bisformamide. This is also known as triforine (CAS registry number 26644-46-2) and the structure is shown in Figure 1. The "inactive" ingredients in this formulation are listed as cyclohexanone, N-methyl pyrrolidone and Atlox 3406-F. The Atlox 3406-F is an agricultural dispersant that contains ionic and nonionic surfactants and mixed aromatic solvents. Electrospray ionization liquid chromatography/mass spectrometry (LC/MS) analysis has shown that the triforine can be easily separated and identified in the formulation [1].

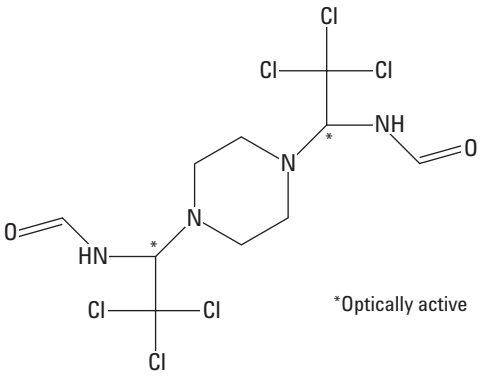

Figure 1. Chemical structure of triforine, the active ingredient in some commercial fungicide formulations.

Experimental

A 10% (v/v) solution of the fungicide formulation was made in acetonitrile. This solution was run on the Agilent 1100 Series LC System. This system included a vacuum degasser, a binary pump, an autoinjector, a thermostated column compartment, and a diode array UV/VIS detector. LC instrument conditions for this analysis are shown in Table 1.

Table 1. LC Analysis Conditions

Liquid chromatograph conditions	
Column:	Zorbax® XDB-C8,150 × 4.6 mm, 5 µm (p/n 993967-906)
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	Acetonitrile
Mobile phase gradient:	30% B at 0 min, 50% B at 7 min, 95% B at 10 min
Flow rate:	1.0 mL/min
Injection volume:	1 mL
Column temperature:	30 °C
Detector:	Diode array
Signal wavelength:	254 nm
Signal bandwidth:	10 nm
Reference wavelength:	500 nm
Reference bandwidth:	40 nm

Results and Discussion

Figure 2 shows the chromatogram of the fungicide formulation. The active ingredient in the formulation, triforine, elutes as two chromatographic peaks between 7.5 minutes and 7.8 minutes. The presence of two triforine peaks is due to the stereochemistry of the structure. Figure 3 shows the four triforine stereoisomers. These four configurations can be grouped into two pairs of mirror images that are diastereoisomers. The S,R and R,S configurations are mirror images that are superimposable, resulting in a meso compound that exhibits no optical activity or differences in physical properties. Therefore, because the S,R and R,S configurations are identical, they will elute as one chromatographic peak. The second pair of mirror images are the R,R and S,S configurations. These are not superimposable and are, therefore, enatiomers that will have different optical activity, but identical physical properties. Conventional reverse-phase liquid chromatography cannot separate these enantiomers, and they will co-elute as a single peak. However, these enantiomers can be separated from the meso compound by reverse-phase LC. This is why there are two triforine peaks, one for the meso compound and one for the enatiomers. Without pure standards of the stereoisomers, it is not possible to determine which configurations can be attributed to the observed chromatographic peaks.

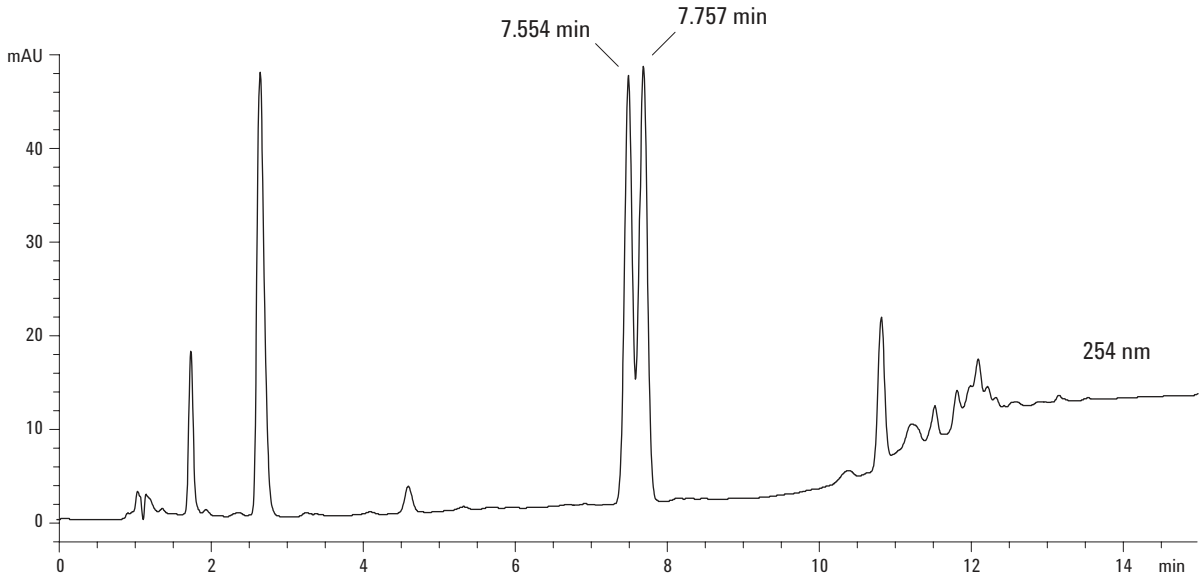

Figure 2. LC of an agricultural fungicide formulation containing the active ingredient triforine. The two peaks at 7.554 min and 7.757 min were shown to be optical isomers of triforine.

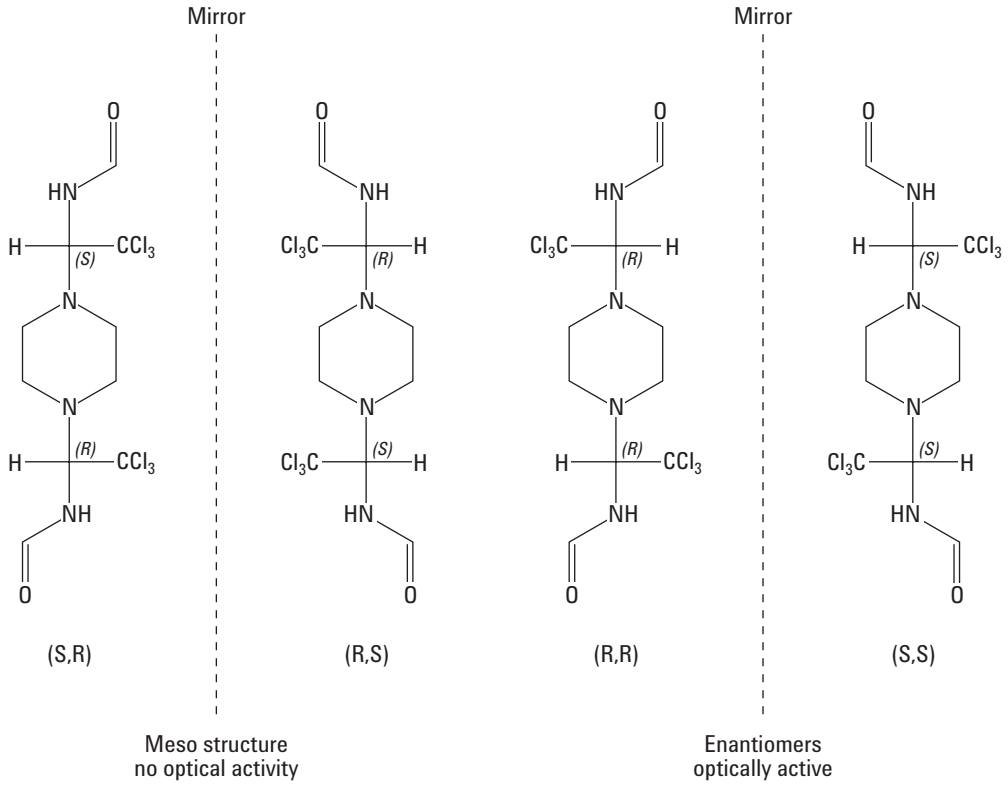

Figure 3. The four triforine stereoisomers arising from the two chiral carbons in the structure. These two pairs of mirror images account for the two triforine peaks observed in the chromatogram.

Conclusions

Liquid chromatography was shown to be an excellent tool for the routine analysis of the active ingredient in an agricultural fungicide formulation. No extensive sample preparation or cleanup was required to remove the active ingredient from the rest of the sample matrix. The active ingredient was easily separated and detected using conventional reverse-phase conditions with a UV/VIS detector.

References

1. McCurry, J.D., and Zavitsanos, P., "Analysis of Components, Contaminants, and Impurities in Fungicide Chemical Formulations by GC/MS and LC/MS," Agilent Technologies Application Note, Publication 5988-6085EN, April 2002.

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Printed in the USA
May 14, 2002
5988-6686EN

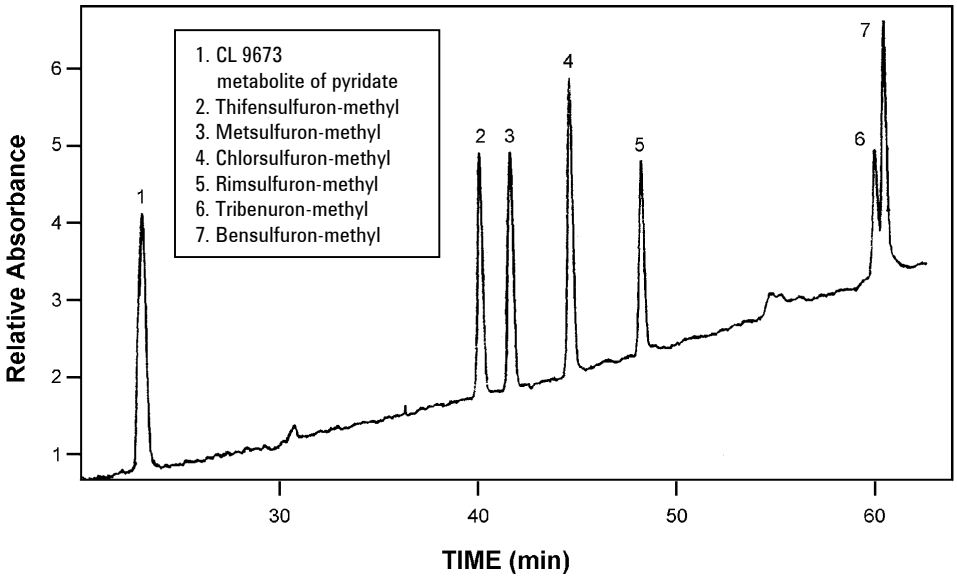
Agilent Technologies

High-Resolution Separation of Sulfonylurea Pesticides

Application

Agrichemical

Robert Ricker



Courtesy of Dr. rer.nat. Claus Schlett, Gelsenwasser AG

Highlights

- *An example of excellent selectivity and peakshape for a new family of pesticides.*
- *ZORBAX SB-C18 has a sterically protected, bonded phase that permits reliable results run-after-run.*

Conditions:
Column: ZORBAX SB-C18, 3.0 x 250 mm (Agilent Part No. 880975-302)
Mobile Phase:
A 0.01% Acetic Acid in H2O
B Acetonitrile, 0.01% Acetic Acid
Inj. Vol.: 50µl UV: (230, 270 nm); Flow: 0.5 mL / min.; 40°C

Gradient Time	%A	%B
2	90	10
70	55	45
85	55	45
89	10	90
94	10	90
95	90	10
110	90	10

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Sample preparation

The sulfonylureas are extracted from water as follows:

1. *The samples (1L) are filtered through a glass-fiber filter and are brought to a pH of 3-4 using hydrochloric acid. Then, 10 ml of methanol are added.*
2. *Solid-phase extraction is carried out using 2 g of sorbent.*
3. *The cartridges are conditioned with 6 bed-volumes of H_2O (adjusted to pH 3-4) followed by 6 bed-volumes of methanol.*
4. *The samples are passed through the cartridge at a rate not exceeding 500 ml/hr.*
5. *The cartridges are dried for 45 min with nitrogen gas at a rate of 90 ml/min.*
6. *The samples are eluted from the extraction column using acetone (3 washes of 3 ml each).*
7. *The acetone is carefully evaporated from the eluted sample, and the sample re-dissolved in 100µl acetonitrile, 400µl H_2O, 0.01% acetic acid.*

Robert Ricker is an application chemist based at Agilent Technologies, Wilmington, Delaware.

ACKNOWLEDGMENT

Agilent Technologies Inc. wishes to thank Dr. rer.nat. Claus Schlett, Gelsenwasser AG, who developed the method and provided this chromatogram.

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Printed in the USA
April 25, 2002
5988-6287EN

Agilent Technologies

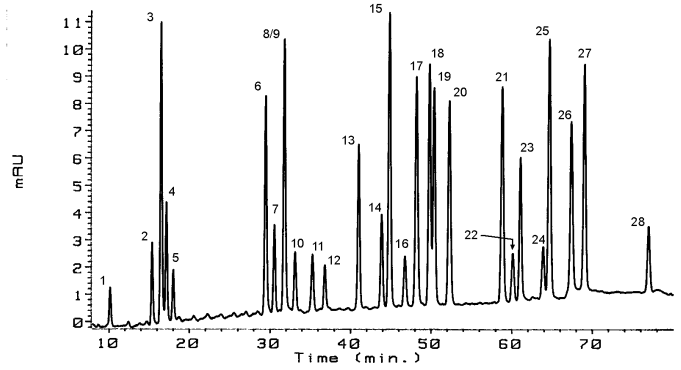
Pesticides
Analysis of Pesticides in Drinking Water

Application

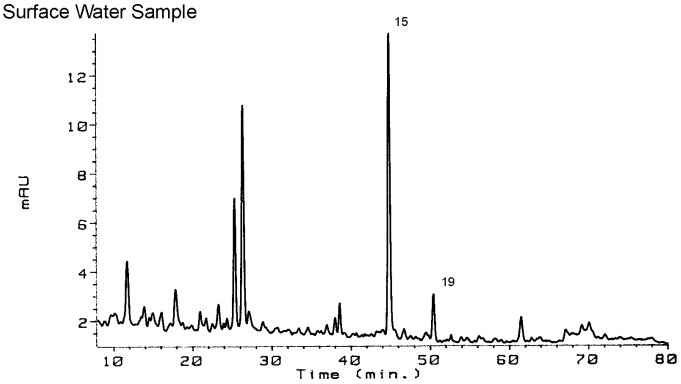
Environmental

Robert Ricker

Standard (500ng/L)



Surface Water Sample



Courtesy of Dr. rer.nat. Claus Schlett, Gelsenwasser AG

1. Desisopropylatrazine
2. Metamitron
3. Fenuron
4. Chloridazon
5. Desethylatrazine
6. Metoxuron
7. Carbetamid
8. Bromacil
9. Hexazinon
10. Simazine
11. Metribuzin
12. Desethylterbutylazine
13. Carbutilat
14. Methabenzthiazuron
15. Chlortoluron
16. Atrazine
17. Monolinuron
18. Diuron
19. Isoproturon
20. Metobromuron
21. Metazachlor
22. Buturon
23. Propazine
24. Dimefuron
25. Terbuthylazine
26. Linuron
27. Chlorbromuron
28. Chloroxuron

Highlights

- *The 3mm-diameter ZORBAX Low-Volume Columns offer significant advantages over standard 4.6 mm i.d. columns:*

  - A 2-fold increase in detection sensitivity -- less sample required
  - A 50% solvent savings -- and reduced solvent-disposal costs

- *ZORBAX SB-C18 has a sterically protected bonded phase that permits reliable results run after run.*

- *28 pesticides are separated with good resolution and peak shape in a single run using simple mobile phases.*

Conditions:
ZORBAX SB-C18 (3.0 x 250 mm) (Agilent P/N: 880975-302)
Mobile Phase: A=2mM Sodium Acetate (pH 6.5) with 5% ACN
B=100% Acetonitrile (ACN)
Gradient Elution: 2min, 10% B; 10 to 45% B in 70 min.
Injection volume 25µl, 0.35 mL/min, 40°C, Detect. UV (245 nm)

Agilent Technologies

SUMMARY

A variety of pesticides have had extensive use in many countries around the world over the last twenty years. These chemicals are currently present in surface water in very low concentrations, and need to be analyzed. High-Performance Liquid Chromatography with diode-array detection is an excellent tool for analysis of these compounds.

TECHNICAL DETAILS

Drinking-water regulations have been developed in many locations that set limits for maximum allowable levels of pesticides. A reliable method of analysis is required to monitor these levels, preferably in a single run. HPLC using diode-array detection after solid-phase extraction can meet this need. Generally, substances can be detected in concentrations less than 0.1 mg/L (i.e., the maximum level set in the drinking-water regulation of Germany).

ACKNOWLEDGMENT

Agilent Technologies, Inc. wishes to thank Dr. rer.nat. Claus Schlett, Gelsenwasser AG who developed the method and provided this chromatogram.

Robert Ricker is an application chemist based at Agilent Technologies, Wilmington, Delaware.

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Printed in the USA
April 25, 2002
5988-6341EN

Agilent Technologies

Analysis of Components, Contaminants, and Impurities in Fungicide Formulations by GC/MS and LC/MS

Application

Agriculture, Specialty Chemical, Environmental, Ag Chem

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Abstract

A commercially available fungicide formulation was analyzed by both gas chromatography/mass spectrometry (GC/MS) and electrospray ionization liquid chromatography/mass spectrometry (ESI-LC/MS). The GC/MS analysis provided a detailed look at the volatile components in the formulation, but did not yield any results for the active ingredient, triforine. The ESI-LC/MS provided information on the stereoisomers of triforine as well as the nonvolatile surfactants and contaminants in the formulation. This paper demonstrates the complementary nature of these two analytical techniques when trying to fully characterize a complex chemical formulation containing a broad range of components.

Introduction

Gas chromatography/mass spectrometry (GC/MS) is an indispensable tool for solving complex problems in the chemical industry. This fast and powerful technique yields detailed information about the expected compounds in the mixture along with any unexpected impurities and breakdown products that can affect product quality. However GC/MS can only provide meaningful information for compounds that are volatile, nonionic, thermally stable, and have relatively low molecular weight. Liquid chromatography is much better suited to analyzing compounds that are nonvolatile, ionic, polar, thermally labile, or have high molecular weight. This includes about 80% of all known organic compounds [1]. When coupled with a modern atmospheric pressure ionization (API) mass spectrometer, LC/MS offers a complementary tool to GC/MS in the chemical diagnostic laboratory.

Commercial pest control formulations contain one or more active compounds along with a recipe of ingredients that can play an important role in the product's efficacy. These "inactive" ingredients are often a combination of solvents and surfactants that allow for easy application and dispersal of the active ingredient onto the target substrate. For this work, an over-the-counter fungicide formulation was purchased at a local home products store. The active ingredient in this product is 6.5 % (wt) of N,N-[1,4-piperazinediylbis(2,2,2-trichloroethylidene)] bisformamide. This is also known as triforine (CAS registry number 26644-46-2), and the structure is shown in Figure 1. The "inactive" ingredients in this formulation are listed as cyclohexanone, N-methyl pyrrolidone, and Atlox 3406-F. The Atlox 3406-F is an agricultural dispersant that contains ionic and nonionic surfactants and mixed aromatic solvents.

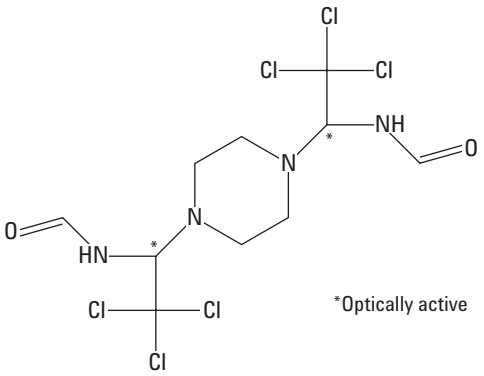

Figure 1. Chemical structure of triforine, the active ingredient in some commercial fungicides. The nominal molecular weight is 432, and the structure contains two optically active carbons.

A complete analysis of this formulation requires GC/MS to separate and identify the volatile components and LC/MS for the surfactants and polar components. Analysis of the active ingredient, triforine, presents a separate challenge. References for triforine analysis cite gas chromatography as the method of choice when analyzing environmental residues [2]. However, the melting point is reported to be 155 °C with decomposition, indicating that gas chromatography may only be possible with on-column injection.

Experimental

Gas Chromatography/Mass Spectrometry (GC/MS)

A 1% (v/v) solution of the triforine formulation was made in acetonitrile and the GC/MS analysis was performed with an Agilent 5973 GC/MS system. The components in this system were a 6890N gas chromatograph, a 7683 autoinjector, and a 5973 mass spectrometer. A cool-on-column inlet in the Agilent 6890 GC was used to avoid decomposition of the triforine. Instrument conditions for the GC/MS analysis are listed in Table 1.

Table I. GC/MS Analysis Conditions

Gas chromatograph conditions	
Column:	30 m × 0.25 mm HP5-MS, 0.25 µm (p/n 19091S-433)
Carrier gas:	Helium at 13.00 psi
Flow rate:	1.6 mL/min., constant flow mode
Inlet:	Cool on-column at 50 °C, oven track mode
Oven temperature program:	50 °C for 3 min 10 °C/min to 275 °C 275 °C for 4 min
MS Transfer line:	280 °C
Injection volume:	1 µL
Mass spectrometer conditions	
Electron multiplier:	1400 V
Solvent delay:	3 min
Scan range:	30 to 800 *m/z*
Scan threshold:	50 counts
A/D Samples:	2
Scan rate	1.95 scans/s

Electrospray Ionization Liquid Chromatography/Mass Spectrometry (ESI-LC/MS)

The same fungicide sample was run on the Agilent 1100 Series LC/MSD. This system included a vacuum degasser, a binary pump, an autoinjector, a thermostatted column compartment, and the LC/MSD SL quadrupole mass spectrometer. LC/MS instrument conditions for this analysis are shown in Table 2.

Results and Discussion

Gas Chromatography/Mass Spectrometry (GC/MS)

The complex nature of this fungicide formulation is revealed when one looks at the GC/MS data. Figure 2 shows the total ion chromatogram (TIC) of the fungicide sample. The volatile components

Table 2. LC/MS Analysis Conditions

Liquid chromatograph conditions	
Column:	150 × 4.6 mm Zorbax® XDB-C8, 5 µm (p/n 993967-906)
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in acetonitrile
Mobile phase gradient:	30% B at 0 min; 50% B at 7 min; 95% B at 10 min
Flow rate:	1.0 mL/min
Column temperature:	30 °C
Injection volume:	1 µL
Mass spectrometer conditions	
Source:	Electrospray
Drying gas flow:	12 L/min
Nebulizer:	40 psig
Drying gas temperature:	350 °C
V_{cap}:	3500 V (positive) and 3000 V (negative)
Stepsize:	0.1 amu
Peak width:	0.1 min
Time filter:	On
Scan range	120 to 1200 *m/z*
Fragmentor	Fixed at 60 V

in the formulation are easily identified from the mass spectral data. The major solvents, cyclohexanone and N-methyl-2-pyrrolidone, dominate the chromatogram while smaller amounts of C9 aromatics, C10 aromatics, and substituted napthalenes are easily separated and identified.

There were no peaks in the TIC whose spectra matched the triforine reference spectra from the Wiley mass spectral library. An extracted ion profile using the triforine base peak of 203 *m/z* did not produce any chromatographic peak indicating the presence of triforine. From this data, it appears that the triforine did not elute from the column into the mass spectrometer. However, a spectral average of the large hump between 18 and 20 minutes shows an isotope pattern indicating one chlorine atom (Figure 3A). Since no chlorine-containing species other than triforine are components in the formulation, the presence of chlorine and the broad peak shape indicates triforine decomposition in the gas chromatograph. The peak at approximately 20-minute retention time also has a mass spectrum containing an isotope pattern indicating the presence of two chlorine atoms in the structure (Figure 3B). This peak could be a decomposition product or a contaminant in the formulation.

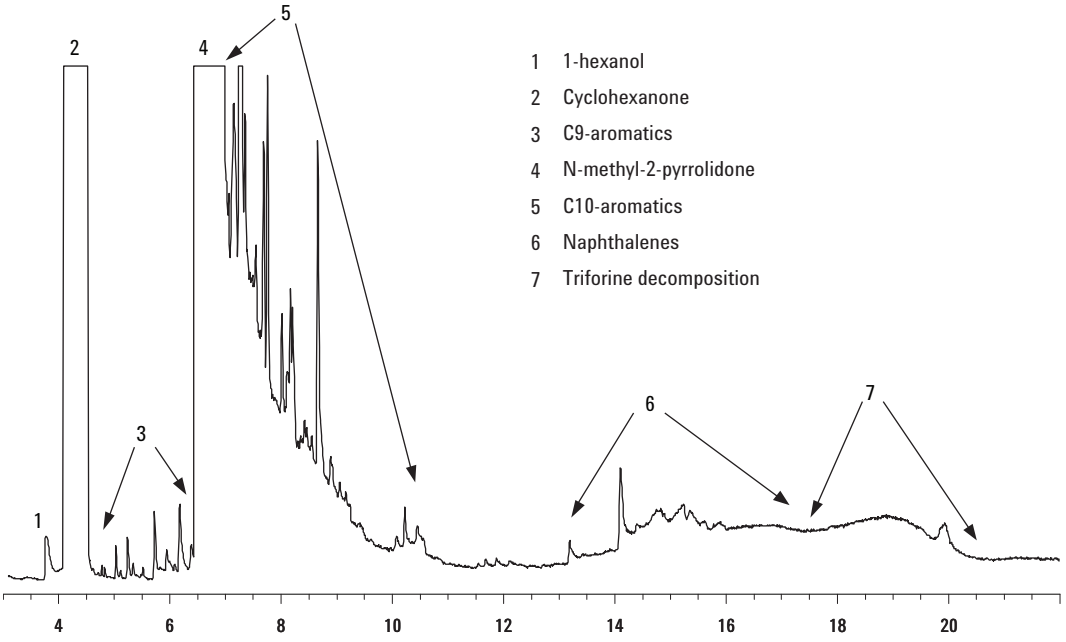

Figure 2. GC/MS TIC showing the complex volatile components in the commercial fungicide formulation.

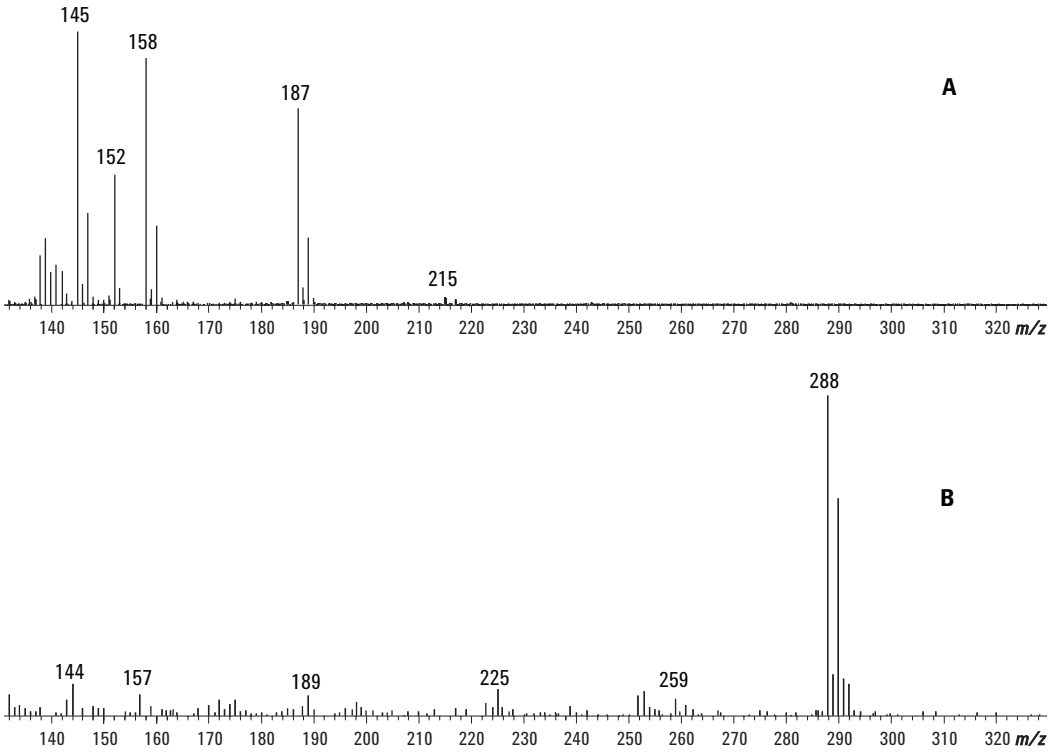

Figure 3. (A) Average mass spectrum of broad hump between 18 and 20 minutes of TIC. Isotope patterns of the peaks at *m/z* 145, 158 and 187 indicate the presence of one chlorine atom. (B) Mass spectrum of the peak at 20 minutes shows the presence of two chlorine atoms in the structure.

Electrospray Ionization Liquid Chromatography/Mass Spectrometry (ESI-LC/MS)

The positive ion ESI-LC/MS chromatogram is shown in Figure 4. Several major peaks are observed along with several minor components. The spectra of the three peaks eluting between 0 and 2 minutes are shown in Figure 5. Since electrospray is a "soft" ionization technique, these spectra do not exhibit the detailed fragmentation needed to interpret structures for these three compounds. However, peak number 2 does have an isotopic pattern indicating the presence of two chlorine atoms in the structure. This compound could be a contaminant related to triforine production or a triforine decomposition product. Figure 6 shows the spectra of the three peaks between 10.5 and 13 minutes. These compounds are the various surfactants that make up the agricultural dispersant used in the formulation.

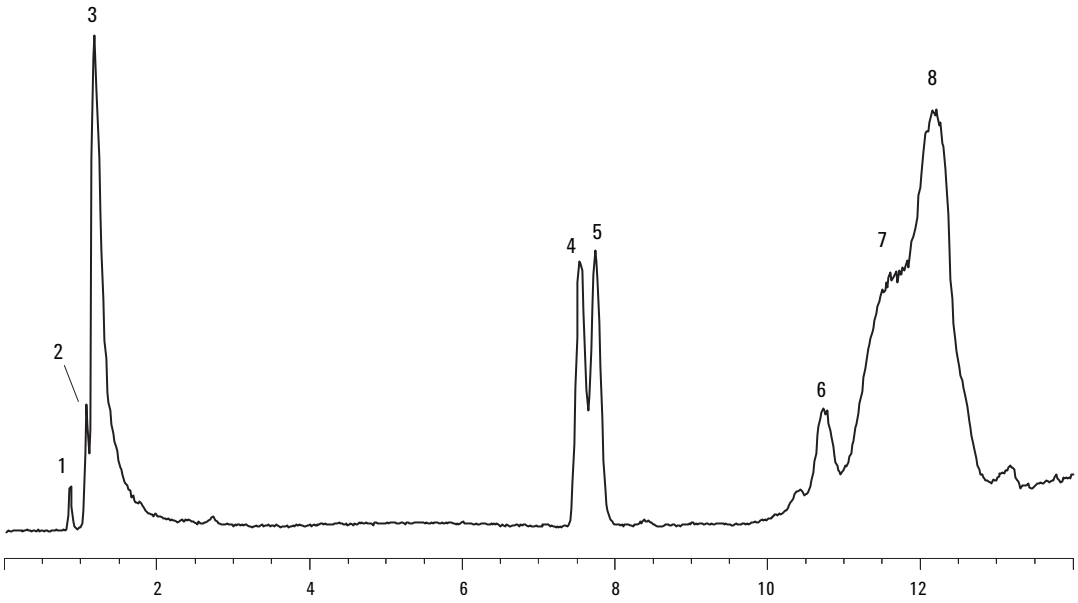

Figure 4. TIC from positive ion ESI-LC/MS of fungicide formulation.

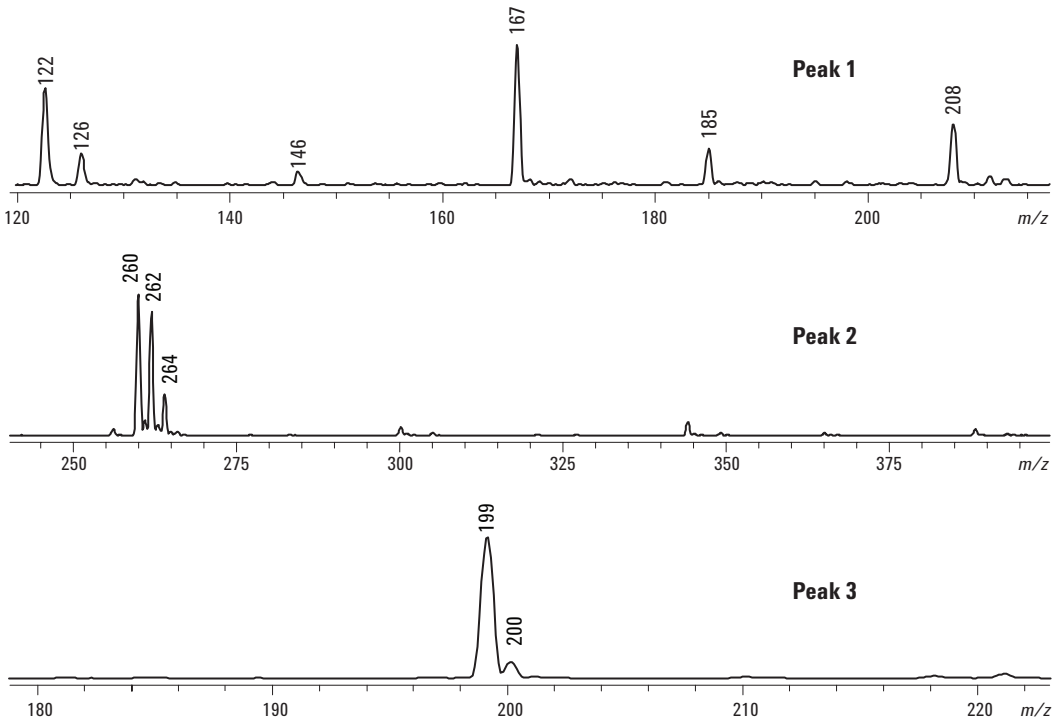

Figure 5. Electrospray spectra from LC/MS peaks 1, 2, and 3. The spectra from peak 2 shows an isotope pattern indicating two chlorine atoms in this structure. This compound may be a contaminant in the formulation from the active ingredient triforine.

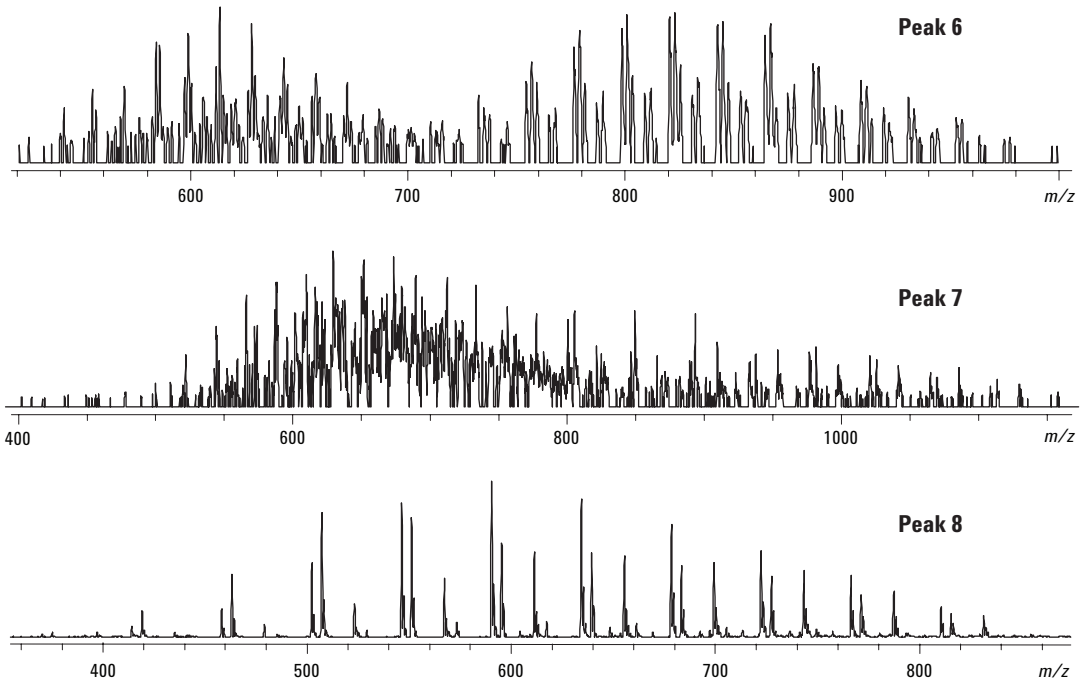

Figure 6. Electrospray mass spectra of LC/MS peaks 6, 7, and 8 from Figure 4. These compounds are the surfactants used in the formulation.

The spectra of LC/MS peaks 4 and 5 (Figure 7) are identical and correspond to the active ingredient, triforine. The protonated molecular ion is observed at *m/z* 433 along a sodium adduct at *m/z* 455. The multiplets for *m/z* 433 to 439 and *m/z* 455 to 461 exhibit an isotopic pattern consistent with six chlorine atoms. The ion at *m/z* 388 is due to a rearrangement and subsequent loss of a formamide group from the protonated molecular ion (*m/z* 433). This is also confirmed by the isotopic pattern indicating six chlorine atoms (*m/z* 388 to 396).

The presence of two triforine peaks in Figure 4 can be explained by the stereochemistry of the structure. Triforine contains two optically active carbons that give rise to four stereoisomers. Figure 8 shows the four configurations that can be grouped into two pairs of mirror images that are diastereomers. The S,R and R,S configurations are mirror images that are superimposable, resulting in a meso compound that exhibits no optical activity or differences in physical properties. Therefore, because the S,R and R,S configurations are identical, they will elute as one chromatographic peak. The second pair of mirror images are the R,R and S,S configurations. These are not superimposable and are, therefore, enatiomers that will exhibit different optical activity, but identical physical properties. Conventional reverse-phase liquid chromatography cannot separate these enantiomers, and they will co-elute as a single peak. However, these enantiomers are not mirror images of the meso compound and can be chromatographically separated from the meso compound. This is why there are two triforine peaks, one for the meso compound and one for the enatiomers. Without pure standards of the stereoisomers, it is not possible to determine which configurations can be attributed to the observed chromatographic peaks.

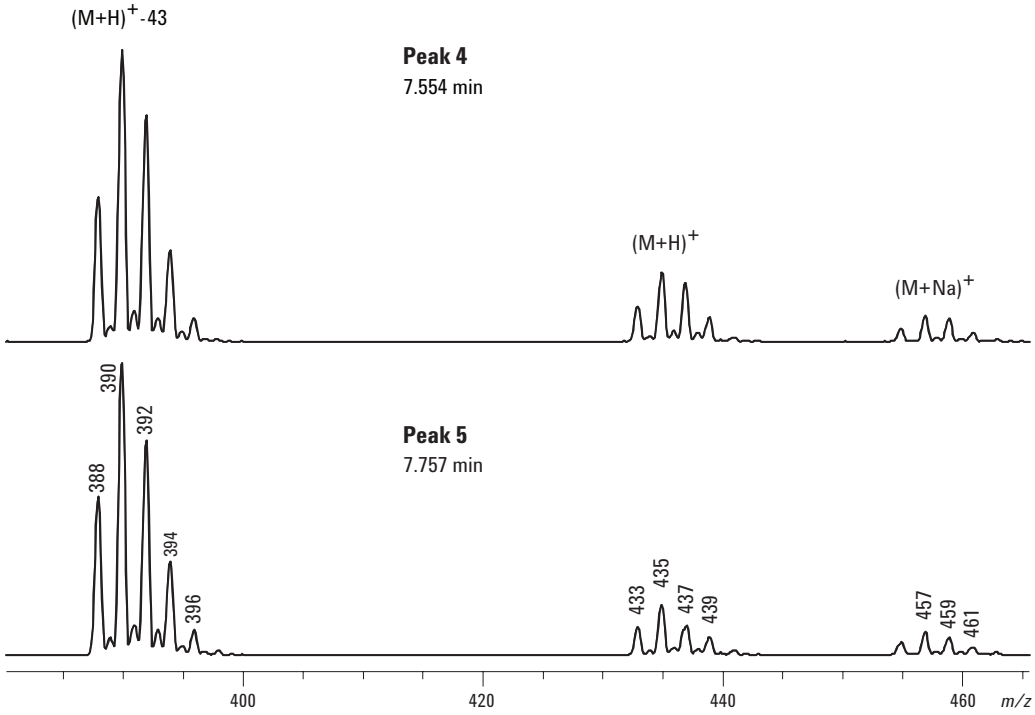

Figure 7. Electrospray mass spectra of peaks 4 and 5 from Figure 4. Both spectra show a protonated molecular ion at *m/z* 433 representing the active ingredient triforine. There is also a sodium adduct (*m/z* 455) of triforine observed for both peaks. A rearrangement and loss of a formamide group from the protonated molecular ions give rise to the multiplet at *m/z* 388 to 396.

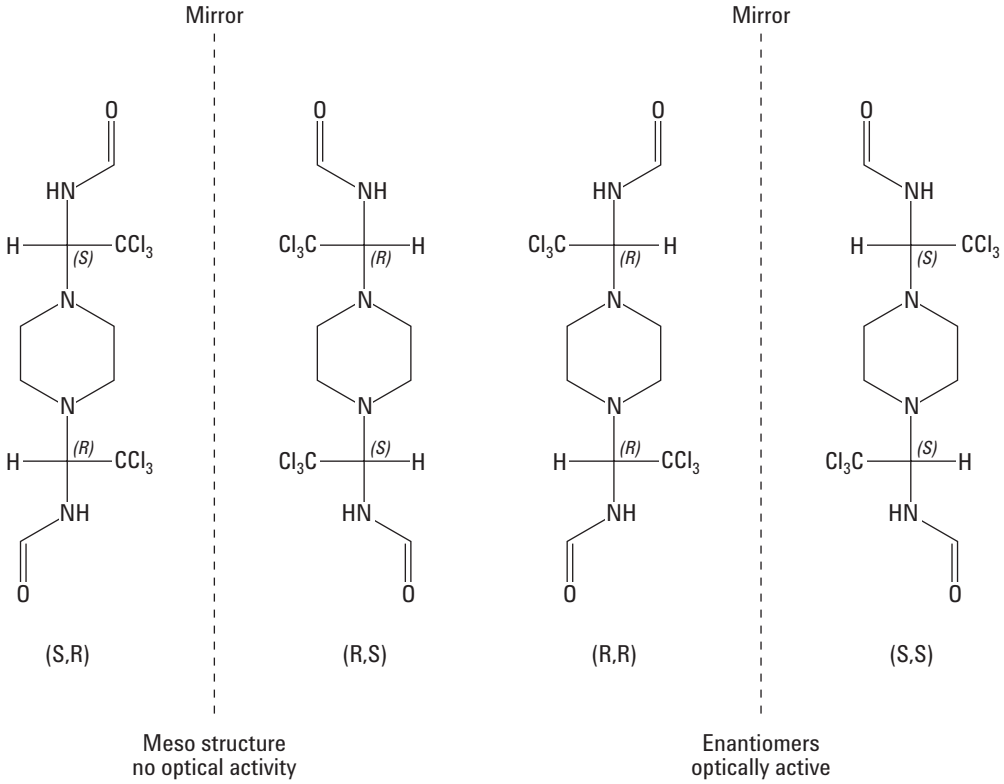

Figure 8. The four triforine stereoisomers arising from the two chiral carbons in the structure. These two pairs of mirror images account for the two triforine peaks observed in the chromatogram (Figure 4).

The fungicide formulation was also run by ESI-LC/MS in the negative ion mode. The results of this analysis are shown in Figure 9. The negative ion mass spectra for these two peaks are shown in Figure 10. For both triforine peaks, the most stable negative ion species is the chloride adduct (*m/z* 467). However, the spectra for the first peak contains a deprotonated molecular ion (*m/z* 431) and a formate adduct (*m/z* 477) that is not observed in the spectra of the later eluting peak. This selective adduct formation is likely related to the stereochemstry of the triforine, but again, without pure standards, the correct configurations cannot be assigned to the chromatographic peaks.

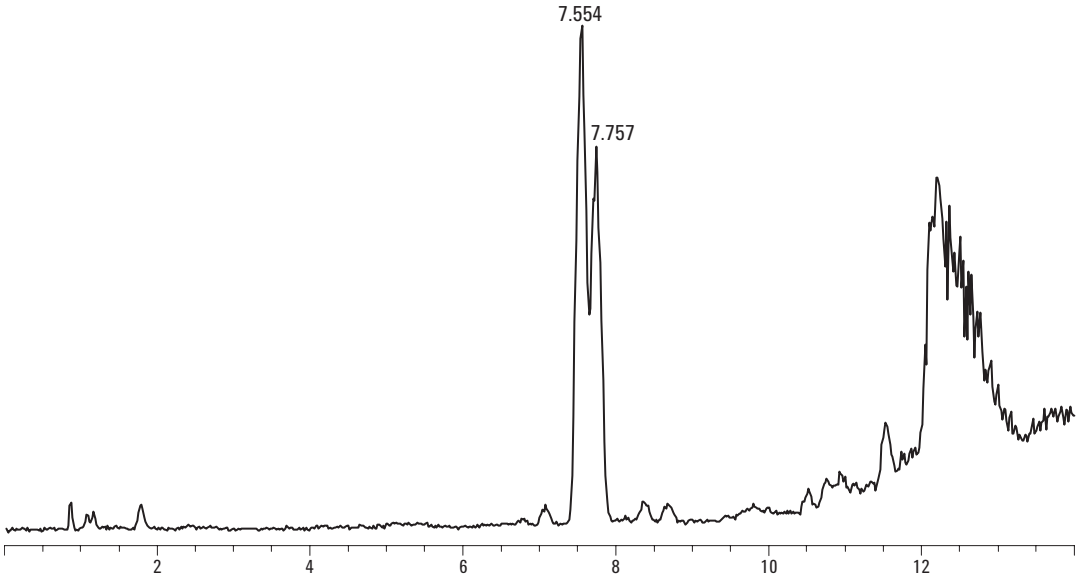

Figure 9. TIC from negative ion ESI-LC/MS of fungicide formulation.

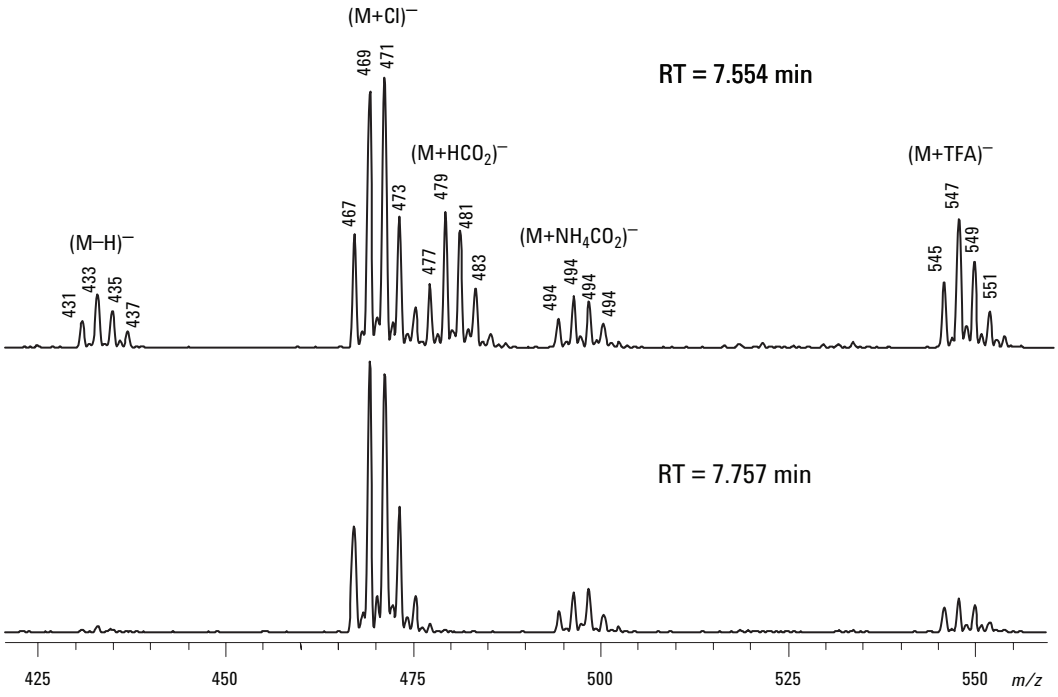

Figure 10. Negative ion electrospray mass spectra of the two triforine peaks. The spectra from peak at 7.554 minutes shows a deprotonated molecular ion (*m/z* 431) and a formate adduct (*m/z* 477) that is not seen in the later eluting peak (7.757 minutes).

Conclusions

This paper demonstrates the complimentary nature of GC/MS and LC/MS when trying to characterize a formulation that is composed of many different chemical species. The volatile compounds in the formulation can be easily separated and identified by GC/MS. In this case, polar solvents such as cyclohexanone and N-methyl-2-pyrrolidone were the major components while 1-hexanone, C9 aromatics, C10 aromatics, and substituted naphthalenes were present as minor components or contaminants. However, GC/MS did not yield any information on the active fungicidal ingredient, triforine, a hexachlorinated compound. This was most likely due to thermal decomposition during GC/MS analysis. Evidence for this was seen in a broad chromatographic hump containing chlorine-containing constituents.

The nonvolatile components in this fungicide were quickly analyzed by ESI-LC/MS. This analysis yielded information on several polar contaminants, some containing chlorine, which may be by-products of triforine production or triforine breakdown products. Also observed were several surfactants that are used in agricultural products as dispersants. The LC/MS analysis did yield significant information on the triforine active ingredient, showing a distribution of stereoisomers in the formulation.

References

1. Willoughby, R., Sheehan, E, and Mitrovich, S. A., Global View of LC/MS: How to Solve your Most Challenging Problems, p. 80, Global View Publishing, 1998.
2. The Pesticide Manual 9th Edition, Worthing, C.R. and Hance R.J. eds., p. 853, The British Crop Protection Council, 1991.

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Printed in the USA
April 24, 2002
5988-6085EN

Agilent Technologies

Determination of Acidic Herbicides in Groundwater and Potable Water by LC/MSD Using Selective Ion Monitoring

Application

Environmental

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Abstract

A validated liquid chromatography/mass spectrometry method for acidic herbicides in groundwater and potable water is described using atmospheric pressure electrospray ionisation in negative ion mode and selective ion monitoring. No derivatisation step is required and only small volumes of sample are used. Detection levels of 10 to 20% of the Prescribed Concentration or Value for an individual herbicide in drinking water in the UK are attained.

Introduction

A validated method is described for the analysis of 19 acidic herbicides and one amide herbicide, Propyzamide, as a single analytical suite. The various herbicides cannot be characterized as a single class but belong to several different groups: chlorophenoxy acids such as mecoprop, are well represented.

The Prescribed Concentration or Value (PCV) for an individual herbicide in drinking water in the UK, as defined by the Water Supply (Water Quality) Regulations, is set at 0.1 µg/L. Ideally, the method of analysis is capable of detecting 10 to 20% of the PVC, that is, 0.01 to 0.02 µg/L. This method clearly attains these lower limits of detection.

Standard analytical methods for these herbicides in water matrices involve either liquid-liquid extraction or solid phase extraction (SPE), followed by a derivatisation using diazomethane or pentafluorobenzyl bromide and analysis by gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS). The derivatisation step involves use of potentially hazardous chemicals.

This application note gives details of a validated liquid chromatography/mass spectrometry (LC/MS) method requiring no derivatisation step and which uses a relatively small volume of sample.

Experimental

All analysis was performed using an Agilent 1100 series LC/MS quadrupole system coupled to an Agilent 1100 series LC system consisting of a quaternary pump, autosampler, thermostated column compartment and vacuum degasser. A diode array detector, in-line before the mass spectrometer was used as a troubleshooting tool. The quadrupole mass spectrometer was operated with the Agilent atmospheric pressure electrospray ionisation (API-ES) source in negative ion mode.

Sample Preparation

1. Fully automated SPE used Oasis HLB, 60 mg, 3 mL cartridges and a series of solutions: #1) 90:10 TMBE: methanol (0.01% formic acid), #2) methanol (0.01% formic acid), #3) HPLC grade water, and #4) HPLC grade water (0.25% hydrochloric acid).
2. Each cartridge was initially conditioned with sequential additions of 5 mL of solution #1, 5 mL of solution #2, 3 mL of solution #3, and 3 mL of solution #4.
3. An aqueous sample of 50 mL was diluted to 200 mL with deionised water and acidified with 0.75 mL of hydrochloric acid.
4. A diluted sample of 100 mL was pumped through the conditioned cartridge at 10 mL/min.
5. Cartridge was dried 10 minutes, using forced air.
6. Cartridge was eluted with solution #1 twice using 1.5 mL and once using 1 mL.
7. The extract was evaporated to dryness in a 45 °C heated block, using a gentle air stream.
8. Residue was dissolved in 250 µL of solution #1.

LC conditions

- Column: Zorbax Eclipse XDB-C18, 150 mm long, 2.1 mm id, 3.5 µm particles, 60 °C
- Pre-column quaternary pump
- Mobile phase A: 0.01% Formic acid in water
- Mobile phase B: Acetonitrile
- Gradient program:

	A	B
Initial	90%	10%
37.5 min	43%	57%
38.0 min	90%	10%
48.0 min	90%	10%

- Pre-column flow rate: 0.3 mL/min
- Sample size injected: 50 µL
- Binary pump with diode array detector (DAD)

Instrument: Agilent 1100 LC/MS with API-ES in Negative Ion Mode

- Drying gas temperature and flow: 300 °C, 11 L/min
- Nebulizer gas pressure: 30 psig
- V_{cap}: 2500 Volts
- Fragmentor voltage: Variable, see Table 1
- SIM ions: See Table 1

Table 1. SIM Parameters

Compound	Time	Group number	SIM ions Quantitation, Qualification (q)	Fragmentor voltage
2,3,6-TBA	1.00	1	178.9, 180.9 q	90
Clopyralid			190.1, 192.0 q	75
Picloram			238.9, 240.9 q	120
Imazapyr			260.1, 261.1 q	75
Dicamba	12.0	2	174.9, 177.0 q	85
Benazolin	17.0	3	169.9, 171.9 q	140
Fluroxypyr			195.0, 196.9 q	145
Bentazone			239.0, 240.1 q	140
Bromacil			259.0, 261.0 q	135
MCPA	24.5	4	199.0, 201.0 q	150
2,4-D			219.0, 220.9 q	150
Bromoxynil			275.9, 278.0 q	140
Dichloroprop	28.0	5	160.9, 162.9 q	150
2,4,5-T			195.0, 196.9 q	150
Triclopyr			195.9, 198.0 q	150
Mecoprop			213.0, 215.0 q	150
Ioxynil			369.9. 371.0 q	145
2,4-DB	33.5	6	160.9, 163.0 q	150
MCPB			227.1, 229.1 q	70
Propyzamide	36.25	7	254.1, 256.0 q	120

The SIM ions and fragmentor voltages listed in Table 1, page 2, were all optimized using Flow Injection Analysis (FIA). Standard solutions of 10 mg/L of each herbicide were injected using scan mode range 150 to 400 amu, and fragmentor voltage ramped from 70 to 150 V in 5 V steps. A typical FIA pattern for dicamba appears in Figure 1, and the peak area relationship appears in Figure 1A.

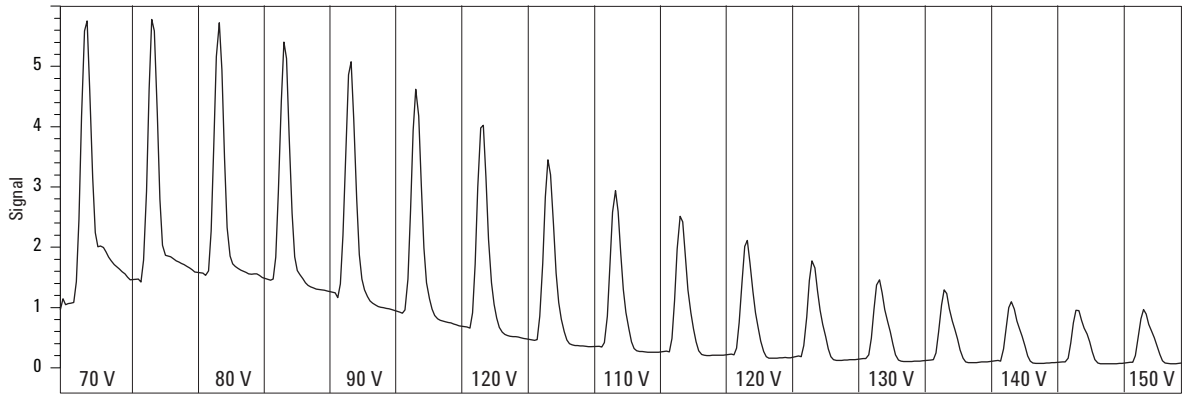

Figure 1. Dicamba TIC versus fragmentor voltage.

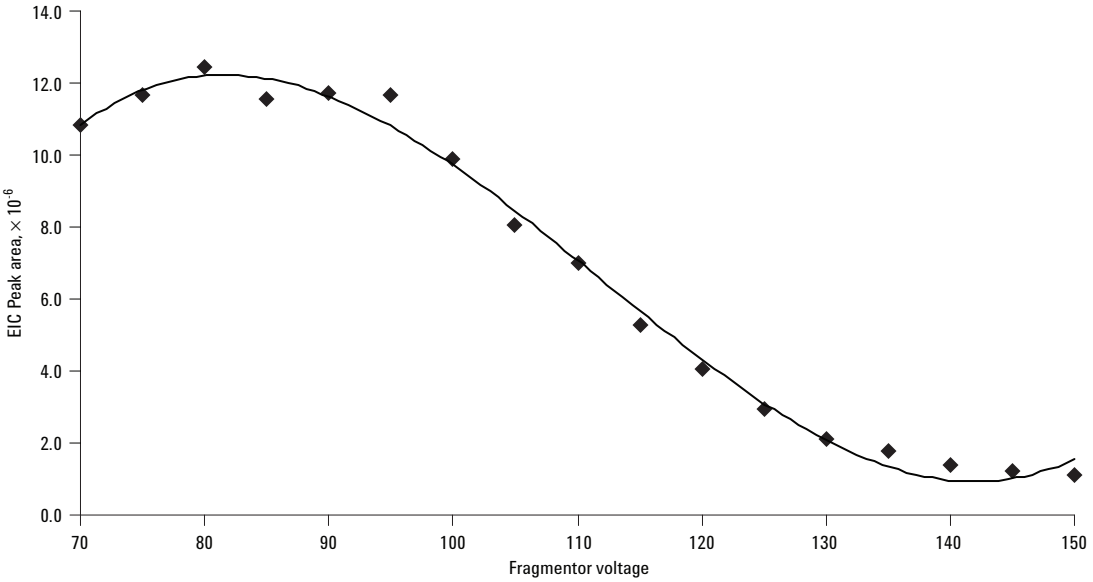

Figure 1A. Dicamba 174.9 *m/z* area vs. fragmentor voltage.

Using the FIA data, the fragmentor voltage generating the maximum response was selected. For dicamba, this corresponded to 80–85 V.

The mass spectrum of each herbicide was obtained at the optimum fragmentor voltage. The mass spectrum of dicamba, which has a molecular weight of 220, appears in Figure 2.

This shows the M-H ion at 219 with the expected two chlorine isotope pattern, but with a much stronger 174.9 ion, corresponding to the additional loss of CO_2. For maximum sensitivity the 174.9 ion was chosen for quantitation and the 177.0 ion as the qualifier ion (q). This process was repeated for all 20 herbicides.

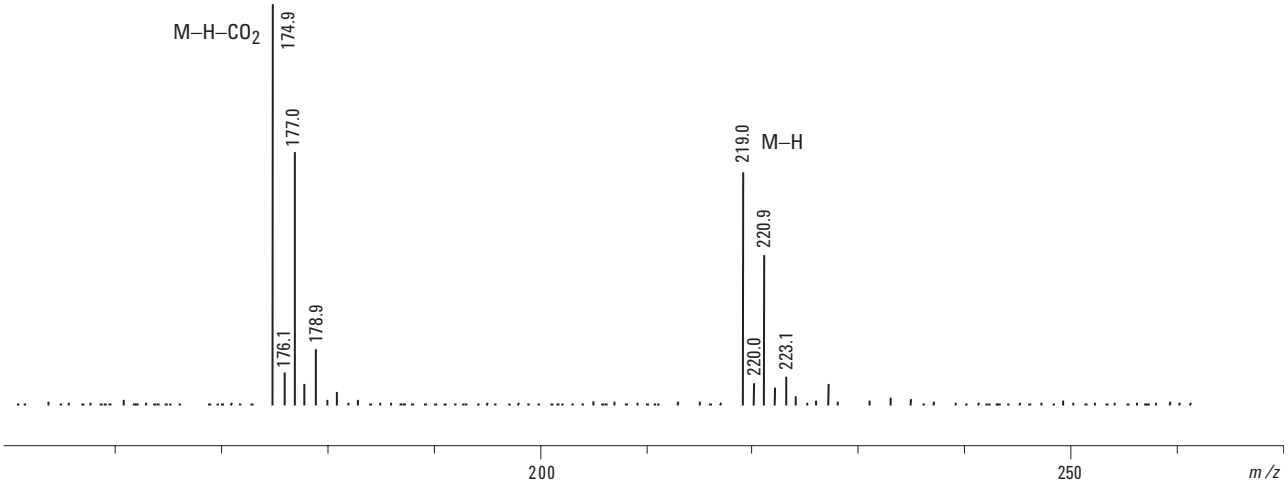

Figure 2. Mass spectrum of dicamba at 85 fragmentor voltage.

Results

A typical chromatogram for the low level standard is shown in Figure 3. This is equivalent to a concentration of 0.1 µg/L.

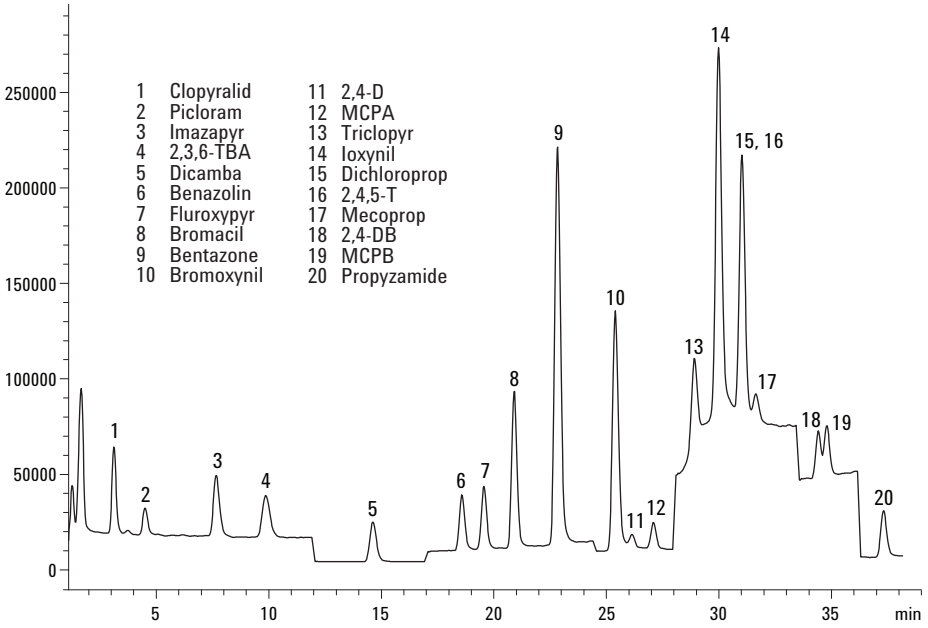

Figure 3. Low level standard chromatogram equivalent to 0.1 µg/L.

Figure 4 shows an extracted ion chromatogram from a real sample found to contain bentazone at a concentration of 0.08 µg/L. The mass spectrum is also shown.

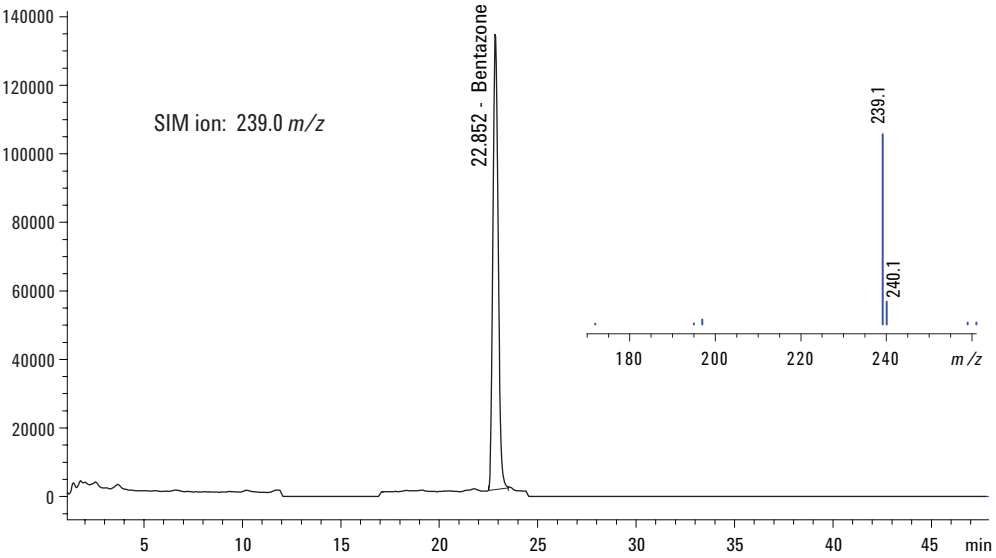

Figure 4. Borehole sample containing 0.08 µg/L bentazone.

Calibration curves were also produced using three calibration levels at 0.1, 0.3 and 0.5 µg/L. The calibration curves are all produced using a quadratic fit and forced through the origin. Typical correlation values are 0.9999 or better for all the herbicides in the suite. A typical calibration curve is shown in Figure 5.

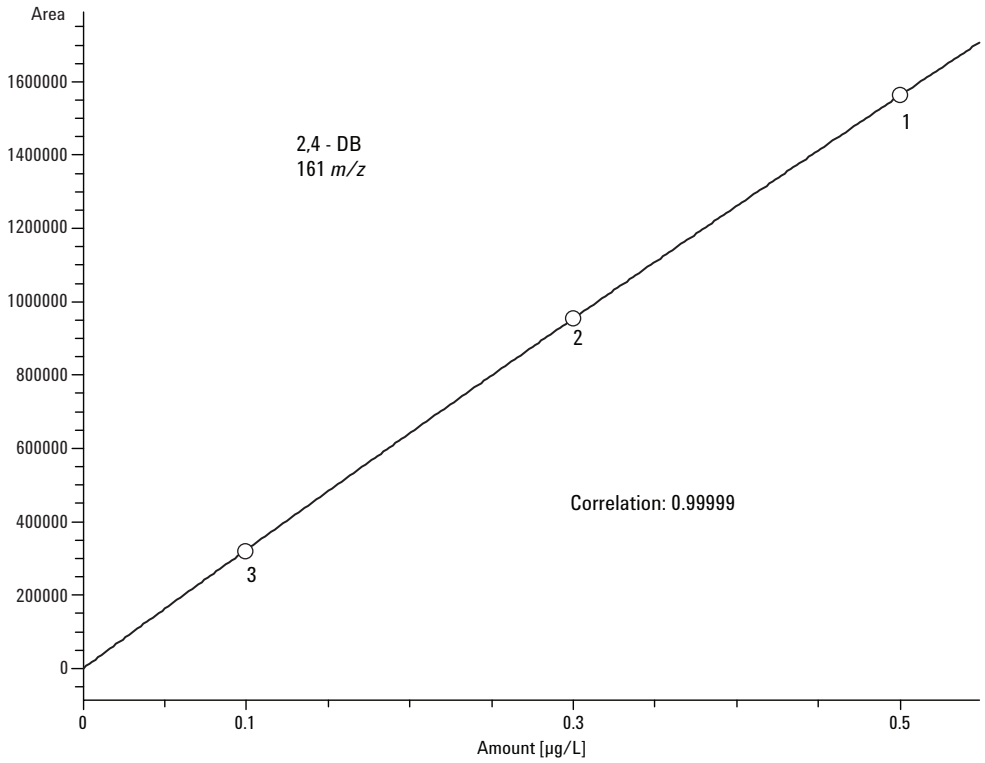

Figure 5. Typical calibration plot.

Validation of the method was carried out on 11 batches of samples. The borehole groundwater was spiked at three levels: 0.01 µg/L, 0.10 µg/L, and 0.40 µg/L. The potable tap water (which was from a surface water source) was spiked at two levels: 0.01 µg/L and 0.10 µg/L. Each batch of samples was analysed in duplicate and in a random order. The limit of detection (LOD) for each herbicide was calculated from the within-batch standard deviation of the borehole sample spiked at 0.01 µg/L. Recovery for both the groundwater and potable water samples was calculated from the 0.10 µg/L spike after subtraction of the 0.01 µg/L standard. Hence the recovery value is based on 0.09 µg/L.

Experimental results are shown in Table 2.

Table 2. Comparison of Spike Recoveries from Groundwater and Potable Water Samples

	Groundwater samples		Potable water samples		
Compound	**Recovery %**	**RSD %**	**Recovery %**	**RSD %**	**LOD µg/L**
Clopyralid	89.4	10.1	61.1	12.9	0.00658
Picloram	67.1	9.4	67.7	9.3	0.00477
Imazapyr	73.1	11.0	47.5	13.6	0.00631
2,3,6-TBA	93.4	6.4	83.5	9.0	0.00335
Dicamba	92.6	6.5	83.0	7.5	0.00882
Benazolin	83.7	7.4	67.6	7.5	0.00629
Fluroxypyr	84.8	7.6	72.7	7.6	0.00694
Bromacil	89.2	7.9	61.6	9.2	0.00702
Bentazone	106.8	4.1	98.6	4.9	0.01034
Bromoxynil	99.5	4.2	94.0	5.1	0.00666
2,4-D	85.1	8.8	76.7	6.1	0.00603
MCPA	91.5	8.1	83.2	4.9	0.00526
Triclopyr	97.7	7.4	85.0	6.8	0.00704
Ioxynil	100.2	6.3	99.8	4.5	0.00653
Dichloroprop	95.8	6.1	89.8	4.4	0.00545
2,4,5-T	86.7	7.3	83.0	5.7	0.00562
Mecoprop	96.6	5.9	93.9	4.6	0.00593
2,4-DB	90.7	6.3	81.6	5.7	0.00488
MCPB	93.2	5.8	84.2	7.9	0.00647
Propyzamide	86.8	5.9	87.5	5.3	0.00574
Average	90.2	7.1	80.1	7.1	0.00630

Discussion

The data produced clearly show that there is a difference in recovery between the two sample matrices tested. This is in part due to different extraction efficiencies from the two different water types, with the potable water in general showing a lower recovery. This potable water sample typically has a Total Organic Carbon (TOC) content of about 4 mg/L, compared to the borehole sample with a typical TOC of about 0.5 mg/L. The higher organic content of the potable water may well lead to slightly lower spiked recoveries.

The other factor involved, especially with the earlier eluting compounds (for example, clopyralid and imazapyr), is suppression of the ionisation. This occurs where there is competition for ionisation in the spray chamber from other compounds in the sample. In the case of potable waters, in particular waters derived from surface water sources, these are likely to be due to humic and fulvic acids, which elute very early in the analytical run. Hence ionisation suppression will lead to an apparent reduction in recovery values obtained. Consequently, when analyzing samples routinely, the recovery correction should be made from reference values obtained from an appropriate matrix, or adjusted by the use of an internal standard(s) where available.

Conclusion

The data shows that the method presented is capable of quantitative analysis for the 20 herbicides in single analytical suite. The performance requirements set by the Drinking Water Inspectorate (DWI) for standard deviation, bias and total error are all met. Although spiked recovery targets of 90 to 110% are not achieved in all cases, this can be compensated for by the application of recovery factors which are calculated from the performance data. The method was granted UKAS accreditation at the laboratory where the method was validated.

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Printed in the USA
April 19, 2002
5988-5882EN

Agilent Technologies

Analysis of Glyphosate and Aminomethyl Phosphonic Acid by Liquid Chromatography/Mass Spectrometry

Application

Environmental

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Abstract

A liquid chromatography/mass spectrometry method using an electrospray ionization source in positive ion mode was developed for the analysis of glyphosate (N-phosphonomethyl glycine) and its metabolite, aminomethyl phosphonic acid in water. Both glyphosate and its metabolite were derivatized using 9-fluorenylmethyl chloroformate in buffer solution prior to reversed phase high performance liquid chromatography separation. The method cleanly resolved both target molecules with excellent sensitivity in both positive and negative ion modes.

Background

Glyphosate (N-phosphonomethyl glycine), $(HO)_2P(O)\text{-}CH_2\text{-}NH\text{-}CH_2CO_2H$, is a global herbicide widely used in forest management, agricultural applications, and urban landscape management. Glyphosate is recognized as a benign, environmentally friendly herbicide with low toxicity [1]. However, there are health and environmental concerns over its toxicity, which are documented in a recent review [2].

Judging from the scope and quantity of glyphosate in use worldwide, it is important that a rugged, routine method be available for the accurate determination of both glyphosate and its metabolite, aminomethyl phosphonic acid (AMPA), $(HO)_2P(O)\text{-}CH_2\text{-}NH_2$, in environmental matrices. Any analytical method must analyze for both, since an absence of glyphosate may have been caused by its conversion to AMPA.

Because of the highly polar nature of these compounds, organic solvents cannot extract them from environmental matrices. This has made their determination a difficult challenge. Many analytical methods for these molecules were reported by Stalikas and Konidari [3] listing diverse techniques including gas chromatography (GC), high performance liquid chromatography (HPLC), ion chromatography (IC), enzyme-linked immunosorbent assays, and capillary electrophoresis (CE). Although, gas chromatography/mass spectrometry (GC/MS) based methods are economical and have good selectivity and sensitivity, they use complicated derivatization processes and require highly specialized personnel. HPLC methods suffer from the relatively poor response of these molecules to ultraviolet (UV) detection.

The present method, offering a liquid chromatography/mass spectrometry (LC/MS) approach with pre-column derivatization, combines simplicity and sensitivity, adequate for a 10–50 ppb determination, given a 100-mL water sample.

*Corresponding author

Method

Sample Preparation

1. Prepare reagent solution: 10 mg/mL 9-fluorenylmethyl chloroformate (FMOC) in acetonitrile.
2. Prepare buffer solution: 5% sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10\ H_2O$), pH = 9.1
3. To 50 µL of sample extract, add 50 µL of buffer solution and mix.
4. Add 50 µL of reagent solution.
5. Let stand 4 hrs.

Instrument: Agilent 1100 LC/MS with electrospray ionization (ESI) in Positive Ion Mode

- Drying gas: 12 L/min, 350 °C
- Nebulizer gas: 60 psi
- V_{cap}: 3500 V
- Fragmentor: 100 V for both scan and SIM runs
- SIM ions: 334 and 392 *m/z*
- Scan range: 120 to 1000 *m/z*

LC conditions

- Column: ZORBAX XDB-C8, 4.6 mm id × 50 mm long, 5 µm particles, 40 °C
- Precolumn pump: Agilent 1100 binary
- Mobile phase A: 50 mM ammonium acetate, aqueous
- Mobile phase B: acetonitrile, 0 to 95% in 5 min, hold 3 min
- Pre-column flow rate: 0.7 mL/min
- Sample size injected: 1 µL
- Post-column pump: Agilent 1100 isocratic. Flow: 0.3 mL/min of 0.6% formic acid
- Binary pump with diode array detector (DAD) and well-plate sampler

Results

Figure 1 shows molecular structures for the starting compounds and their derivatized products. It is these derivatives that are analyzed and depicted in subsequent figures.

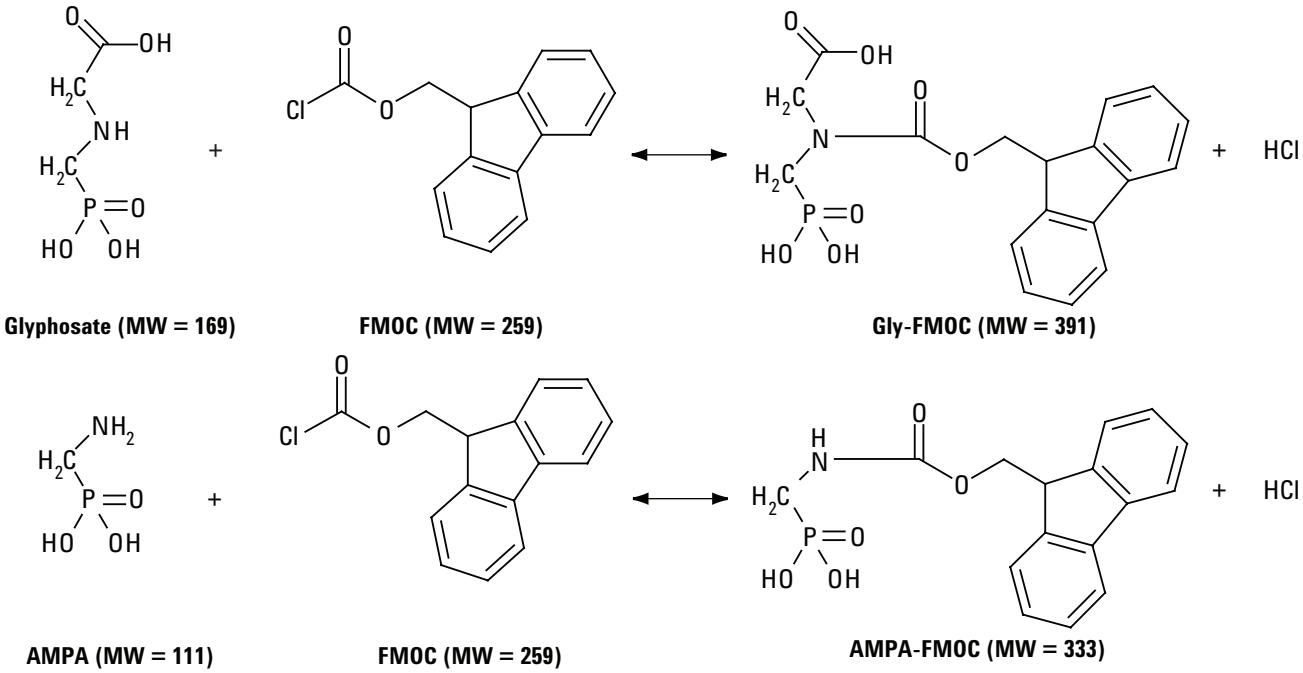

Figure 1. Derivatization reactions for glyphosate and AMPA with FMOC.

Figure 2 displays the mass spectra of both derivatized molecules. Positive ions 392 and 334, representing glyphosate and AMPA respectively, were chosen for further analysis.

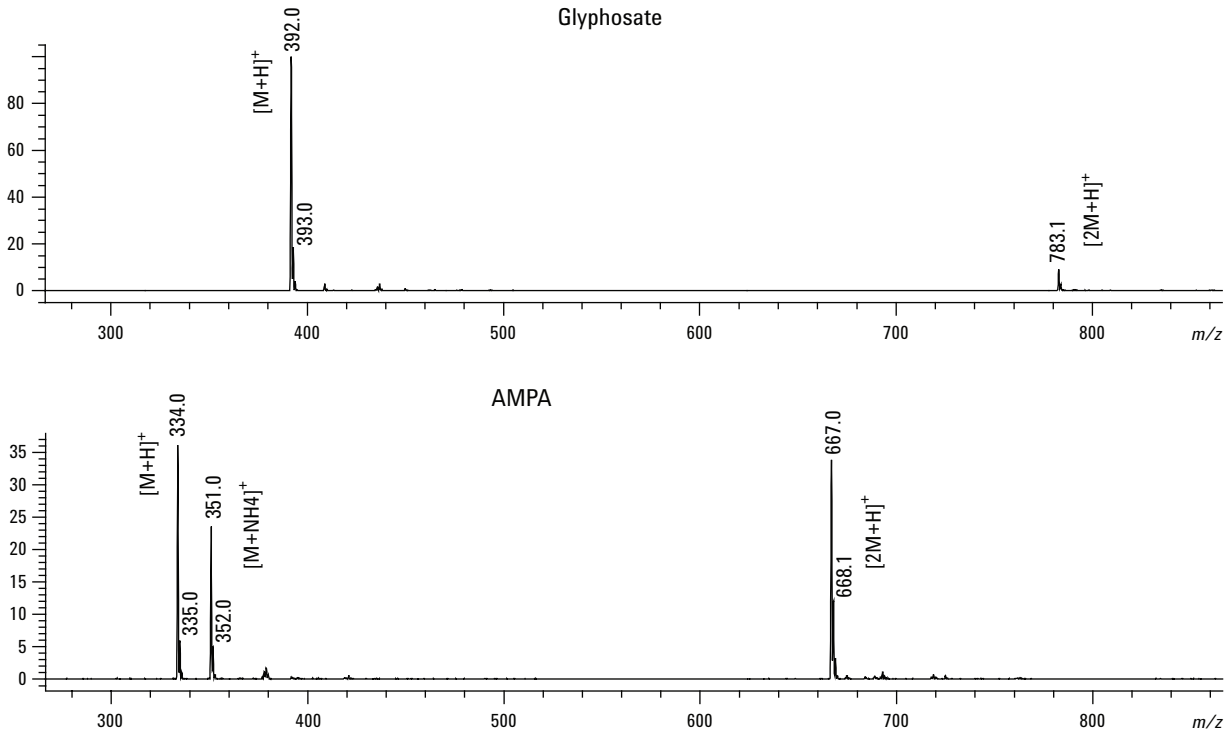

Figure 2. Mass spectra of target molecules.

Figure 3 is a stacked extracted ion chromatogram of the derivatized target molecules at low concentration.

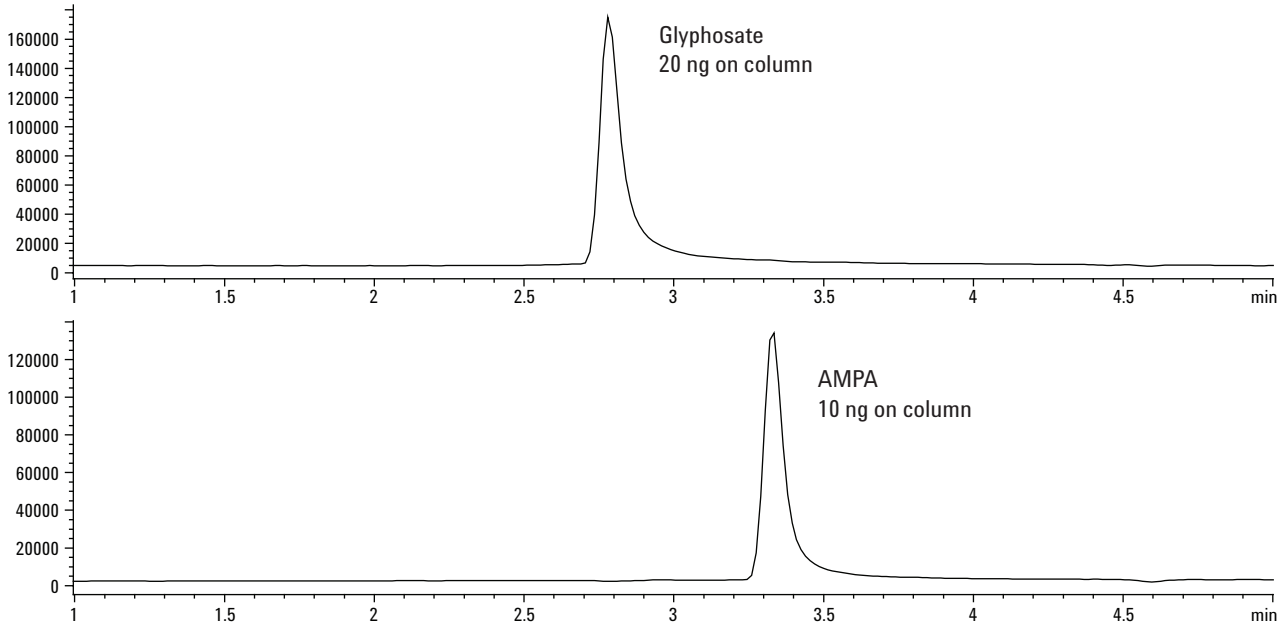

Figure 3. Low level extracted ion chromatograms for both target molecules.

Figure 4 is a similar stacked plot, but at a higher concentration.

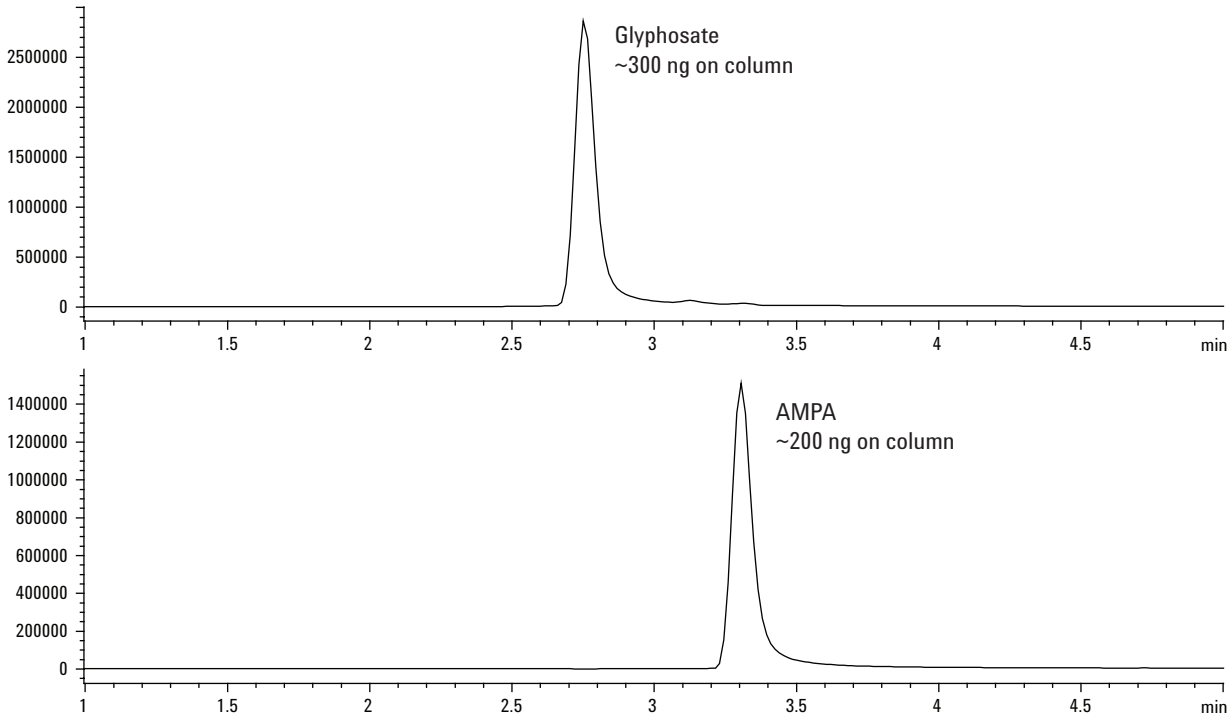

Figure 4. Higher level extracted ion chromatograms for both target molecules.

Figures 5 and 6 are abundance vs. concentration plots for the derivatized target molecules, as the positive ions for glyphosate and AMPA, respectively.

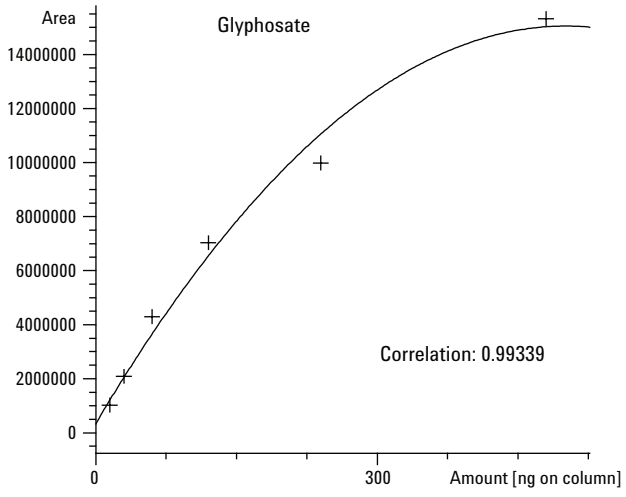

Figure 5. Abundance vs. concentration for derivatized glyphosate, detected as $[M+H]^+$, 392 *m/z*.]

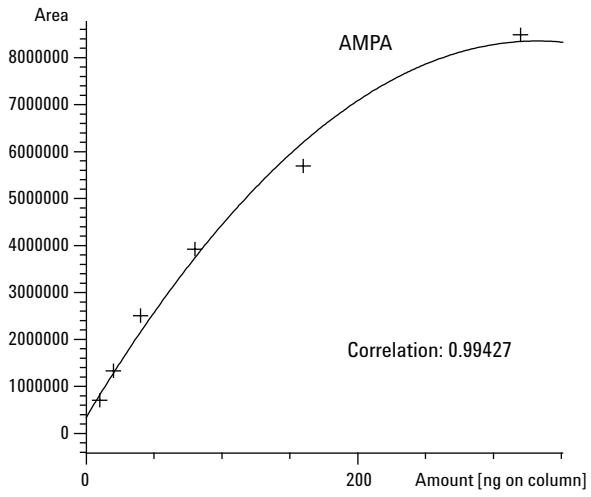

Figure 6. Abundance vs. concentration for derivatized AMPA, detected as $[M+H]^+$, 334 *m/z*.]

The non-linearity of the positive ion experiments at the higher concentrations is believed due to insufficient FMOC used in this initial experiment. This belief is strengthened by the work of Yang, et al [4] where linearity was achieved over a comparable concentration range with an optimized derivatization, also using FMOC, but analyzed in negative ion mode.

Figures 7 and 8 show abundance vs. concentration data, per Yang [4], for the derivatized target molecules as the negative ions, 390 and 332 *m/z* for glyphosate and AMPA, respectively.

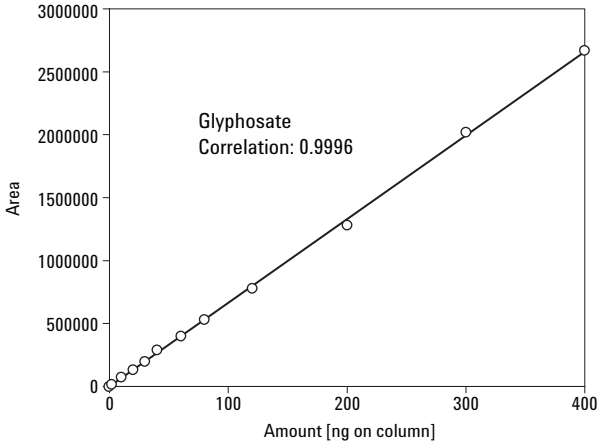

Figure 7. Abundance vs. concentration for derivatized glyphosate, detected as [M-H]⁻, 390 *m/z*.

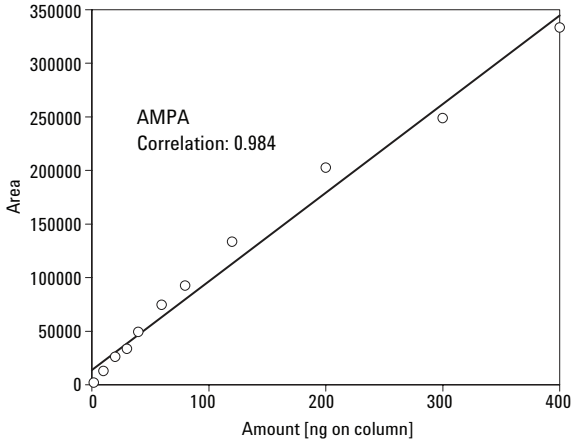

Figure 8. Abundance vs. concentration for derivatized AMPA, detected as [M-H]⁻, 332 *m/z*.

Conclusions

- Both glyphosate and AMPA, as FMOC derivatives, can be readily and sensitively detected using LC/MS with electrospray ion source in positive or negative ion mode.
- Chromatography is excellent with good separation between parent and breakdown product.
- Instrument can easily measure 10–20 ng on column.
- Sensitivity is adequate for a 10–50 ppb determination given a 100 mL water sample size.
- In positive ion mode, linearity is good below 200 ng on column. Non-linearity at higher concentrations is caused by depletion of FMOC. A drop in FMOC's UV response at higher target concentrations supports this, even though absorbencies were well within the UV detector linear range.
- The present positive ion work can be a starting point for further method development to increase both linear range and applicable matrices.
- The negative ion method shows what a fully engineered method can accomplish.
- Full sample extraction procedures can be found in Reference 4.
- This work represents an example of how derivatization can enhance the power of LC/MS. Generally, derivatization is not thought of as an LC/MS option.
- Other derivatization strategies can be studied for similar compounds.

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1. Glyphosate: A unique global herbicide. *ACS Monograph 189*, Washington D.C., American Chemical Society.
2. Cox, C., Herbicide Fact Sheet. Glyphosate (Roundup). *Journal of Pesticide Reform*, 1989. 18, 3-17.
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Printed in the U.S.A.
February 20, 2002
5988-4981EN

Agilent Technologies

Identification and Quantitation of Pesticides in the Parts-per-Trillion Range Using Retention Time Locking and GC/MS

Application

Environmental, Food

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Abstract

The typical pesticide quantitation limit for a mass spectrometer in the Scan mode is in the sub-ppm range. By using a selected ion monitoring method, a lab can lower the target compound quantitation limit to the low parts-per-billion (pg/µL) range using a retention time locked gas chromatography/mass spectrometry method. By adding large volume injection capability to the method, target compounds at parts-per-trillion can be quantified.

A specially developed 567-compound retention time locking pesticide mass spectral library can automatically screen an acquired sample's data file for all 567 compounds in seconds. The library can also be applied for rapid screening of samples acquired in selected ion monitoring method. Using the compound library information, a selected ion monitoring method for 80 target compounds was created in less than 2 hours without running any analyses.

Introduction

Most pesticides are typically analyzed on a gas chromatograph (GC) with element-selective detectors (ESDs). Although these ESDs provide low ppb detection limits and are easy to operate, the data do not provide sufficient information to confirm a compound's presence with confidence. Due to the universal nature of mass spectrometric detection, a mass spectrometer (MS) provides additional information and increased confidence in the assignment of compound identity. With recent advances in GC/MS hardware and software and the decrease in cost of ownership, more and more laboratories are routinely analyzing pesticide residue samples with MS detection.

To match the GC/ESD detection limits and/or to eliminate sample concentration steps, a user must lower the MS detection limit by 2 to 3 orders of magnitude. This application note, discusses the following approaches.

- Run the MS in single ion monitoring (SIM) mode
- Make large volume injections (LVIs)
- Use higher electron multiplier voltage (EMV)

For compound identification, a specially developed 567-compound retention time locking (RTL) [1] pesticide library could perform the entire 567-compound screening in seconds using Scan data. A subset of the library could be screened in seconds from SIM data.

Experimental

A pesticide standard mixture was used to compare the lowest detection limits of splitless injection and LVI under Scan and SIM modes.

System Configuration for Screening and Quantitation:

- 6890 GC with a programmable temperature vaporizer (PTV) [2,3] inlet
- 5973 Mass Selective Detector (MSD)
- 7683 Automatic Liquid Sampler (ALS) tray and autoinjector
- HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm), P/N 19091S-433
- G1701BA version B.00.00 MSD ChemStation software or higher
- G1049A MSD RTL Pesticide Database/Library

Table 1. GC Method Parameters

Oven	70(2)/25/150(0)/3/200(0)/8/280(10) = 41.87 min
Inlet	PTV
Inlet pressure	17.30 psi (locked to methyl chlorpyrifos at 16.593 min), constant pressure mode

Table 2. Injection Parameters

Injection mode	Solvent vent	Splitless
Injection volume (syringe)	25 µL (50-µL syringe, P/N 5183-0318)	1 µL (10-µL syringe, P/N 9301-0713)
Injection speed	Inject @ 100 µL/min Draw @ 300 µL/min Dispense @ 4500 µL/min	Fast
Inlet temp	40(0.35)/600/320 (3)/50/200 (Hold until end)	280 °C
Vent	Vent time = 0.29 min Vent flow = 150 mL/min Vent pressure = 0.00 psi	
Purge	60 mL/min @ 2 min	60 mL/min @ 2 min
Liner	Deactivated, Multi Baffled (P/N 5183-2037)	Deactivated, Multi Baffled (P/N 5183-2037)
Inlet cooling	Liquid CO_2	None

Table 3. MS Method Parameters

Solvent delay	3 min
Tune file	Atune.u
Transfer line	280 °C
MS Quad	150 °C
MS source	230 °C
Threshold	150
Sample #	2
Scan range	35 to 500 amu (in Scan mode)
Forty (40) SIM groups (in SIM mode)	

Table 4. Pesticide Screening Parameters for the SIM Method

Extraction window	±0.100 minute
Qualifier mode	Absolute
Qualifier %	30
Zero qualifiers	Included
Subtraction mode	Average start/stop
Screen database	Rtlpest.SCD

Results and Discussion

RTL [1] was used to:

1. Expedite data comparison in overlay format
2. Achieve lower target compound detection limit
3. Allow rapid pesticide screening using the RTL pesticide database/library
4. Help to differentiate isomers by their retention time (RT) differences
5. Eliminate the tedious SIM method RT updating process after column maintenance
6. Simplify the editing of the SIM ion groups

A mixture from the California Department of Food and Agriculture (CDFA) of 80 pesticides at 5000 pg/µL each was used as the stock solution for this study. The mixture contained carbamate, organochlorine, organophosphorus, and organonitrogen pesticides. Figure 1 is an offset overlay of three total ion chromatograms (TIC) with 50, 100, and 500 pg of each of the pesticides injected. These TICs were obtained in the Scan mode from 1-µL spiltless injections. For many of these pesticides the quantitation limit in the Scan mode is about 500 pg on column.

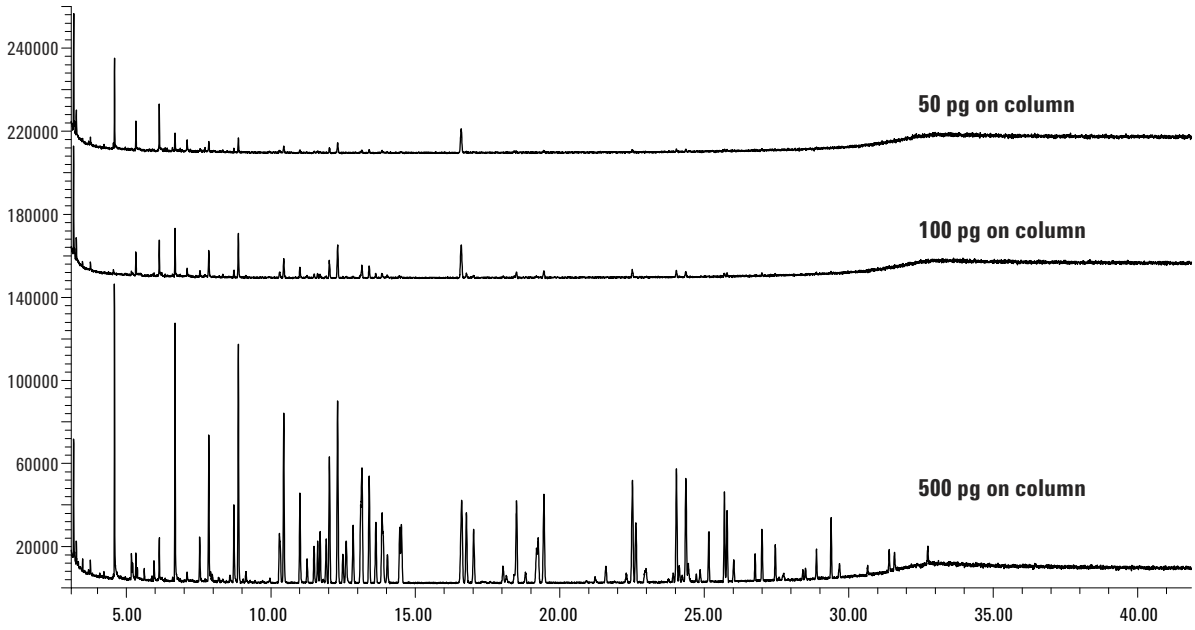


Figure 1. Total ion chromatograms from 1-µL splitless injections of 80 pesticides with 50, 100, and 500 pg of each compound injected.

SIM Mode

To lower the detection limit, a SIM method was created. Instead of the traditional way of making a SIM method, a user can use the information in the RTL Pesticide Database to build a SIM method without running an analysis. Here are the steps for editing SIM ion group parameters:

1. List the MSD RTL Pesticide Database from the ChemStation (Figure 2 is a partial listing) and paste the complete listing into a spreadsheet.

Scdlist.txt - Notepad

File Edit Search Help

SCD Compound List Report

Screen Database : C:\DATABASE\RTLPEST.SCD
Total SCD Cpnds : 567

Cpd#	Compound Name	TIon	Exp_RT	Q1	Q2	Q3
1	Diethylene glycol	45	3.39	75	76	44
2	Aniline	93	3.55	66	65	92
3	p-Dichlorobenzene	146	3.88	148	111	75
4	Dicyclopentadiene	66	4.00	132	67	65
5	Dimefox	44	4.01	110	154	42
6	o-Dichlorobenzene	146	4.09	148	111	75
7	2-Methylphenol	108	4.25	107	77	79
8	4-Methylphenol	107	4.42	108	77	79
9	m-Cresol	108	4.42	107	79	77
10	1,2-Dibromo-3-chloropropane	157	4.53	155	75	159
11	2,4-Dimethylaniline	121	5.19	120	106	77
12	2,6-Dimethylaniline	121	5.20	106	120	77
13	2,4-Dichlorophenol	162	5.19	164	63	98
14	1,2,4-Trichlorobenzene	180	5.29	182	184	145
15	Ethiolate	100	5.41	72	161	44
16	3-Chloroaniline	127	5.45	129	65	92
17	4-Chloroaniline	127	5.48	129	65	92
18	2-Ethyl-1,3-hexanediol	56	5.51	55	73	57
19	p-Nitrotoluene	91	5.57	137	65	107
20	Methamidophos	94	5.66	95	141	47
21	Dichlorvos	109	5.83	185	79	187
22	Allidochlor	41	6.18	56	138	132
23	2,3,5-Trichlorophenol	196	6.63	198	200	160
24	2,6-Dichlorobenzonitrile	171	6.75	173	136	100

Figure 2. A partial listing of the pesticide screener database. The listing includes the compound number, compound name, target ion, expected retention time, and three qualifier ions.

2. In the spreadsheet, delete the rows of the compounds not needed in the method.
3. Separate target compounds into groups (see the added "Group #" column on Figure 3) using these criteria:
   - One to three compounds in each group, and
   - The RTs of the adjacent compounds in adjacent groups are at least 0.2 minute apart. For example, compounds 42 and 51 are more than 0.2 minute apart, so they are in different groups. Compounds 51 and 55 are less than 0.2 minute apart, so they are in the same group.
4. Use the average RT of the adjacent compounds in adjacent groups as the SIM group RT (see the added "Group RT" column on Figure 3). For example, the average retention time of compound 42 (7.91 min, in group 2) and compound 51 (8.78 min, in group 3) is 8.35 minute which is used as the starting retention time of group 3. When all the group numbers and respective starting retention times are determined, make a hardcopy of the spreadsheet for easy entry into the "MS SIM/Scan Parameters" in the next step.
5. Enter the target ion and qualifier ion(s) (Q1, Q2, and/or Q3) of all compounds into the respective ChemStation SIM group (Figure 4). Notice that all the information for building the SIM groups came from Figure 3.

#	Compound Name	MSD_RT	T	Q1	Group #	Group RT
24	2,6-Dichlorobenzonitrile	**6.75**	171	173	**1**	**3.00**
35	Mevinphos	**7.60**	127	192		
42	Propham	**7.91**	93	179	**2**	**7.75**
51	o-Phenylphenol	**8.78**	170	169	**3**	**8.35**
55	Pentachlorobenzene	**8.95**	250	252		
76	Propoxur	**10.35**	110	152	**4**	**9.60**
82	Diphenylamine	**10.52**	169	168		
92	Chlorpropham	**11.05**	127	213	**5**	**10.76**
98	Ethalfluralin	**11.28**	276	316		
102	Bendiocarb	**11.54**	151	126	**6**	**11.41**
103	Trifluralin	**11.64**	306	264		
104	Benfluralin	**11.73**	292	264		
111	Phorate	**11.96**	75	121	**7**	
113	BHC alpha isomer	**12.09**	181	219		
117	Hexachlorobenzene	**12.38**	284	286		
120	Dicloran	**12.56**	206	176		
122	Demeton-S	**12.63**	88	60		
124	Dimethoate	**12.68**	87	93		
129	Simazine	**12.91**	201	186		

Figure 3. A spreadsheet of target compounds separated into different SIM groups with RTs of the adjacent compounds in adjacent groups at least 0.2 minute apart. The starting retention time of each group was determined by calculating the average RT of the adjacent compounds in adjacent groups.

The number of qualifier ions used in a SIM method depends on the number of analytes of interest. For a method monitoring 20 to 30 compounds, all three qualifier ions should be used in the SIM method. As the list of target compounds grows, fewer qualifier ions should be used in the method to maintain a reasonable and comparable ion dwell time and sampling rate.

In general, 10 scans (cycles) per peak are recommended for quantitation purposes. For example, if an analyte peak is 6 seconds wide, about 1.7 cycles per second should be maintained for that SIM ion group. Once the number of cycles per second is determined, the dwell time of the ions can be varied to meet that. As the dwell time is entered for each ion, the ChemStation automatically shows the number of cycles per second. In Figure 4, Group 6 has 3.03 cycles per second.

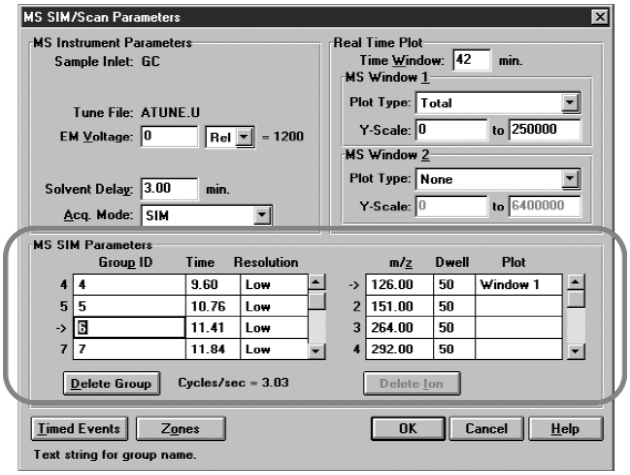


Figure 4. A screen capture of the MSD ChemStation showing the MS and SIM parameters. The SIM parameters (group ID, group retention time, and ions) were all derived from Figure 3.

Figure 5 shows two chromatograms obtained from 1-µL splitless injections at 50 pg/µL using both Scan and SIM modes. The Scan mode has significantly higher baseline noise than the SIM mode. Some of the compounds, especially the late eluters, were not detected in the Scan mode. When the Scan method was changed to a SIM method at this concentration, the signal-to-noise ratio (S/N) increased by a factor of 100. It is worth pointing out that a SIM method does not record background ions from the sample matrix, therefore minimizing the baseline noise and improving the S/N.

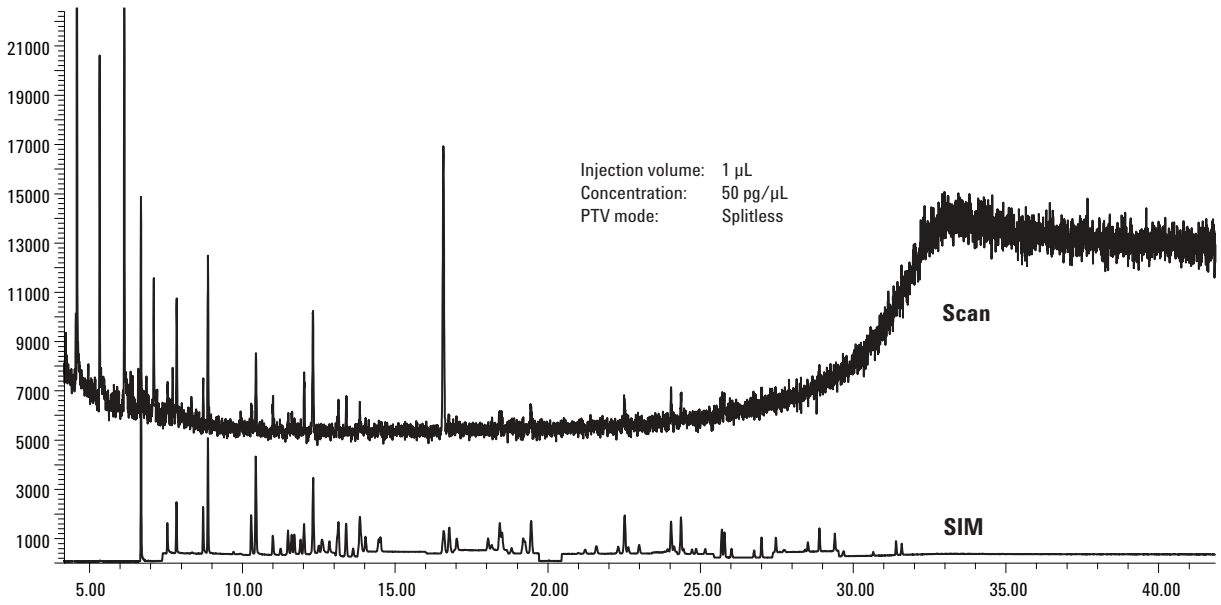


Figure 5. Chromatograms of 1-µL splitless injections at 50 pg/µL from Scan and SIM modes.

In a SIM method, the retention times of the ion groups normally need updating after column maintenance. By using RTL, a user can not only eliminate the tedious RT updating process [4] but also decrease the detection limit. With reproducible known RTs of target compounds, the start and end time of each ion group can be determined optimally. By narrowing the time windows of an ion group to monitor only one or two compounds at a time, the MS can monitor fewer ions in each window, allowing more sampling time for the target ions.

Ideally, a SIM method will have the maximum number of ion groups and the minimum number of ions in each group. In this way, each ion group can get more scans per unit time resulting in better peak shape and more accurate quantitation.

LVIs

To decrease the detection limit further, a user can put more sample on column using the LVI technique. The typical “solvent-vent” approach is to inject the sample slowly into a PTV inlet at a temperature just below the solvent boiling point and let solvent evaporate before ramping up the inlet temperature to move the compounds onto the capillary column. Figure 6 compares a 1-µL splitless injection with a 25-µL solvent-vent injection. Both injections resulted in 50 pg per compound on column. Note that the solvent-vent ion chromatogram is plotted upside down for ease of comparison with the splitless ion chromatogram. It is obvious from the figure that the two techniques provide very similar results. This demonstrates that the solvent-vent technique is a viable approach for sample introduction.

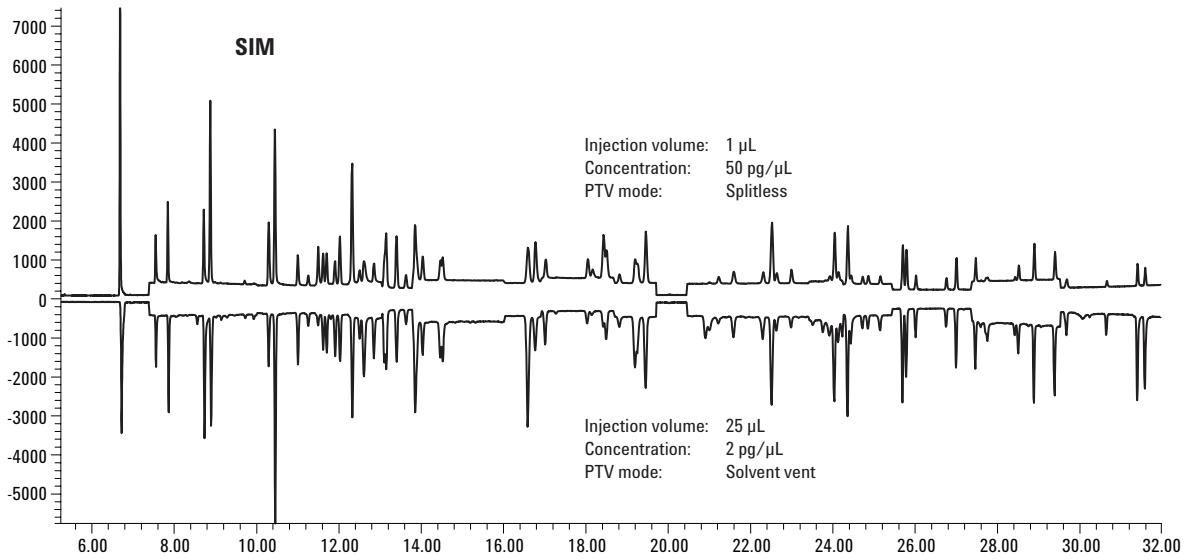


Figure 6. SIM results of 50 pg on column using either a 1-µL splitless or a 25-µL solvent-vent injection.

Higher EMV

It is known that the signal increases with higher EMV on the MS. In Figure 7, the upper signal, after 10-fold magnification, is a 25-µL LVI of 0.5 pg/µL at tune voltage. The bottom signal is the same injection with the electron multiplier set to tune +400 V. Adding 400 V to the EMV increases the signal by 10X, which makes the integration more accurate. However, the baseline noise also increases by 10X, so the S/N stays the same.

Although increasing the EMV does help to bring small peaks over the detection threshold, it shortens the life of the multiplier. In general, the EMV should be kept at the tune voltage.

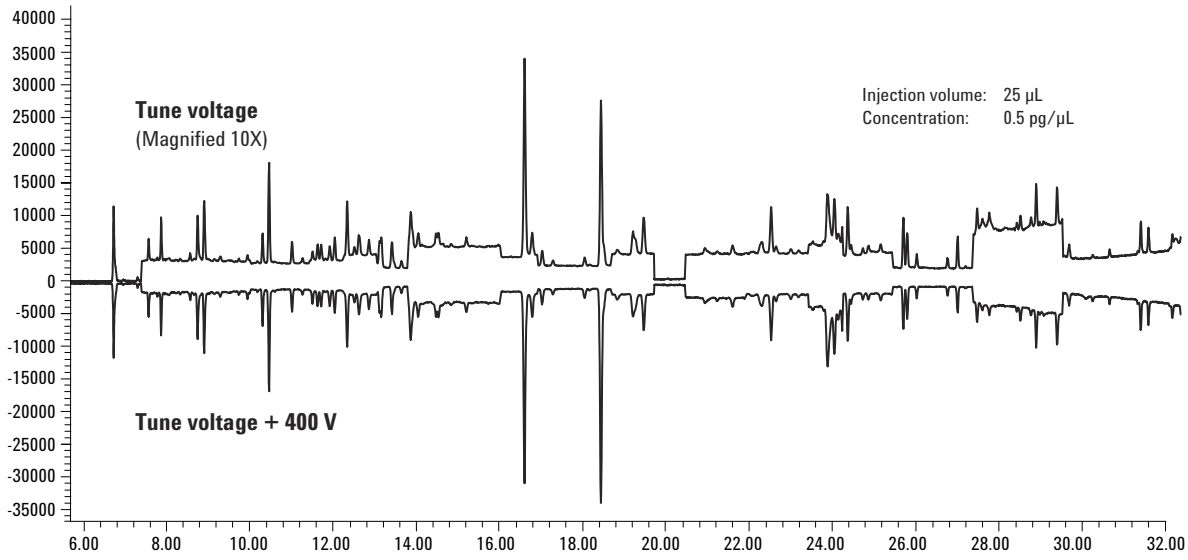


Figure 7. SIM results of 12.5 pg on column using either EMV at Tune voltage or Tune +400 V.

LVIs in Combination with SIM Methods

Combining LVI and SIM, Figures 8 and 9 show quantifiable peaks of three compounds at as low as 5 pg on column. In Figure 8, ion chromatograms of endosulfan sulfate and p,p'-DDT at 0.2 and 500 pg/µL are shown. The top chromatogram was from a 25-µL solvent-vent SIM method and the bottom chromatogram was from a 1-µL splitless Scan method. By using LVI and SIM, it is interesting to see that similar S/N ratios were achieved even with a 2500-fold decrease (from 500 to 0.2 pg/µL) in sample concentration.

By increasing the injection volume to 100 µL, samples at concentration as low as 0.05 pg/µL can also be quantified as shown in Figure 9. The top portion shows the chlorthal-dimethyl extracted ion chromatograms (EIC) of mass 299 and 301 from a 100-µL full Scan run. The bottom portion shows the same ions from a 100-µL SIM run. The SIM method shows better peak shape and lower baseline noise.

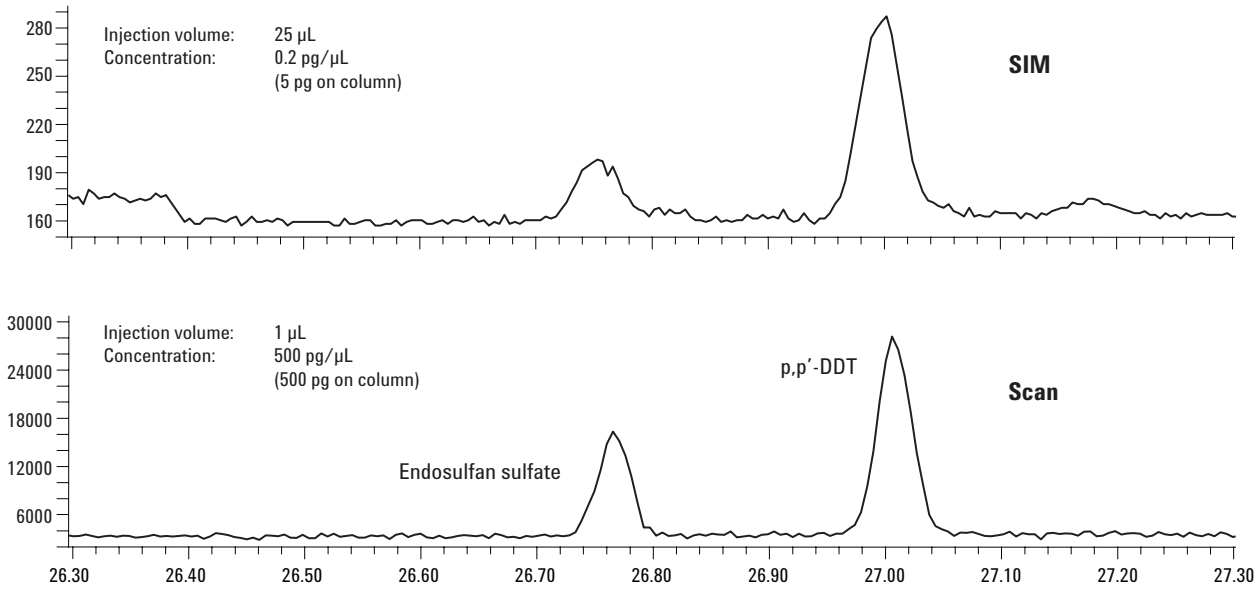


Figure 8. Ion chromatograms of endosulfan sulfate and p,p'-DDT at 0.2 and 500 pg/µL. The top chromatogram was from a 25-µL solvent-vent SIM method and the bottom chromatogram was from a 1-µL splitless Scan method.

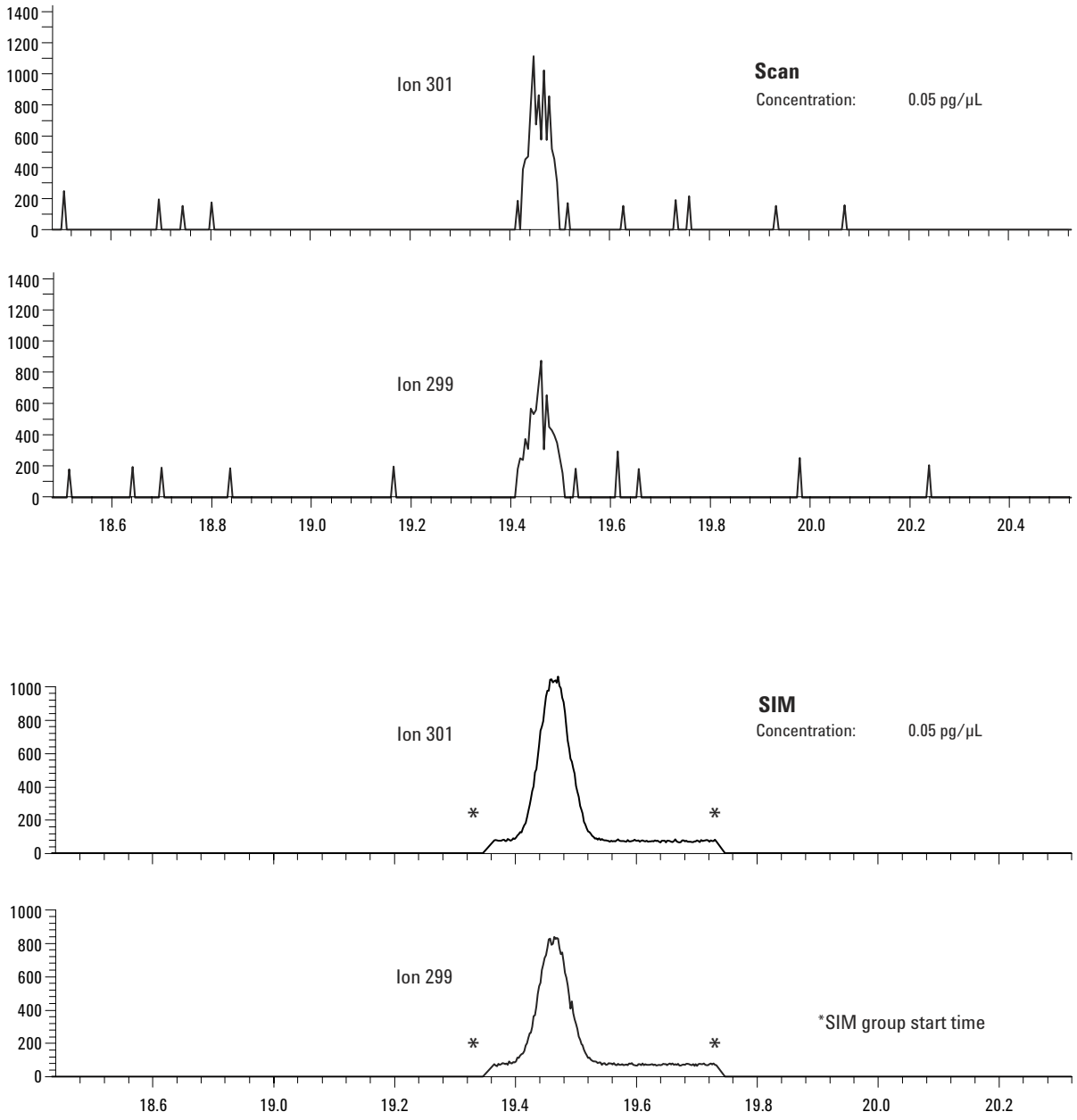
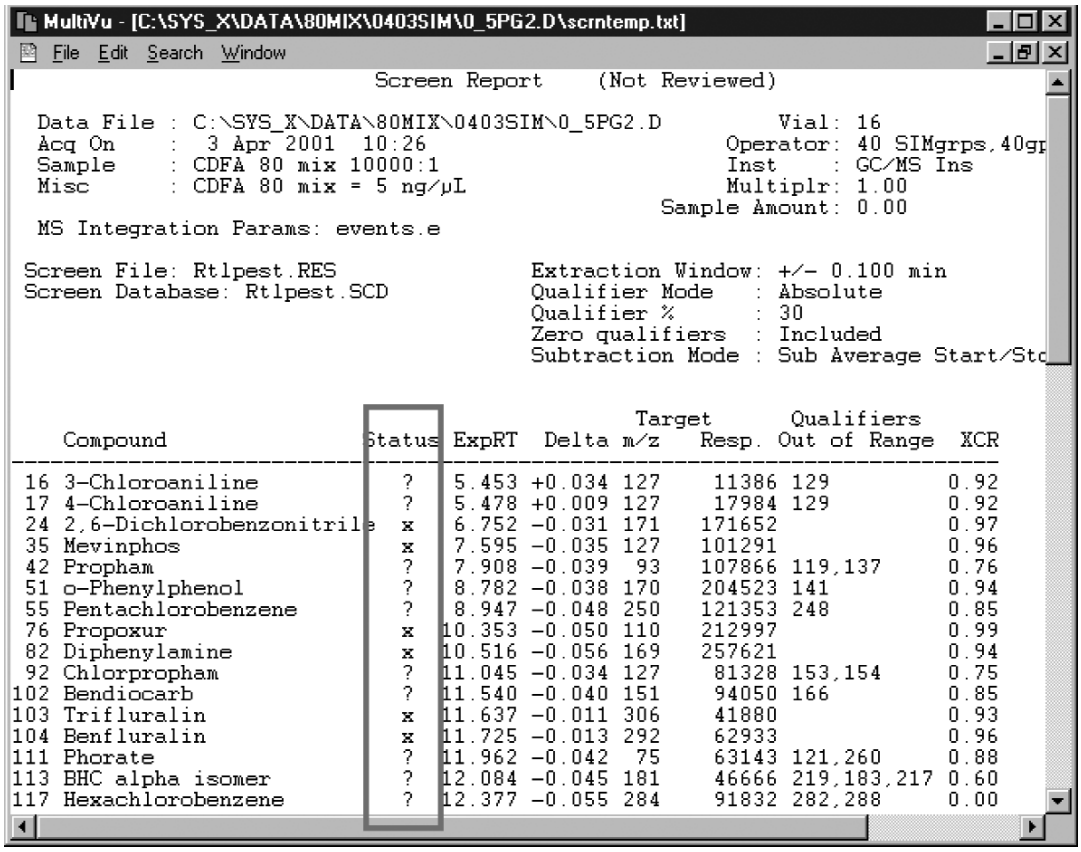


Figure 9. Ion chromatograms of 100-µL chlorthal-dimethyl injected at 0.05 pg/µL. The top portion was from a full Scan run and the bottom portion was from a SIM run.

Target Compound Screening

Combing RTL and the G1049A MSD RTL Pesticide Database/Library, a user can screen for 567 pesticides and suspected endocrine disrupters from any Scan run [5]. A user can screen a subset of the library with improved sensitivity using a SIM method. The MSD ChemStation can generate a 567-compound screening report automatically in less than 30 seconds. Figure 10 is a report of the 0.5 pg/µL sample (25 µL injected in SIM mode) that lists the "probable hits" (marked with an x) and "possible hits" (marked with a ?). All target compounds at this 12.5 pg on column level were found by the software.



MultiVu - [C:\SYS_X\DATA\80MIX\0403SIM\0_5PG2.D\scrntemp.txt]

File Edit Search Window

Screen Report (Not Reviewed)

Data File : C:\SYS_X\DATA\80MIX\0403SIM\0_5PG2.D Vial: 16
Acq On : 3 Apr 2001 10:26 Operator: 40 SIMgrps,40gp
Sample : CDFA 80 mix 10000:1 Inst : GC/MS Ins
Misc : CDFA 80 mix = 5 ng/µL Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: events.e

Screen File: Rtlpest.RES Extraction Window: +/- 0.100 min
Screen Database: Rtlpest.SCD Qualifier Mode : Absolute
Qualifier % : 30
Zero qualifiers : Included
Subtraction Mode : Sub Average Start/Sto

	Compound	Status	ExpRT	Delta	Target m/z	Resp.	Qualifiers Out of Range	XCR
16	3-Chloroaniline	?	5.453	+0.034	127	11386	129	0.92
17	4-Chloroaniline	?	5.478	+0.009	127	17984	129	0.92
24	2,6-Dichlorobenzonitrile	x	6.752	-0.031	171	171652		0.97
35	Mevinphos	x	7.595	-0.035	127	101291		0.96
42	Propham	?	7.908	-0.039	93	107866	119,137	0.76
51	o-Phenylphenol	?	8.782	-0.038	170	204523	141	0.94
55	Pentachlorobenzene	?	8.947	-0.048	250	121353	248	0.85
76	Propoxur	x	10.353	-0.050	110	212997		0.99
82	Diphenylamine	x	10.516	-0.056	169	257621		0.94
92	Chlorpropham	?	11.045	-0.034	127	81328	153,154	0.75
102	Bendiocarb	?	11.540	-0.040	151	94050	166	0.85
103	Trifluralin	x	11.637	-0.011	306	41880		0.93
104	Benfluralin	x	11.725	-0.013	292	62933		0.96
111	Phorate	?	11.962	-0.042	75	63143	121,260	0.88
113	BHC alpha isomer	?	12.084	-0.045	181	46666	219,183,217	0.60
117	Hexachlorobenzene	?	12.377	-0.055	284	91832	282,288	0.00

Figure 10. Typical report from the GC/MS pesticide screener showing probable "hits" (marked with an x) and possible hits (marked with a ?). Other information includes the library retention time followed by the RT difference in this chromatogram, the target ion, its abundance, out of range qualifier(s), and a cross correlation value with the library spectrum.

Conclusions

Using the information (compound names, retention times, and ion masses) in the RTL pesticide database, a SIM method of 80 target compounds can be created in less than 2 hours without running any analyses. The examples show that both LVI and SIM are effective techniques to decrease the quantitation limit of target compounds from sub-ppm to ppt.

Any lab can decrease the quantitation limit by a factor of 100 without any hardware modification. Lowering the quantitation limit from 500 pg down to 5 pg on column can be done using a SIM method and RTL. By adding LVI to the system, target compounds in femtogram/µL can be quantified.

Acknowledgement

The author would like to acknowledge Alex Chung and Mark Lee at CDFA for providing the pesticide mixture used in this study.

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Printed in the USA
November 14, 2001
5988-4392EN

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Analysis of Simazine, Thiobencarb, and Thiuram by Liquid Chromatography/Mass Spectrometry

Application

Environmental

Author

Hiroki Kumagai

Abstract

A liquid chromatography/mass spectrometry method using electrospray ionization in positive ion mode was successfully applied to the sensitive and simultaneous determination of the pesticides Simazine, Thiobencarb, and Thiuram.

Background

In recent years, the potential contamination of water supplies by runoff of many kinds of pesticides from golf courses and agricultural fields has become a societal problem. Many governments have established guidelines for pesticide use and water quality standards to limit such contamination. In Japan, the concentration limits in drinking water for the pesticides Simazine, Thiobencarb, and Thiuram are 3, 20, and 6 ppb, respectively.

Typically, gas chromatography-mass spectrometry (GC/MS) is used to determine Simazine and Thiobencarb in drinking water, while Thiuram is determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. However, the Thiuram method used to date has problems with both selectivity and sensitivity. A better method of analysis is needed for this chemical. Such a method is described below.

Method

- Instrument: Agilent 1100 Liquid Chromatograph/Mass Spectrometer (LC/MS) with electrospray ionization (ESI) positive ion mode
  Drying gas: N_2 (8 L/min, 350 °C)
  Nebulizer: N_2 40 psi
  Fragmentor: 40 V (Thiuram), 70 V
  Mass range: 100–500 amu
- LC Conditions:
  Mobile phase A: CH_3OH/30 mM CH_3COONH_4 (50/50)
  Mobile phase B: CH_3OH
  Gradient: 0 % to100 % B in 20 min
  Flow rate: 0.2 mL/min
  Oven temperature: 40 °C
  Injection volume: 50 µL
- Column: Inertsil ODS3, 3.1 mm id × 250 mm long × 5 µm

Sample Analysis

All three pesticides were determined simultaneously using the Agilent 1100 LC/MS. The following figures illustrate both the sensitivity and applicability of this method.

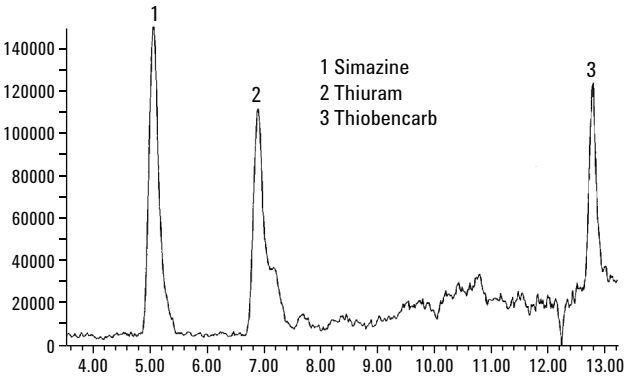

Figure 1. Total ion chromatogram of target pesticides, each at 5 ng.

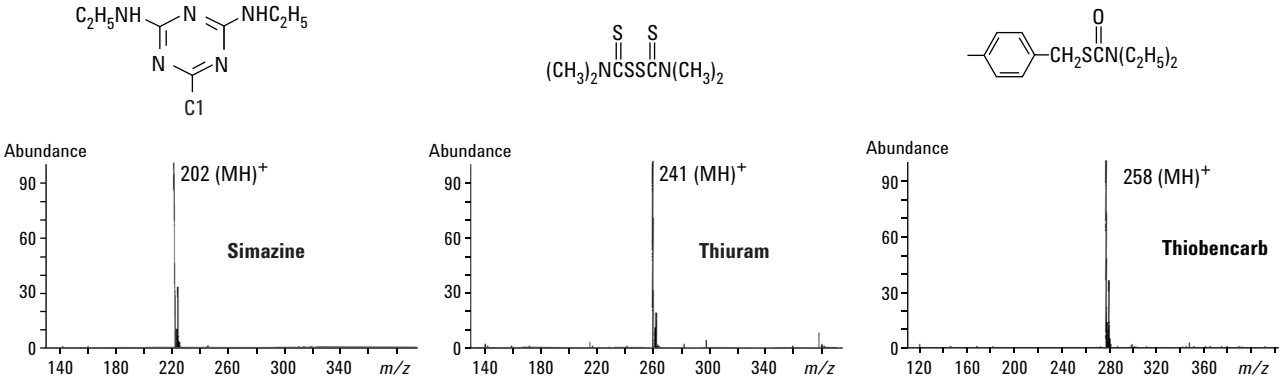

Figure 2. Mass spectra of target pesticides.

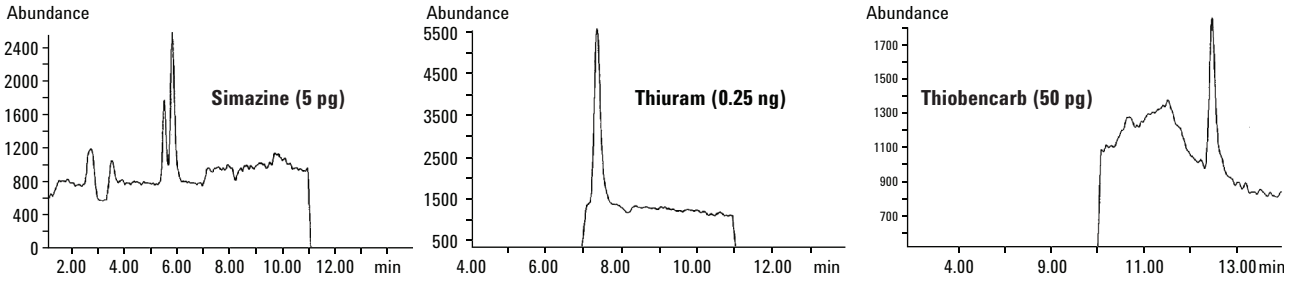

Figure 3. SIM chromatograms of target pesticides.

Conclusion

The LC/MS method described above is suitable for the simultaneous determination of the pesticides Simazine, Thiuram, and Thiobencarb. The peaks are well separated with detection limits of 0.02, 2.5, and 1 ppb, respectively, approximately 1/10 of the Japanese concentration limits.

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Printed in the USA
October 18, 2001
5988-4233EN

Agilent Technologies

The Analysis of Organophosphate Pesticides by LC/MS

Application

LC-MS

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Abstract

Organophosphate pesticides were readily analyzed using liquid chromatography-mass spectrometry with electrospray ion source. Sensitivity and selectivity were significantly better than using a diode-array UV detector.

Overview

Liquid chromatography-mass spectrometry (LC-MS) is rapidly becoming a routine technique for efficient trace analysis of polar pesticides in various types of samples. In comparison to existing methodologies, such as gas chromatography-mass spectrometry (GC-MS) and ultraviolet (UV) detection, LC-MS considerably simplifies cleanup procedures, reducing both time of analysis and method development time.[1]

LC-MS with an electrospray ion (ESI) source avoids the thermal degradation of labile pesticides encountered with GC and eliminates the need for preliminary derivatization to increase compound volatility.

Additionally, LC-MS provides unequivocal identification of each pesticide, even if the pesticide was not completely resolved from neighboring eluants. Traditional UV detection cannot provide the required specificity because many of the pesticides within the same class exhibit similar UV spectra.

Sample case

A mixture of organophosphate pesticides and an internal standard were analyzed using an Agilent 1100 LC/MS with an ESI source (Table 1).

Table 1. Mixture of Organophosphate Pesticides

Elution order	Compound	$[M+H]^+$	Concentration µg/mL
1	Mevinphos isomer 1	225	0.2
2	Dimethoate	230	0.5
3	Mevinphos isomer 2	225	0.5
4	Dichlorvos	221	0.5
5	Azinphos methyl	318	0.05
6	Parathion methyl	264	0.2
7	Malathion	331	0.5
8	Diazinon	305	0.2
9	Triphenyl orthophosphate*	327	1.0
10	Parathion ethyl	292	0.1
11	Phorate	261	0.1
12	Reldan	322	0.5
13	Ronnel	321	0.1
14	Terbuphos	289	0.2
15	Dursban	350	0.1
16	Ethion	385	0.2
17	Temephos	467	0.1

* Internal standard

Method summary

- Column 2.1 mm id × 5 cm long, filled with 3.5 µm particles, C18 chemistry
- 20 mM ammonium acetate vs. acetonitrile mobile phase gradient
  - 5 % to 95 % acetonitrile in 4 minutes
  - Hold 2 minutes
- Splitless 400 µL/min flow
- 3 µL injection volume
- Scan data 120 to 600 m/z
- SIM data as per Table 1. 95 msec dwell/ion in two groups

Results

Simultaneous UV (220 nm) and MS detector outputs are compared in Figure 1. The MS plot is a composite of all the individual extracted ion chromatograms. Each was obtained at the $[M+H]^+$ value given in Table 1, and are separated and stacked in Figure 2 for easy comparison.

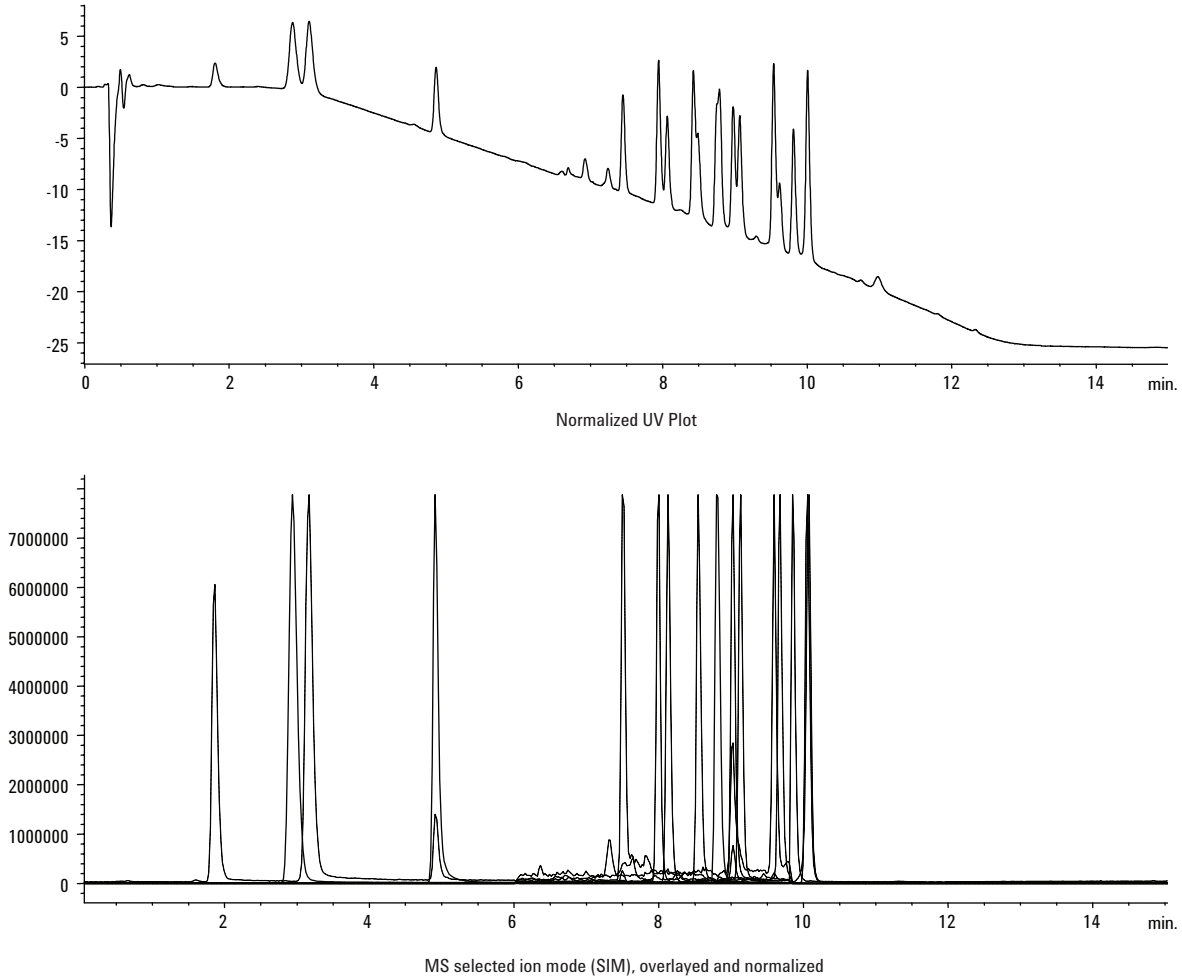

Figure 1. Comparison of UV and MS chromatograms.

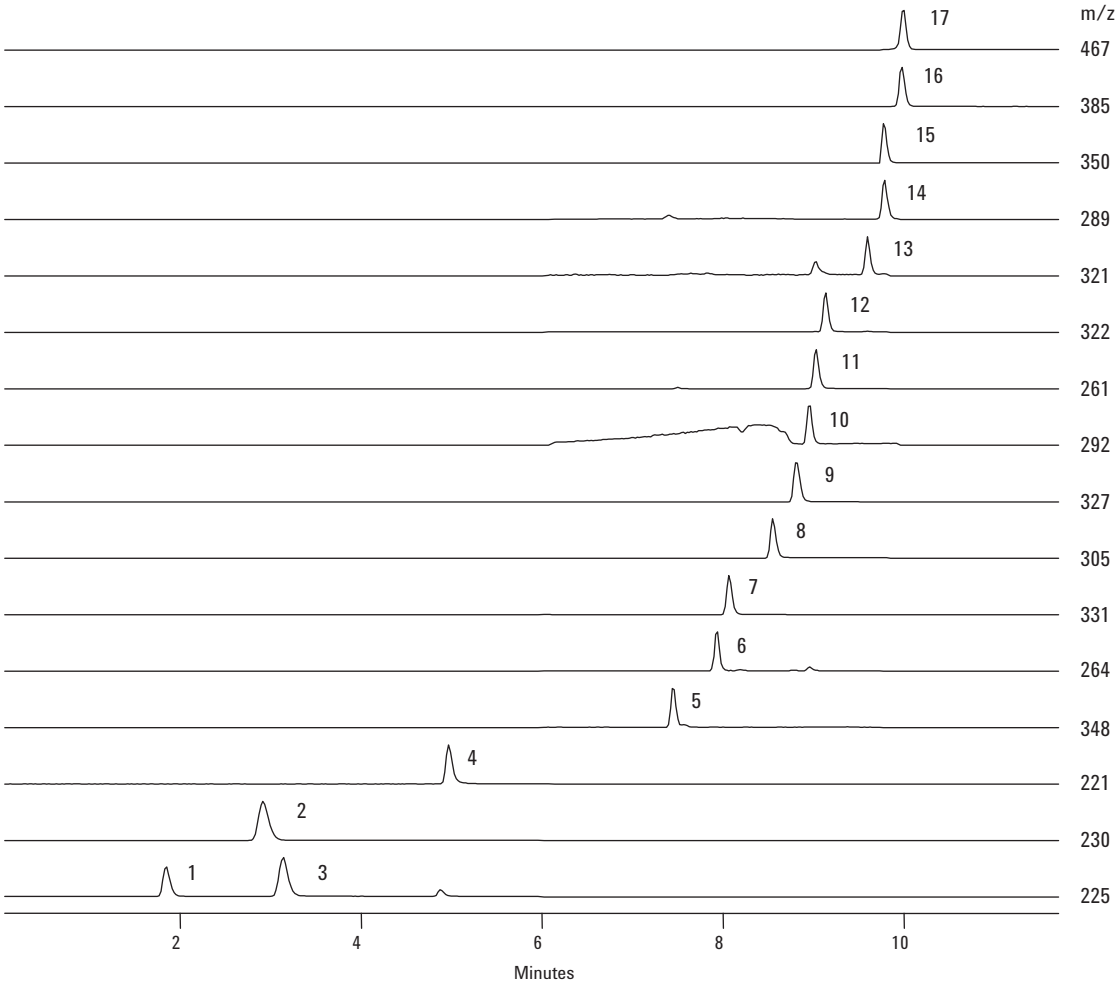

Figure 2. Stacked normalized extracted ion chromatograms for compounds 1 through 17.

Figures 3 through 6 show the resulting normalized mass ion spectra for each compound included in Table 1.

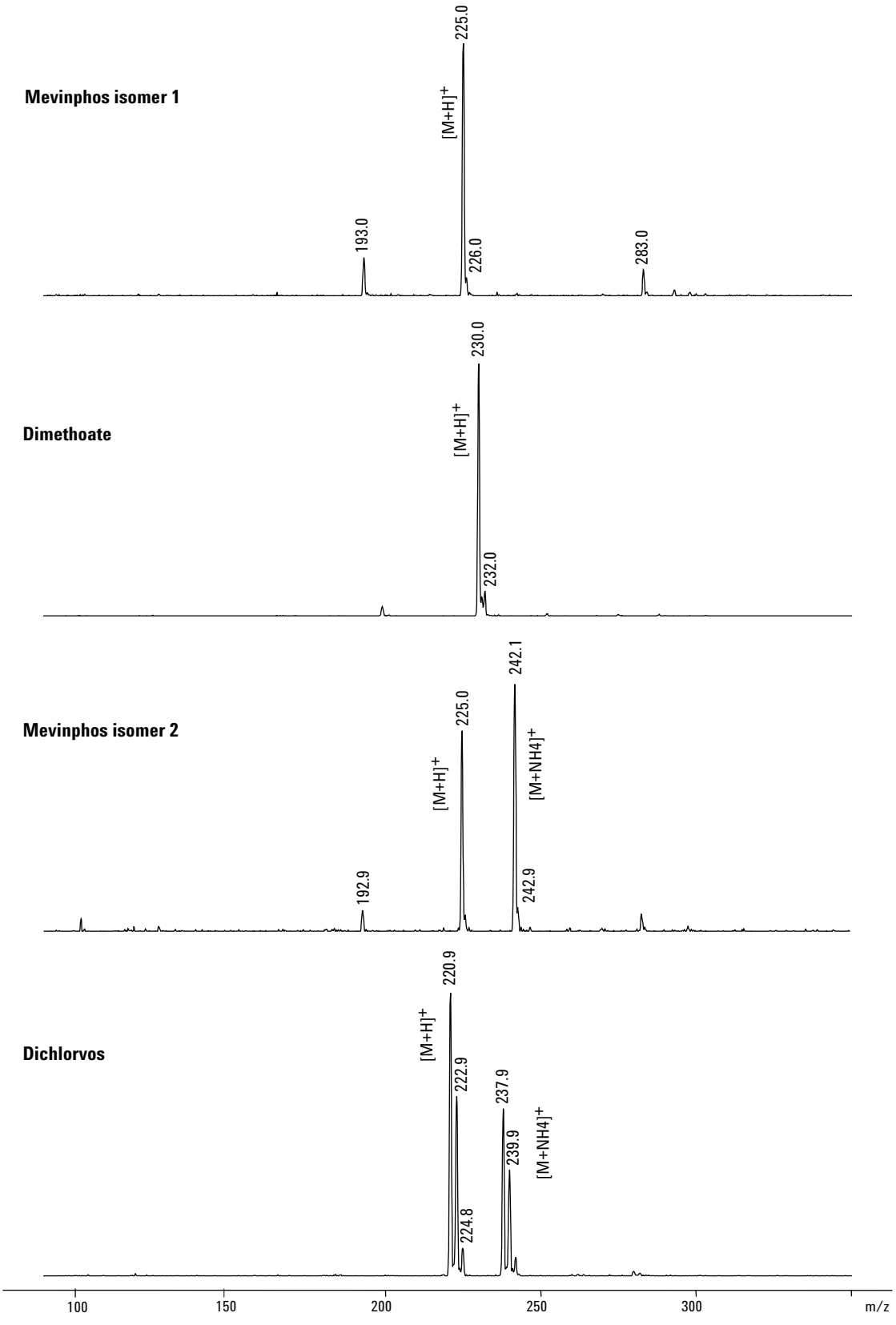

Figure 3. Stacked normalized ion mass spectra for compounds 1 through 4.

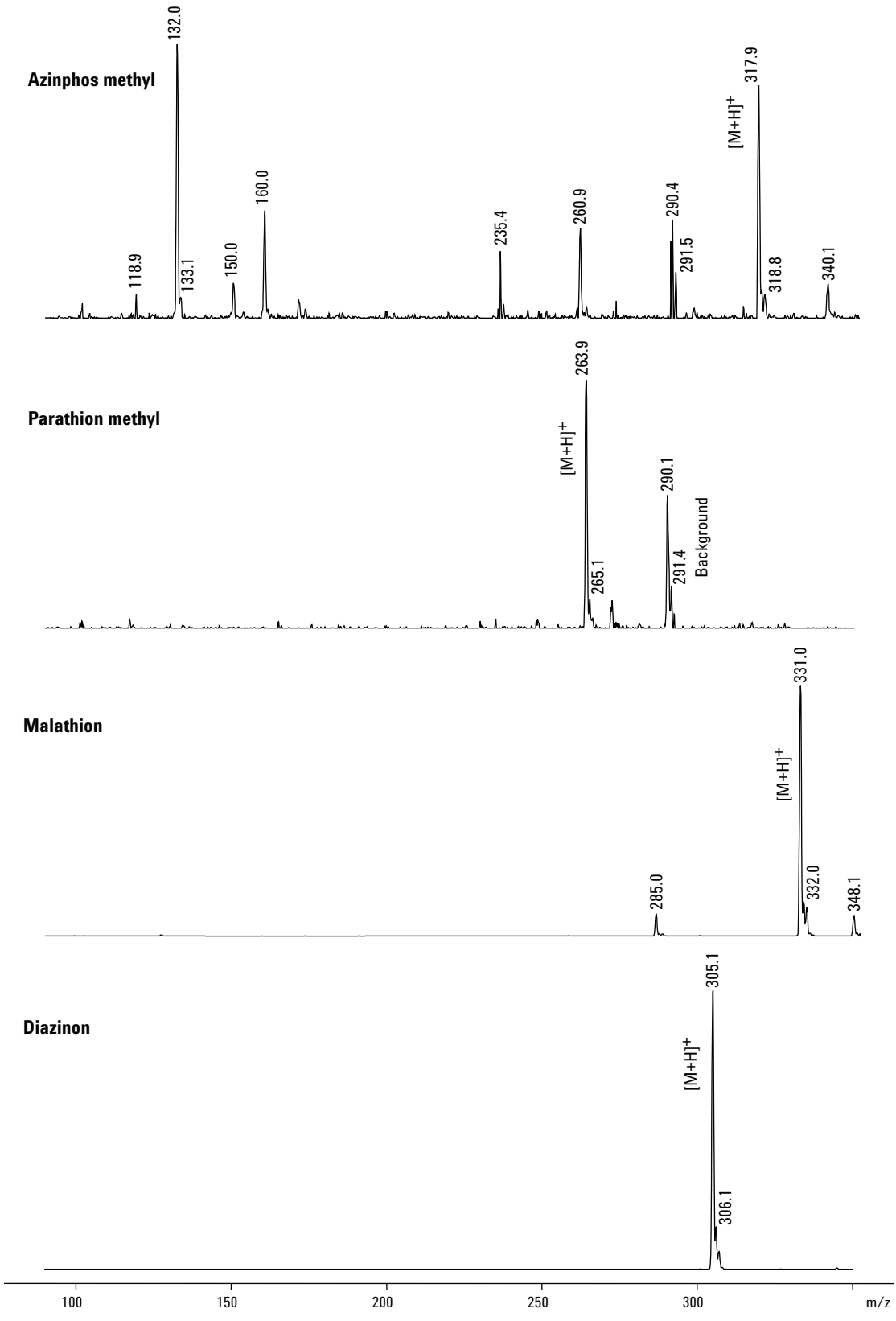

Figure 4. Stacked normalized ion mass spectra for compounds 5 through 8.

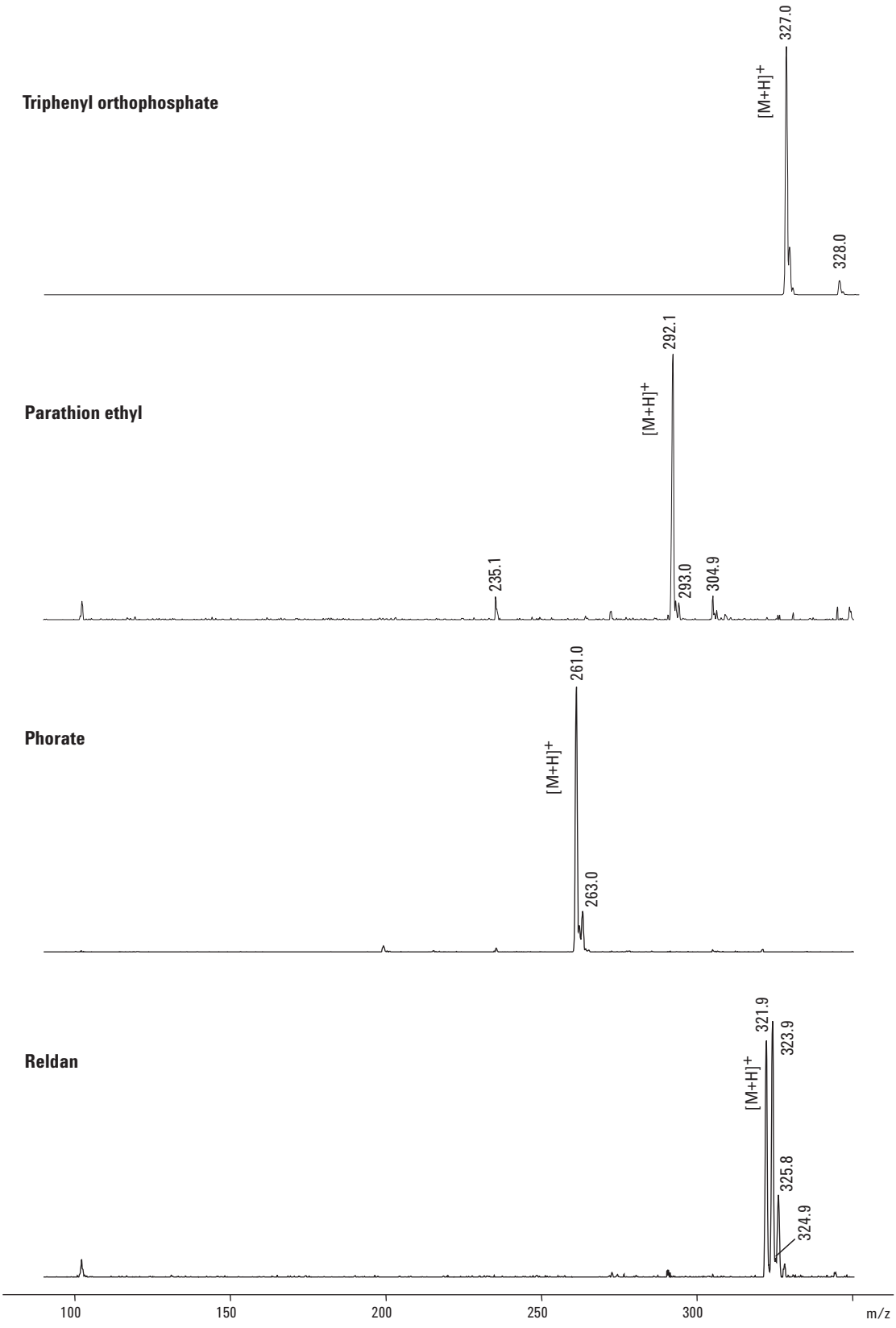

Figure 5. Stacked normalized ion mass spectra for compounds 9 through 12.

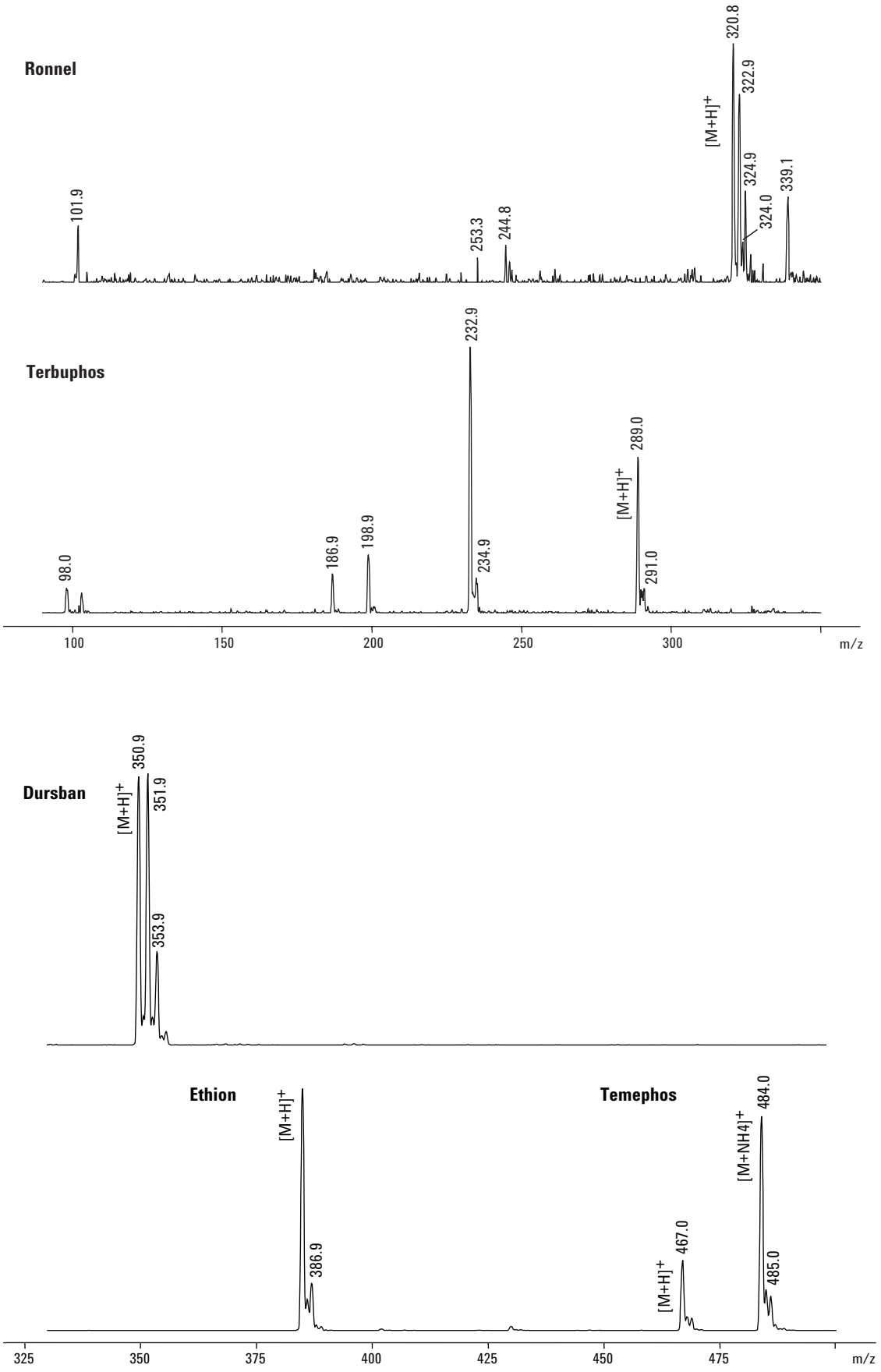

Figure 6. Stacked normalized ion mass spectra for compounds 13 through 17.

Conclusions

When determining organophosphate pesticides using LC-MS with an ESI source:

- All the tested organophosphate pesticides ionized well and gave definite $[M+H]^+$ ions
- Sensitivity and selectivity are significantly better than using diode-array UV detector
- Overall chromatography and analysis is simple and straightforward
- Positive identification and quantification are performed using integrated software

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Printed in the USA
September 13, 2001
5988-3774EN

Agilent Technologies

Application of Online Solid-Phase Extraction Ion Trap Mass Spectrometry in Environmental Matrices

Application Note

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Introduction

Sulfonylureas are a class of potent herbicides that are characterized as acetolactate synthase-inhibiting (ALS). Compared with other herbicides and pesticides, sulfonylureas are considered to be highly potent and are consequently used at much lower application rates for such purposes as agricultural weed control. This ultimately results in lower concentration levels found in real environmental samples, compared with levels found from use of other herbicides/pesticides. Sulfonylureas are thermally labile compounds and are directly metabolized in the environment. While the level of toxicity to animals from these compounds is low, their ability to persist in soil and water can cause damage to crops in later years. Therefore, in order to successfully detect, confirm, and quantify trace levels of these compounds in complex matrices such as environmental samples, LC/MS techniques are needed.

Applications involving the analysis of sulfonylurea herbicides in environmental matrices have already been investigated via several techniques.[1, 2, 3] Typically, the rate limiting steps in these determinations are the time-consuming (~13 hours) and labor-intensive, non-automated extraction and cleanup steps.

This application describes the technique of turbulent-flow solid-phase extraction (SPE) with ion trap mass spectrometric detection for the analysis of sulfonylureas in environmental samples. This whole procedure can be accomplished on line in approximately 45 minutes per sample, by combining sample extraction, cleanup, and detection into one process. This application uses a small-bore (0.5–1 mm) SPE column, typically containing 50 micron C_{18} particles, permitting sample introduction at flow rates from 1 to 10 ml/min (turbulent-flow conditions). These flow conditions minimize the time required for loading, washing, and eluting the analytes onto and off of the extraction column. Under these conditions, lipophilic analytes are efficiently trapped on the extraction column, while salts, biological materials, and other hydrophilic materials are flushed from the column with water. The retained analytes are then quickly eluted off of the extraction

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column and loaded onto the analytical column. Post column addition is used after the extraction column in order to adjust the organic content of the mobile phase to allow for more efficient trapping of the hydrophilic analytes onto the analytical column. Through the use of gradient elution programming, the level of organic content in the mobile phase is gradually increased until the analytes are eluted off the analytical column and are subsequently introduced into the ion trap mass spectrometer. The ion trap offers the advantage of MS/MS to improve specificity and detection limits in dirty matrices, which would not be achievable with UV or single-MS operation.

Experimental

All experiments were conducted using samples obtained from North Carolina sources of surface water. Samples of lake, pond, and runoff water were initially spiked at the 0.2 and 2.0 part-per-billion (ppb) levels using a 1 mg/ml solution of 12 sulfonylureas and 1 sulfonamide herbicide standard prepared in acetonitrile. A method blank was prepared in the same manner from the unspiked well water. Solvent standards were prepared in deionized water and spiked at 0.1, 0.3, 1.0, 3.0 and 10 ppb levels.

Online SPE LC/MS/MS was performed using a modified Cohesive 2300 High Throughput Liquid Chromatography system coupled to an Agilent Technologies ion trap mass spectrometer. The components of the LC system were as follows: an Agilent 1100 Series isocratic pump and autosampler, a Perkin-Elmer series 200 binary pump, and a Cohesive Technologies 2300 valve switching unit. A Cohesive Turbo-C18 (1.0 x 50 mm, 50 µm) column was used as the online extraction column. A Metasil Basic (2.0 x 100 mm, 5 µm) column was used as the analytical column. Four solvents were used to achieve online extraction and chromatography: loading solvent (A) = water (0.1 M ammonium acetate and 0.1% (v/v) acetic acid); extraction solvent (B) = 9:1 acetonitrile/ water; binary solvent (C) = 95:5 water/acetonitrile (v/v) with 1% acetic acid (v/v); and binary solvent (D) = 60:40 acetonitrile/water with 1% acetic acid (v/v).

The Cohesive 2300 valve switching unit has two valves: a column direction valve and a source/destination valve, which are operated independently. The system can be plumbed in either a single-column mode or dual-column mode (Figure 1). In the single-column mode, the first valve controls the direction of flow through the extraction column, while the second valve simultaneously controls the source of flow to the extraction column (binary pump or isocratic pump) and the destination of the extraction column eluent (mass spectrometer or waste). In the dual-column mode, the first valve controls direction of flow through the extraction column and diverts the flow path prior to the extraction column through a 100 µl loop. The second valve selects the destination of the eluent from the first extraction column either to waste or to the second analytical column. Experiments for this application were conducted in dual-column mode.

The online SPE LC/MS/MS process was initiated by the injection of 1.8 ml of the spiked water samples into the analytical system. Each sample was first passed through a 0.45 µm filter and then loaded onto the extraction column with the loading solvent (A). Elution of the analytes from the extraction column was carried out with the extraction solvent (B), which was contained in the 100 µl loop. Although a strong organic content (90%) elution solvent is required to elute the analytes from the extraction column, it presents a problem with the subsequent retention of some analytes on the analytical column. Due to the strong organic content of (B), some of the more hydrophilic analytes will not be retained on the analytical column. Therefore, a binary solvent (C) is added post extraction column, via a mixing tee, to reduce the organic content from 90% to approximately 17%. This allows all analytes to be more efficiently retained. This was followed by a gradient chromatographic separation from the analytical column using binary solvents (C) and (D) and then introduction into the ion trap mass spectrometer for mass analysis. For these MS/MS mass determinations, the $[M+H]^+$ ion for each compound was isolated. In addition to MS/MS mode, the ion trap was also operated in full scan mode.

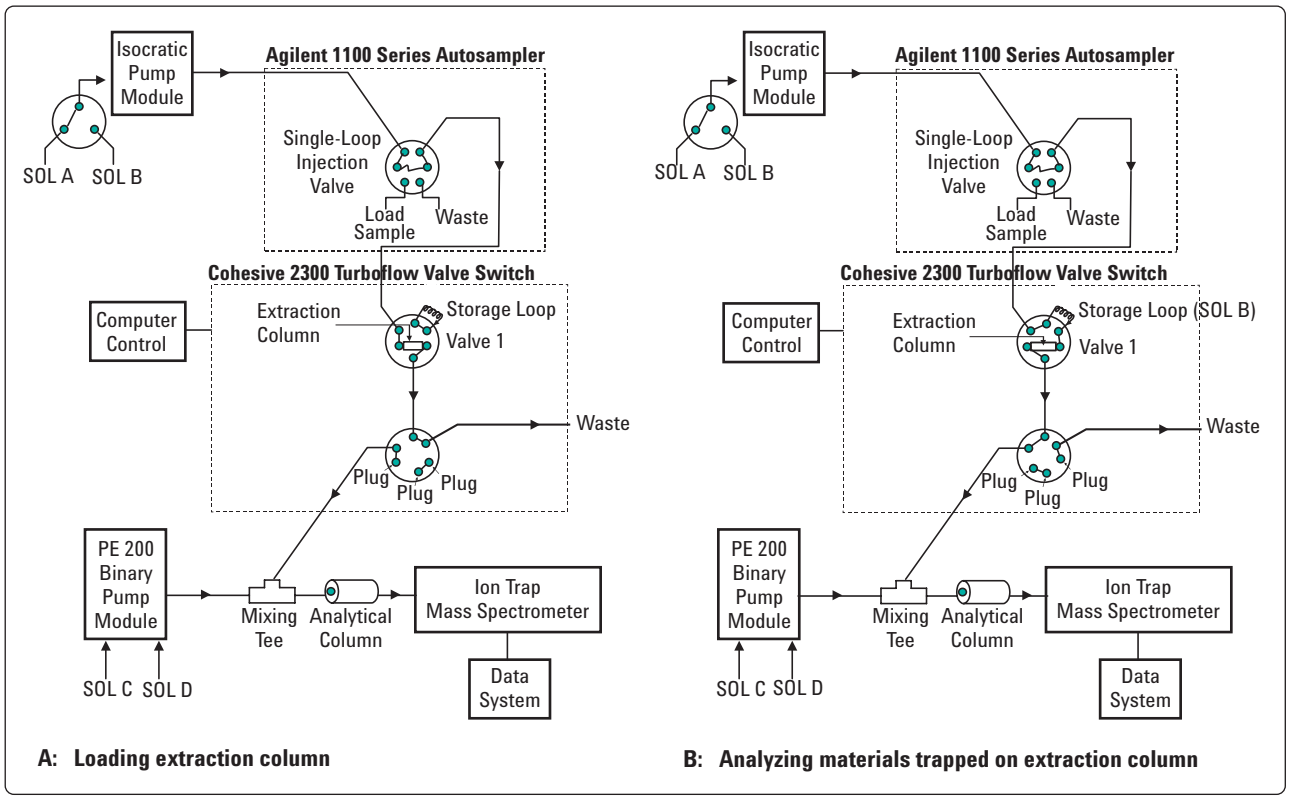

Figure 1. Analytical system with valve switching unit plumbed in dual-column mode

Results and Discussion

Several parameters for the MS/MS experiments on the ion trap had to be optimized in order to maximize the $[M+H]^+$ ion for each compound of interest as observed in full scan mode. Isolation times, fragmentation times, and fragmentation energies were all optimized to maximize the signal-to-noise ratio for a single product ion for each compound. For the detection of numerous target compounds, it was shown that the dual-column SPE approach was superior to the single-column configuration. The time-programmed chromatographic separation provided adequate separation for each compound, allowing MS/MS to occur with sufficient points across each peak to result in the best possible sensitivity and quantitative accuracy.

Figures 2 and 3 show a comparison of LC/UV, LC/MS (selected ion monitoring, single-quadrupole MS), and SPE LC/MS/MS (ion trap MS/MS) chromatograms of all 13 compounds at the limit of quantitation (LOQ)–200 pg/ml. Figure 2 represents the solvent standard, while Figure 3 shows the dirtiest matrix (marsh water sample). Chromatograms (A) and (B) represent 50 ng on column, while chromatogram (C) represents 400 pg on column. It can be seen that MS/MS removes the background noise allowing an increase in specificity and sensitivity for all compounds, especially in the dirtiest matrix. The 200 pg/ml LOQ could not be achieved using LC/UV or LC/MS in this matrix. Figures 4 and 5 show LC/ion trap MS/MS spectra for two representative sulfonylureas in marsh water. Ions observed for each compound, their respective RSDs, and recoveries are shown in Table 1. Table 2 shows the linearity and dynamic range over the calibration range of 100–10,000 pg/ml.

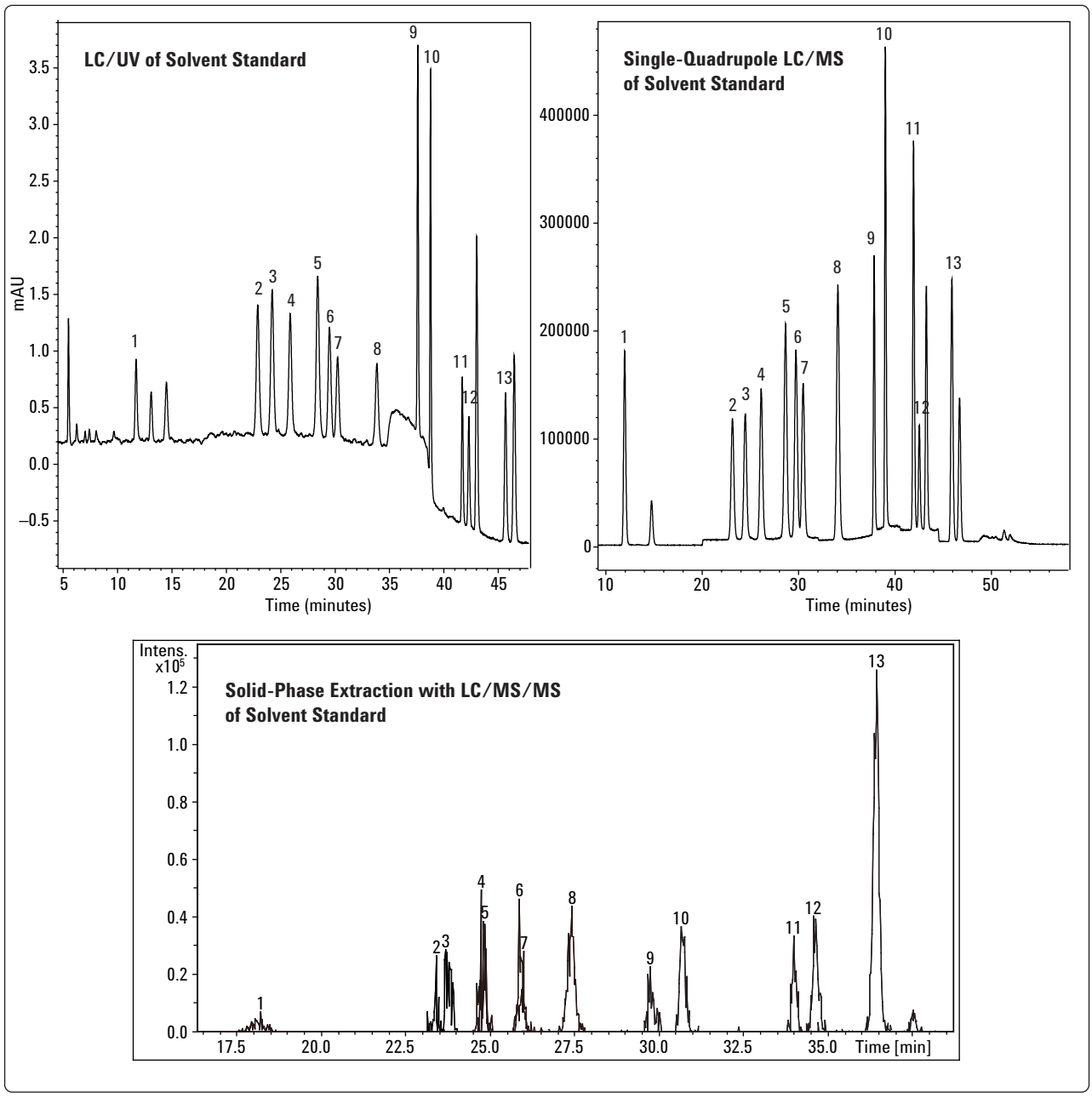

Figure 2. Chromatographic results for LC/UV (A), LC/MS (B), and SPE LC/MS/MS (C) analysis of solvent standard (0.2 ppb)

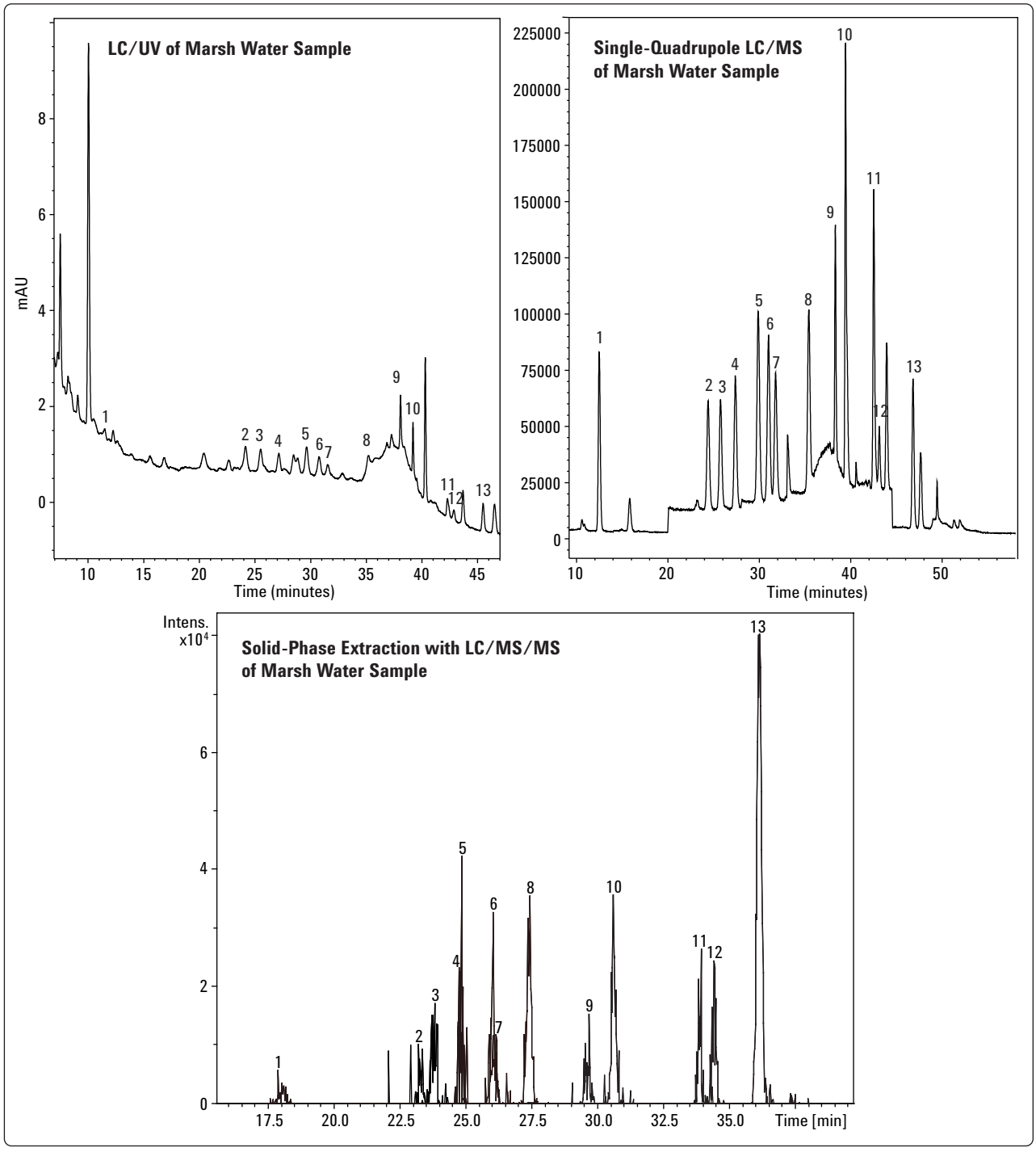

Figure 3. Chromatographic results for LC/UV (A), LC/MS (B), and SPE LC/MS/MS (C) analysis of marsh water sample (dirtiest matrix)

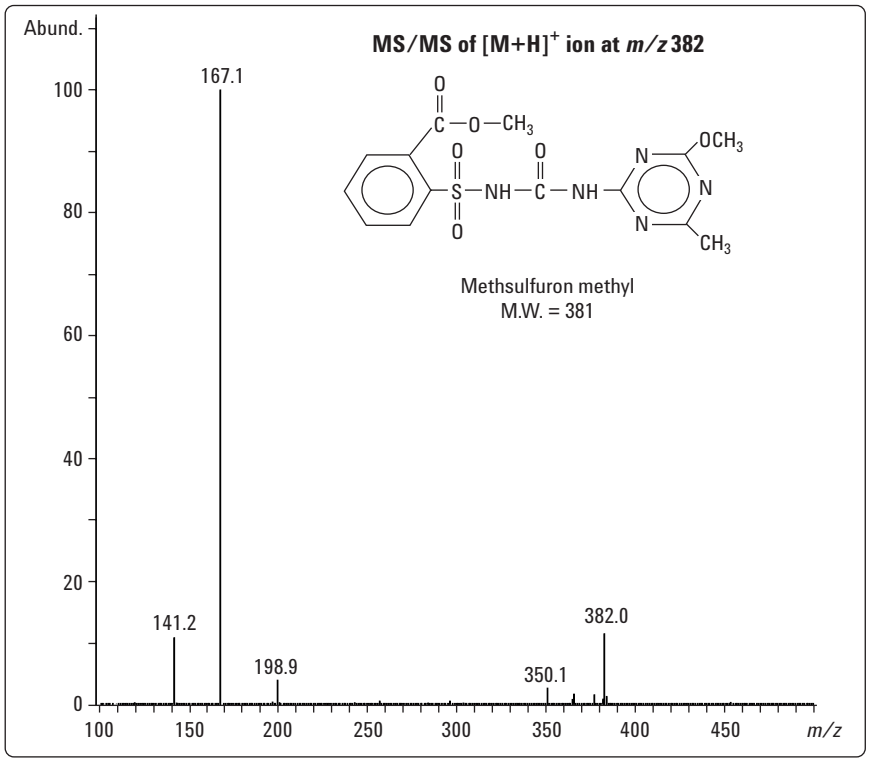

Figure 4. LC/ion trap MS/MS mass spectrum of metsulfuron methyl obtained from a 1 ng/ml spike of sulfonylureas in marsh water. The CID mass spectrum was generated from the $[M+H]^+$ ion.

Figure 5. LC/ion trap MS/MS mass spectrum of chlorimuron ethyl obtained from a 1 ng/ml spike of sulfonylureas in marsh water. The CID mass spectrum was generated from the $[M+H]^+$ ion.

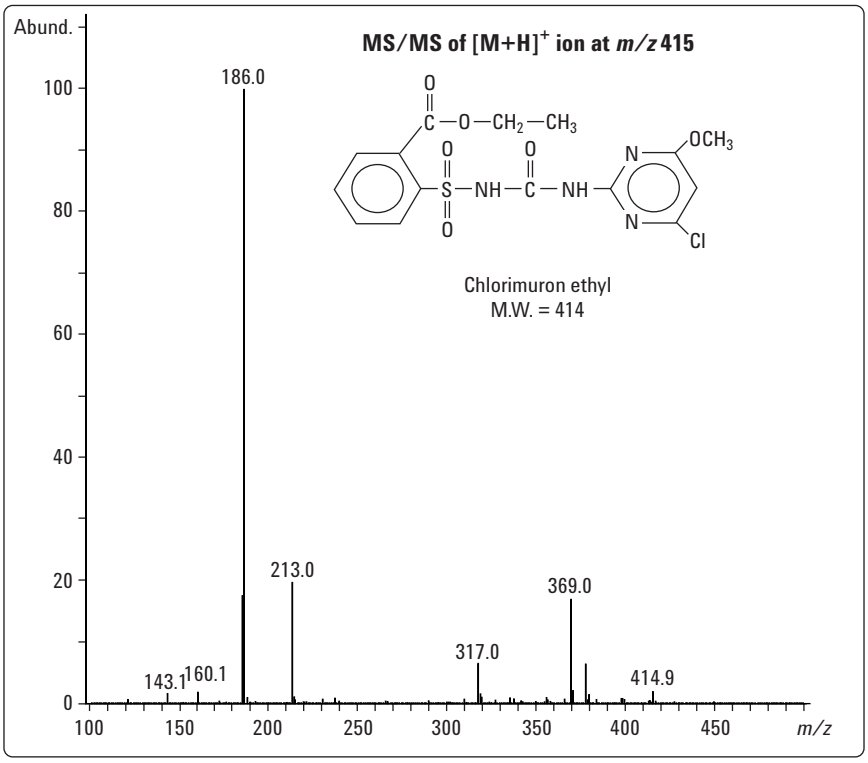

Table 1. SPE LC/MS/MS results (LOQ = 200 pg/ml)

Compound	$[M+H]^+$ (*m/z*)	MS/MS Fragment Ions (*m/z*)	Solvent Standard		Marsh Water	
			% Recovery	% RSD	% Recovery	% RSD
Flumetsulam	326	129*, 192, 262	92	5.9	58	6.7
Oxasulfuron	407	150*, 210, 284, 258	112	9.4	95	8.0
Thifensulfuron methyl	388	141, 167*	104	13.0	62	14.0
Metsulfuron methyl	382	141, 167*	114	32.0	81	23.0
Sulfometuron methyl	365	106, 150*, 199, 333	124	18.0	85	7.2
Triasulfuron	402	141, 167*, 359	108	7.5	87	7.6
Chlorsulfuron	358	167*	73	19.0	55	9.7
Ethametsulfuron methyl	411	142, 168, 196*	110	2.8	97	7.3
Sulfosulfuron	471	165, 211*, 265	100	5.9	88	6.1
Bensulfuron methyl	411	119, 149*, 182, 213	116	5.2	102	7.1
Prosulfuron	420	141*, 167	110	2.0	78	3.3
Chlorimuron ethyl	415	186*, 213, 369	101	12.0	74	6.7
Triflusulfuron methyl	493	238, 264*, 460	98	3.8	95	3.6

*ion used for quantitation

Table 2. Linearity and dynamic range for SPE LC/MS/MS over the calibration range of 100–10,000 pg/ml

Peak Number	Compound	Linear Correlation Coefficient	Residual
1	Flumetsulam	0.9987	2.8
2	Oxasulfuron	0.9988	17.1
3	Thifensulfuron methyl	0.9977	6.9
4	Metsulfuron methyl	0.9987	1.8
5	Sulfometuron methyl	0.9994	4.4
6	Triasulfuron	0.9990	5.2
7	Chlorsulfuron	0.9989	1.3
8	Ethametsulfuron methyl	0.9993	3.5
9	Sulfosulfuron	0.9987	7.8
10	Bensulfuron methyl	0.9971	–4.4
11	Prosulfuron	0.9968	3.6
12	Chlorimuron ethyl	0.9843	–13.6
13	Triflusulfuron methyl	0.9970	8.7

Recoveries from the solvent-standard sample range from 73% to 124%, with percent RSDs for 11 of the 13 compounds < 15%. Both of these parameters are within acceptable EPA tolerances. The few excessively large RSDs are likely due to interfering compounds in the sample extracts. The recoveries from the marsh water sample range from 55% to 102%, with percent RSDs for 12 of the 13 compounds < 15%. It can be seen from Table 2 that the linear correlation coefficients are all < 0.985 with residuals all less than ±18%, using quadratic fits.

In comparison to the method used here, the current North Carolina State Laboratory and EPA methods for the analysis of sulfonylurea and sulfonamide herbicides include an off-line SPE pre-concentration step, a sample cleanup step with anion-exchange and alumina cartridges, and a 50-minute chromatographic separation with detection by UV and/or MS.[1] The methods employed here reduce the time of extraction and analysis by 94%.

Conclusions

This application has shown that turbulent-flow SPE is an excellent time-saving technique when combined with ion trap MS/MS for the analysis of environmental samples. Time of extraction and analysis was reduced dramatically (45 minutes vs. 765 minutes), sample size reduced (1.8 ml vs. 250 ml), and a lower LOQ (0.2 ppb vs, 2–10 ppb) was established over the current EPA method. Fouling of the mass spectrometer or analytical column due to dirty samples was also avoided.

Recovery and reproducibility are acceptable under EPA criteria; sensitivity and specificity were shown to have increased. Some loss in recoveries was noted with the dirtier matrices. This may be due to matrix effects and suppression of ionization. The increase in specificity and sensitivity provided by the use of MS/MS can partially compensate for the loss of recoveries due to matrix effects.

Future work targeting increasing trapping efficiency through the use of other SPE column dimensions and/or packing materials will attempt to further increase the time efficiency of the online analysis and allow applications to multi-residue and multi-matrix analyses.

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Printed in the U.S.A. August 15, 2001
5988-3649EN

Agilent Technologies

The Analysis of Dioxin Using a Benchtop Mass Spectrometer

Application

6890/5973 Gas Chromatograph/Mass Selective Detector

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Abstract

Currently the analysis of dioxins uses high resolution mass spectrometry, (HRMS); often considered a prohibitively expensive technique. To move to a more cost-effective approach, improvements in the analytical method (sample cleanup and chromatographic separation) as well as improvements in sensitivity of benchtop mass spectrometers were needed.[1, 2]

Compared to earlier generations of benchtop mass spectrometers, the 5973 mass selective detector offers measurably greater sensitivity for electron-impact-based detection due to a number of innovative enhancements.[3]

Analysis via the GC/MSD System

This work focused on determining the detection limit for 2,3,7,8-tetrachlorodibenzo-p-dioxin with a GC/MSD system configured as outlined in this note. The desired analytical goal was to detect 0.2 pg.

A submitted sample [5 pg/µL (ppb); 2,3,7,8-TCDD in 95/5 hexane/ether] was diluted by a factor of 100 with pure hexane. (The hexane was analyzed for response at the appropriate masses prior to use to verify its purity with respect to this analyte.)

For both concentrations, the mass ratio 319.9/321.9 was measured to confirm appropriate isotopic performance. Moreover, the response factors for m/z = 321.9 were determined for both levels and compared to verify linearity over a large concentration range.

Results

The mass ratio of 319.9/321.9 is 78%, correctly reflecting appropriate isotopic abundances. Comparing the response ratios of 0.05 pg and 5 pg injections (1 µL each level), we observed that those were nearly equal: 24.6 and 23.0 (2302.6 ÷ 100).

The signal/noise for the 0.05 pg injection is about 4:1 peak/peak, representing an approximate detection limit on the system used.

The conclusion is that the sensitivity of a 5973 MSD operating as an electron impact instrument is well-suited to trace analysis of dioxins, making it a cost-effective instrument for use in EPA Methods 625 and 613.[4] For 2,3,7,8-TCDD, the detection limit with the 5973 is comparable to using HRMS. Note, however, that the ultimate method detection limits will depend on other factors — e.g., the sample matrix, type of sample cleanup used, etc. Additional sensitivity may be possible by using large volume injection techniques.[3] Future experiments will aim at evaluating the NCI (negative chemical ionization) performance of the 5973 for further gains in sensitivity and selectivity.

This will mean that a laboratory manager can choose configurations of both the chromatograph and the MSD to best match the needs of a laboratory workload. The work on the system described here demonstrated greatly enhanced sensitivity provided by cost-effective benchtop mass spectrometry.

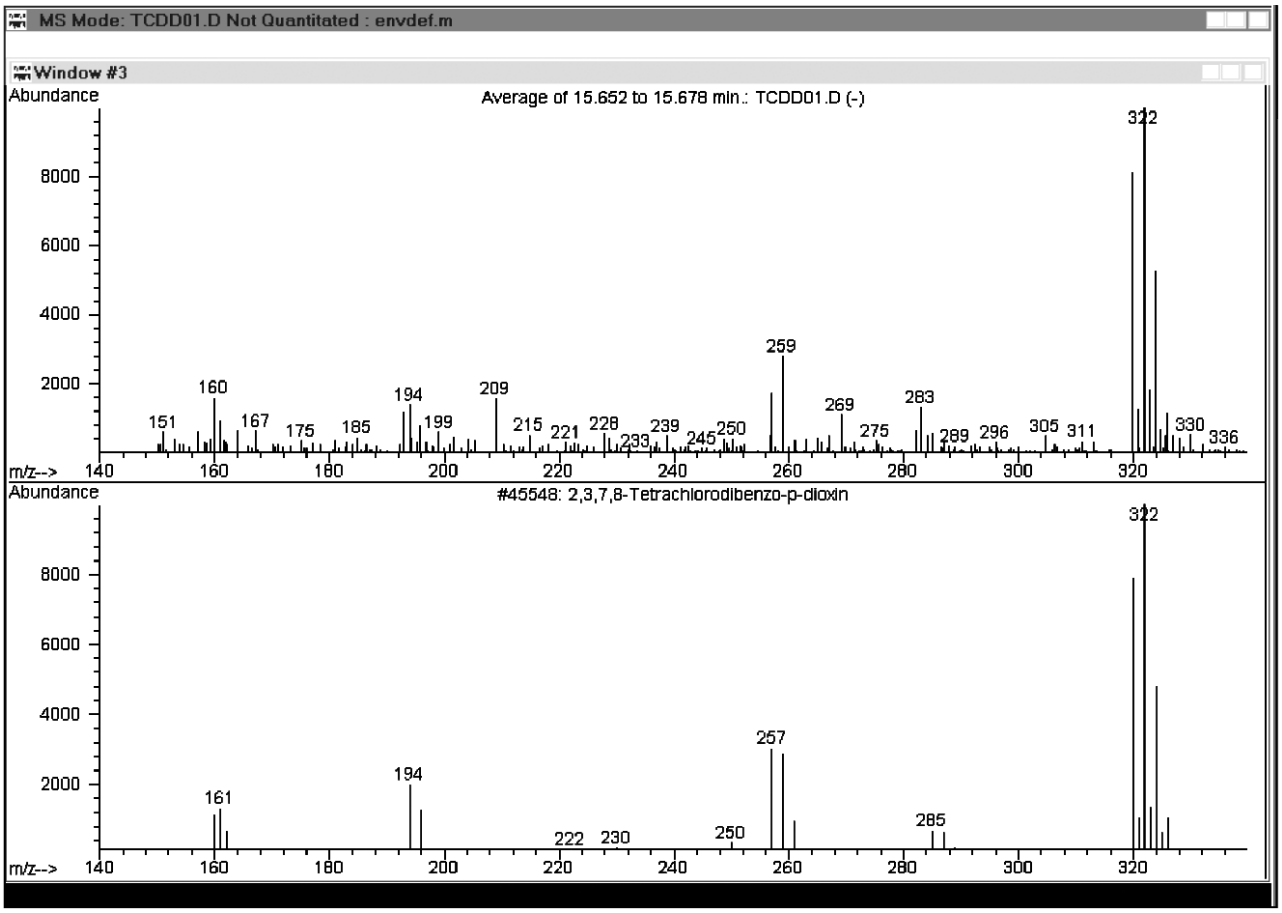

Figure 1. The match of the spectum for 5 pg 2,3,7,8-tetrachlorodibenzo-p-dioxin with the library search (lower panel). The match quality was 90%.

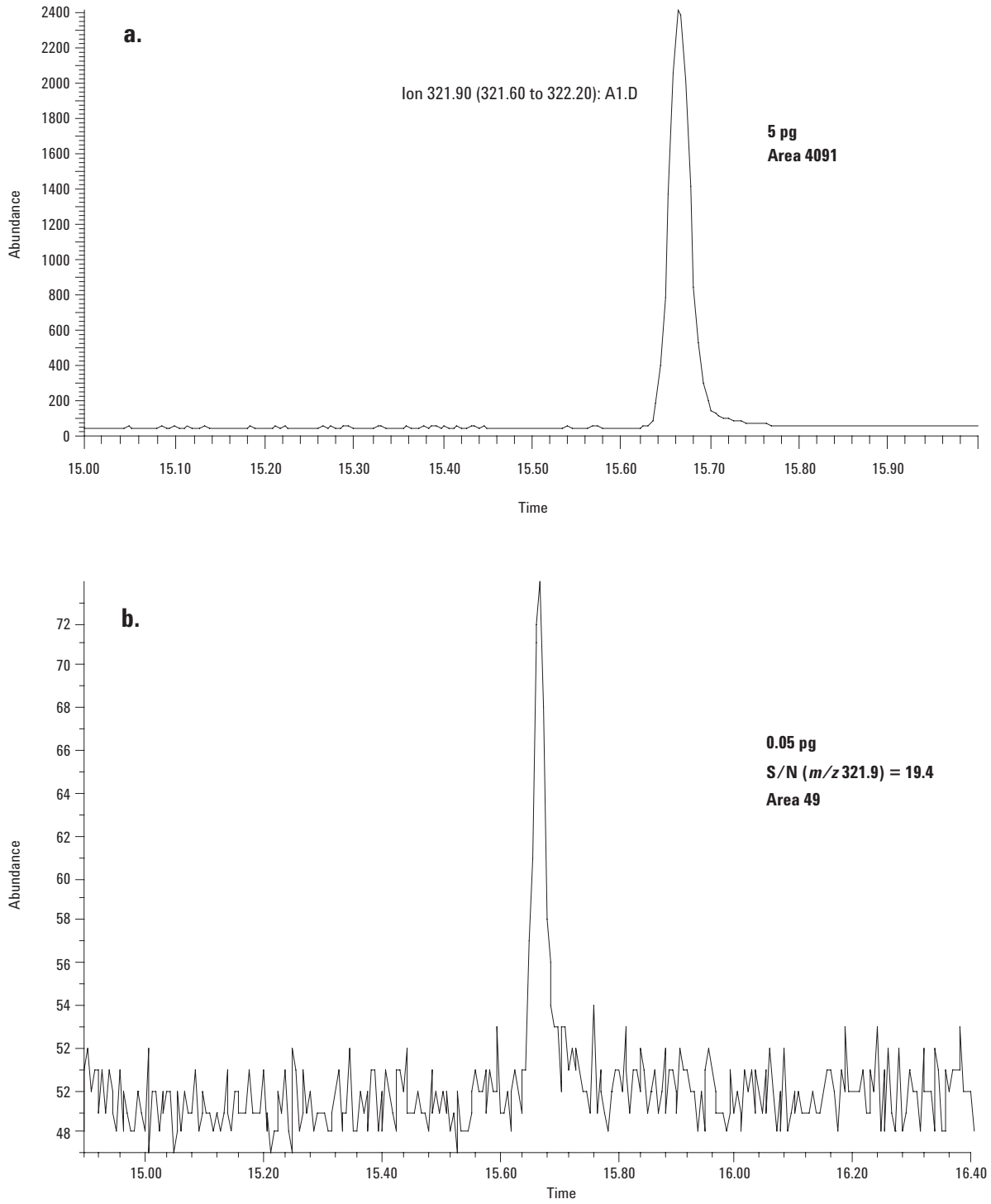

Figure 2. The TIC at *m/z* 321.9 for injection quantities of 5 pg (a.) and 0.05 pg (b.) in SIM mode.

6890 with 5973 MSD

Injection	• Pulsed splitless single taper liner with glass wool plug, P/N 5062-3587. • 250 °C • 1 µL injection volume • Viscosity delay, 1 sec • Sample washes, 3; post-injection solvent washes, 4
Column	HP-5MS: 30 m × 250 µm, 0.25 µm film (crosslinked 5% Ph Me Siloxane), P/N 19091S-433
Carrier	Helium, 37 cm/sec; vacuum compensation, on.
Temperature	Initial: 70 °C for 1.50 min
Program	Rate 1: 25.00 °C/min to 150 °C Rate 2: 10.00 °C/min to 280 °C Final: 280 °C for 0.00 min
Pressure	25.0 psi for 1.50 min; then
Program	1.0 mL/min constant flow rate
MSD	• Temperatures Transfer line = 300 °C Source = 230 °C Quadrupole = 106 °C • Tune = autotune • Emission current = 35 µamp • SIM mode, EMV = Autotune + 400 V • Solvent delay = 14.00 min • Dwell per ion = 125 msec • SIM Ions (*m/z*): 319.9, 321.9
Autosampler	7673B
ChemStation	G1701AA

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1. P. R. Gardinali et al, Chemosphere 32 (1), pp. 1-11 (1996).
2. R. Malisch et al, Chemosphere 32 (1), pp. 31-44 (1996).
3. L. Doherty, "Enhancing Pesticide Analysis with a Highly Sensitive GC/MSD System," Application Note, Pub. No. (23) 5966-0370E (1997).
4. Code of Federal Regulations, Title 40, Vol. 13, Parts 136-149, Appendix A. Revised, July 1, 1997. U.S. Government Printing Office (via GPO Access; CITE: 40CFR136). Method 613 - 2,3,7,8-tetrachloro-dibenzo-p-dioxin by GC/MS (SIM). Method 625 - Base/Neutrals and Acids, Semivolatiles by GC/MS (SCAN).

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Printed in the USA
April 2, 2001
(23) 5966-4294E

Agilent Technologies

Fast Screening of PCB Congeners Using the Agilent 6890/5973N GC/MSD System

Application

Gas Chromatography

May 2000

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Abstract

Agilent Technologies' fast GC/MSD method can significantly speed up the screening of PCB congeners. Agilent's GC method translation software (available free from the Agilent Technologies Web site, http://www.chem.agilent.com/cag/servsup/usersoft/main.html#mxlator) was used in developing the new method based on a standard 42-min method. A 15-m × 0.25-mm × 0.25-µm Agilent HP-5MS column was used to increase analysis speed up to four-fold. The time savings were implemented in increments (down to 10.5 minutes) to verify the predictability of scaling and the affect of scaling on the signal-to-noise ratio.

Key Words

RTL, PCB, polychlorinated biphenyls, congeners, environmental, screening, fast GC, method translation, 5973, 6890, MTL

Introduction

Polychlorinated biphenyls (PCBs) are a group of 209 individual compounds (known as congeners) with varying harmful effects. Chronic (long term) exposure to some PCB formulations by inhalation in humans results in respiratory tract symptoms, gastrointestinal effects, mild liver effects, and effects on the skin and eyes such as chloracne, skin rashes, and eye irritation.

PCBs are no longer produced in the United States and are no longer used in the manufacture of new products. Smaller amounts of PCBs may be released to the air from disposal sites containing transformers, capacitors, and other PCB wastes, incineration of PCB-containing wastes, and improper disposal of the compounds to open areas. Today, PCBs are still detected in water and soil due to the environmental recycling of the compound. PCBs have been detected in foods and they bio-accumulate through the food chain, with some of the highest concentrations found in fish.

The analysis of PCBs normally is accomplished using GC with an electron-capture detector (ECD). Because of the drastically different toxicity of the different congeners, it is of great interest to identify the individual congeners using a mass spectrometer (MS).

Agilent Technologies has developed techniques to solve the peak identification problem based on Agilent's retention time locking (RTL) and a mass spectral library that contains the locked retention times and characteristic ions for all 209 PCB congeners. A GC/MSD method was developed based on a standard 42-min method[1] to screen for all congeners. A specific combination of column stationary phase, carrier-gas flow rate, and oven temperature programming is required to lock all the compounds to an expected retention timetable[2]. Compound identification based only on spectral searching alone is difficult when the isomers have the same mass spectra.

The screening tool, integrated within Agilent's ChemStation for MSD software, searches for all 209 congeners by first checking and integrating the

expected target ion within the expected time window. If the target ion is found, the software will then search and integrate the three qualifier ions within the expected time window. Last, the software will print out a report showing "hits" and "possible hits" (ratios of characteristic ions that do not match the expected values in the library within specified limits).

In order to improve laboratory productivity, we scaled the method for four-fold speed-up. While a 30-m × 0.25-mm × 0.25-µm Agilent HP-5MS column is used for standard speed, a 15-m × 0.25-mm × 0.25-µm Agilent HP-5MS column is used for the four-fold speed. These faster methods were able to be scaled exactly as predicted by using a combination of Agilent's method translation (MTL) and RTL software.

Often, when speeding up GC methods, an analyst trades resolution for increased analysis speed. This loss of resolution can complicate peak identification, even with a mass selective detector (MSD). However, because scaling was exact, the faster methods can be used with precisely scaled congener libraries, making the screening process even more powerful and adaptable to individual needs.

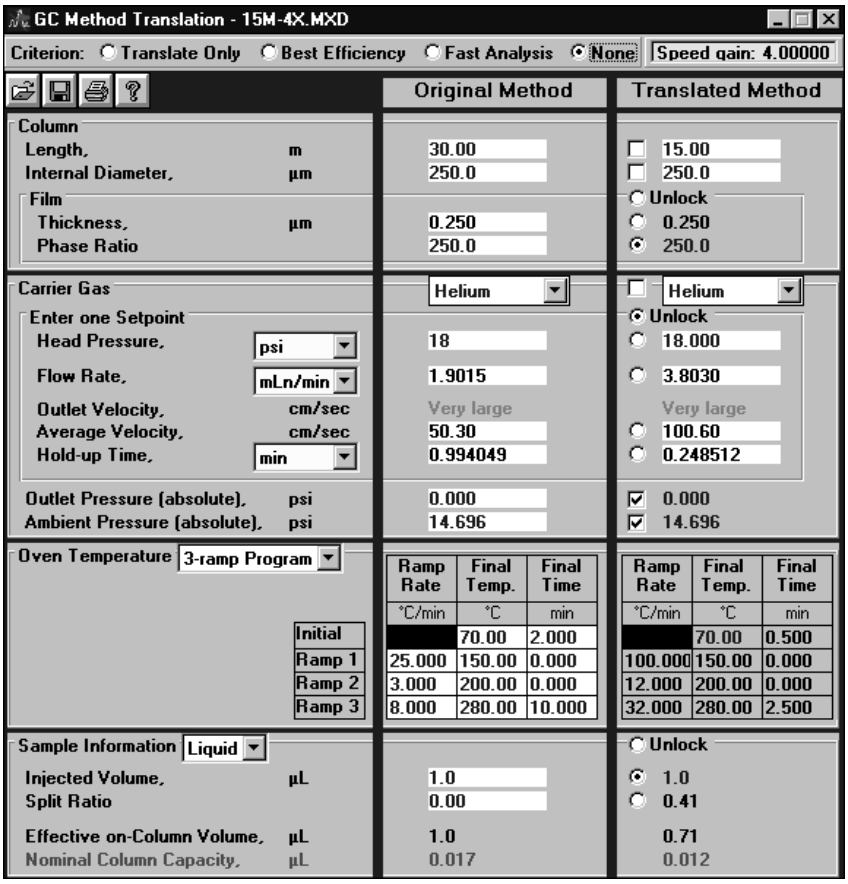

Figure 1. Screen capture showing the method translation (MTL) software data entry used in a 4X speed-gain translation.

Experimental

The GC method translation software tool was used to find operating conditions for the faster methods. Figure 1 is a screen capture of the MTL software data entry showing the original conditions and the new chromatographic conditions for a four-fold speed gain. The column flow rate, which is helpful to avoid exceeding MSD pumping capacity[3], also is found in the table. In this study, a turbo pump that could handle the 3.8 mL/min carrier flow was used. The program also determined the required column head pressure and corresponding oven ramp. The Agilent 6890 GC fast oven option (220/240V in the U.S.) was required for the faster oven ramp used in this study.

General chromatographic conditions are listed in Table 1. The RTL standard used was a mixture of pesticides and PCB congeners at 10 ppm. A 15-m × 0.25-mm × 0.25-µm Agilent HP-5MS column (part number 19091S-431) was used. The head pressure determined by the method translation software (18 psi) was used as the starting point for retention time locking. The column head pressure required to lock retention times of the compounds to the library (the original retention time divided by four) was determined using the automated RTL process integrated within the Agilent ChemStation for MSD.

A very important modification to the MS method is changing the default values of "Use mass range from" to **–0.50** to **+0.50** amu (the default values are –0.3 to +0.7). The changes can be made from the "Extracted Ion Chromatograms…" dialog box selected from the "Chromatogram" on the menu bar.

Figure 2 shows the results of the shortened analysis times. The two chromatograms look extremely similar, except that the time axis is scaled proportionally. It is interesting to note that the last peak in the 4X analysis came out *before the first peak of the 1X analysis.* Because MTL followed by RTL scales methods very precisely, scaled screening libraries for corresponding time

reductions can be obtained by dividing the retention times in the library by the speed gain (which does not have to be an integer).

Conclusion

The highly accurate and reproducible pressure and temperature control of the Agilent 6890 GC allows precise scaling of a standard 42-min GC/MSD method. The run time was shortened to 10.5 minutes using a fast oven ramp rate and a 15-meter 250-micron column. The combination of MTL and RTL facilitated scaling and yielded exact scaling. RTL libraries can be scaled accurately to correspond to the faster analyses. The GC/MSD conditions used are the same as the fast pesticide method[4], which allows for screening pesticides and PCB congeners in a single analysis.

References

1. B. D. Quimby, L.M. Blumberg, M. S. Klee, and P. L. Wylie, "Precise Time-Scaling of Gas Chromatographic Methods Using Method Translation and Retention Time Locking," Application Note 228-401, Agilent publication number 5967-5820E, May 1998.
2. H. Prest, P. L. Wylie, K. Weiner, and D. Agnew, "Efficient Screening for Pesticides and Endocrine Disrupters Using the HP 6890/5973 GC/MSD System," Agilent publication number 5968-4884E, April 1999.
3. H. Prest, "GC Column Selection and Pumping Considerations for Electron and Chemical Ionization MSD operation," Agilent publication number 5968-7958E, November 1999.
4. C. Kai Meng and Michael Szelewski, "Fast Screening of Pesticides and Endocrine Disrupters Using the Agilent 6890/5973N GC/MSD System, Part II", Agilent publication number 5980-1057E, May 2000.

Table 1. Chromatographic Conditions

Speed	Standard	Four-fold
GC	110 V	220/240 V
Column	30-m × 0.25-mm × 0.25-µm Agilent HP-5MS (part number 19091S-433)	15-m × 0.25-mm × 0.25-µm Agilent HP-5MS (part number 19091S-431)
Injection mode	Splitless	Splitless
Column head pressure	18.0 psi	18.0 psi
Column flow (mL/min)	1.9	3.8
Inlet control mode	Constant pressure	Constant pressure
Carrier gas	Helium	Helium
Injector Temperature	250 °C	250 °C
Oven Temperature	70 (2 min)	70 (0.5 min)
Ramp 1	25 °C/min	100
	150 (0 min)	150 (0 min)
Ramp 2	3 °C/min	12
	200 (0 min)	200 (0 min)
Ramp 3	8 °C/min	32
	280 (10 min)	280 (2.5 min)
Oven equilibration	2 min	2 min
Injection volume	1 µL	1 µL
Liner	5183-4647	5183-4647
MS Conditions (Turbo pump)		
Solvent delay	3 min	0.9 min
Tune file	Atune.u	Atune.u
Low mass	50 amu	50 amu
High mass	550 amu	550 amu
Threshold	200	200
Sampling	3	1
Scans/sec	1.52	5.56
Quad Temperature	150 °C	150 °C
Source Temperature	230 °C	230 °C
Transfer line Temperature	280 °C	280 °C
Acquisition mode	Scan (EI)	Scan (EI)

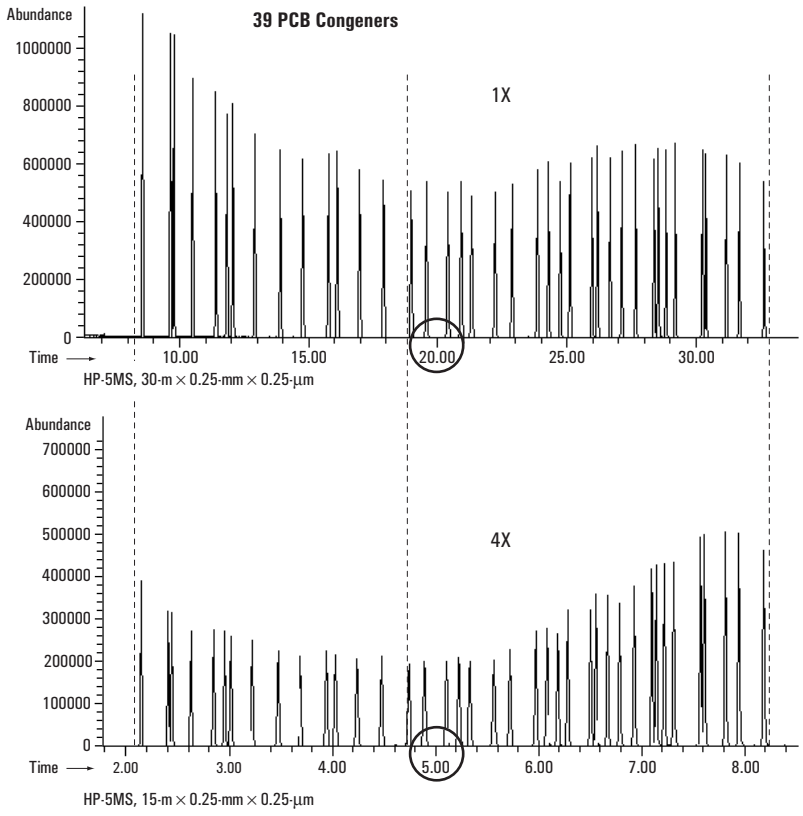

Figure 2. The TICs of the standard speed and fast (4X) analyses. The standard analysis (1X) was 42 minutes long.

Printed in the USA 5/2000
5980-1472E

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Fast Screening of Pesticides and Endocrine Disrupters Using the Agilent 6890/5973N GC/MSD System, Part II

Application

Gas Chromatography
May 2000

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Abstract

Agilent Technologies' new, fast GC/MSD method can significantly speed up the screening of pesticides. Agilent's GC Method Translation software (available free from the Agilent Technologies Web site, http://www.chem.agilent.com/cag/servsup/usersoft/main.html#mxlator) was used in developing the new method based on the standard 42-min method. A 15 m × 0.25 mm × 0.25 µm Agilent HP-5MS column was used to increase analysis speed up to fourfold. The time savings were implemented in increments (down to 10.5 minutes) to verify the predictability of scaling and the effect of scaling on the signal-to-noise ratio.

Key Words

RTL, pesticide, environmental, screening, fast GC, method translation, 5973, 6890, MTL

Introduction

Analysts want faster analyses to improve laboratory productivity. Often, when speeding up GC methods, an analyst will trade resolution for increased analysis speed. This loss of resolution can complicate peak identification, even with a mass selective detector (MSD).

Agilent Technologies has developed new techniques to solve the peak identification problem based on Agilent's retention time locking (RTL) and a new mass spectral library that contains the locked retention times and characteristic ions for 567 of the most common pesticides and endocrine disrupters of concern worldwide. A GC/MSD method was developed based on the standard 42-min method[1] to screen for all 567 of the most common analytes. A specific combination of column stationary phase, carrier gas flow rate, and oven temperature programming is required to lock all the compounds to an expected retention timetable[2]. Compound identification based only on spectral searching alone is difficult when analyzing extracts containing significant sample matrix content because of overlapping peaks and noisy baselines.

The new screening tool, integrated within Agilent's ChemStation for MSD, searches for all 567 compounds. It first checks and integrates four characteristic ions within the expected time window and then prints a report showing "hits" and "possible hits" (ratios of characteristic ions that do not match the expected values in the library within specified limits).

In Part I of the MSD fast screening application brief[3], a 10 m × 0.1 mm × 0.1 µm Agilent HP-5 column was used to increase analysis speed up to fourfold. In this application brief, a 15 m × 0.25 mm × 0.25 µm Agilent HP-5MS column was used. The faster methods were scaled exactly as predicted by using a combination of Agilent's method translation (MTL) and RTL software. Because scaling was exact, these faster methods can be used with precisely-scaled pesticide libraries, making the screening process even more powerful and adaptable to individual needs.

Experimental

The GC method translation software tool was used to find operating conditions for the faster methods. Figure 1 is a screen capture of MTL software data entry showing the original conditions and the new chromatographic conditions for a fourfold speed gain. The column flow rate, which is helpful to avoid exceeding MSD pumping capacity[4], also is found in the table. In this study, a turbo pump was used, which could handle the 3.8 mL/min carrier flow. The program also determined the required column head pressure and corresponding oven ramp. The Agilent 6890 GC fast oven option (220/240V in the U.S.) was required for the faster oven ramp used in this study.

General chromatographic conditions are listed in table 1. The standard used was a mixture of 26 pesticides at 10 ppm. A 15 m × 0.25 mm × 0.25 µm Agilent HP-5MS column (part number 19091S-431) was used. The head pressure determined by the method translation software (18 psi) was used as the starting point for retention time locking. The column head pressure required to lock retention times of the compounds to the library (the original retention time divided by 4) was determined using the automated RTL process integrated within the Agilent ChemStation for MSD.

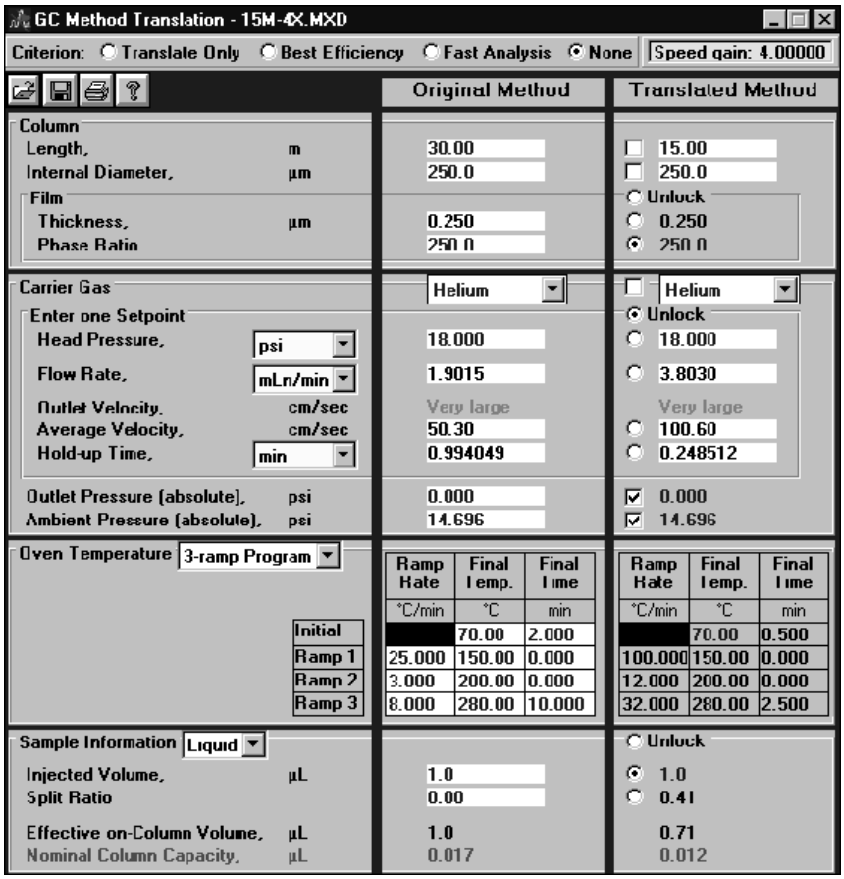

Figure 1. Screen capture showing the method translation (MTL) software data entry used in a 4X speed gain translation.

This process (first translate the method then lock the retention times) was repeated for the 2.5X time reductions.

Figure 2 shows the results of the shortened analysis times. The three chromatograms look extremely similar, except that the time axis is scaled proportionally. Because MTL followed by RTL scales methods very precisely, scaled screening libraries for corresponding time reductions can be obtained by dividing the retention times in the library by the speed gain (which does not have to be an integer). Using the same injection method (1-µL splitless), the peak heights of the faster runs were twice those from the original

Table 1 Chromatographic Conditions

Speed	**Onefold**	**Two and a half fold**	**Fourfold**
GC	110 V	220/240 V	
Column	30 m × 0.25 mm × 0.25 µm HP-5MS (P/N 19091S-433)	15 m × 0.25 mm × 0.25 µm HP-5MS (P/N 19091S-431)	
Injection mode	Splitless	Splitless	
Column head pressure	18.0 psi	5.74 psi	18.0 psi
Column flow (mL/min)	1.9	1.49	3.8
Inlet control mode	Constant pressure	Constant pressure	
Carrier gas	Helium	Helium	
Injector Temp.	250 °C	250 °C	
Oven Temp.	70 (2 min)	70 (0.8 min)	70 (0.5 min)
Ramp 1	25 °C/min	62.5	100
	150 (0 min)	150 (0 min)	150 (0 min)
Ramp 2	3 °C/min	7.5	12
	200 (0 min)	200 (0 min)	200 (0 min)
Ramp 3	8 °C/min	20	32
	280 (10 min)	280 (4 min)	280 (2.5 min)
Oven equilibration	2 min	2 min	
Injection volume	1 µL	1 µL	
Liner	5183-4647	5183-4647	

MS Conditions (Turbo pump)			
Solvent delay	3 min	1.44 min	0.9 min
Tune file	Atune.u	Atune.u	
Low mass	35 amu	35 amu	
High mass	500 amu	450 amu	
Threshold	150	250	
Sampling	2	2	1
Scans/sec	3.15	3.50	6.54
Quad Temp.	150 °C	150 °C	
Source Temp.	230 °C	230 °C	
Transfer line Temp.	280 °C	280 °C	
Acquisition mode	Scan (EI)	Scan (EI)	

analysis. A faster oven ramp and the shorter column made the peaks narrower and higher, so an improvement in the signal-to-noise ratio is realized with the faster methods.

Conclusion

The highly accurate and reproducible pressure and temperature control of the Agilent 6890 GC allows precise scaling of the standard 42-min GC/MSD pesticide method. Run time was shortened to 10.5 minutes using a fast oven ramp rate and a 15-meter, 250-micron column. The combination of MTL and RTL facilitated scaling and yielded exact scaling. RTL libraries can be scaled accurately to correspond to the faster analyses.

References

1. B. D. Quimby, L.M. Blumberg, M. S. Klee, and P. L. Wylie, "Precise Time-Scaling of Gas Chromatographic Methods Using Method Translation and Retention Time Locking," Application Note 228-401, Agilent publication number 5967-5820E, May 1998.
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3. C. K. Meng and M. Szelewski, "Fast Screening of Pesticide and Endocrine Disrupters Using the Agilent 6890/5973N GC/MSD System", Agilent publication number 5968-9220, January 2000.
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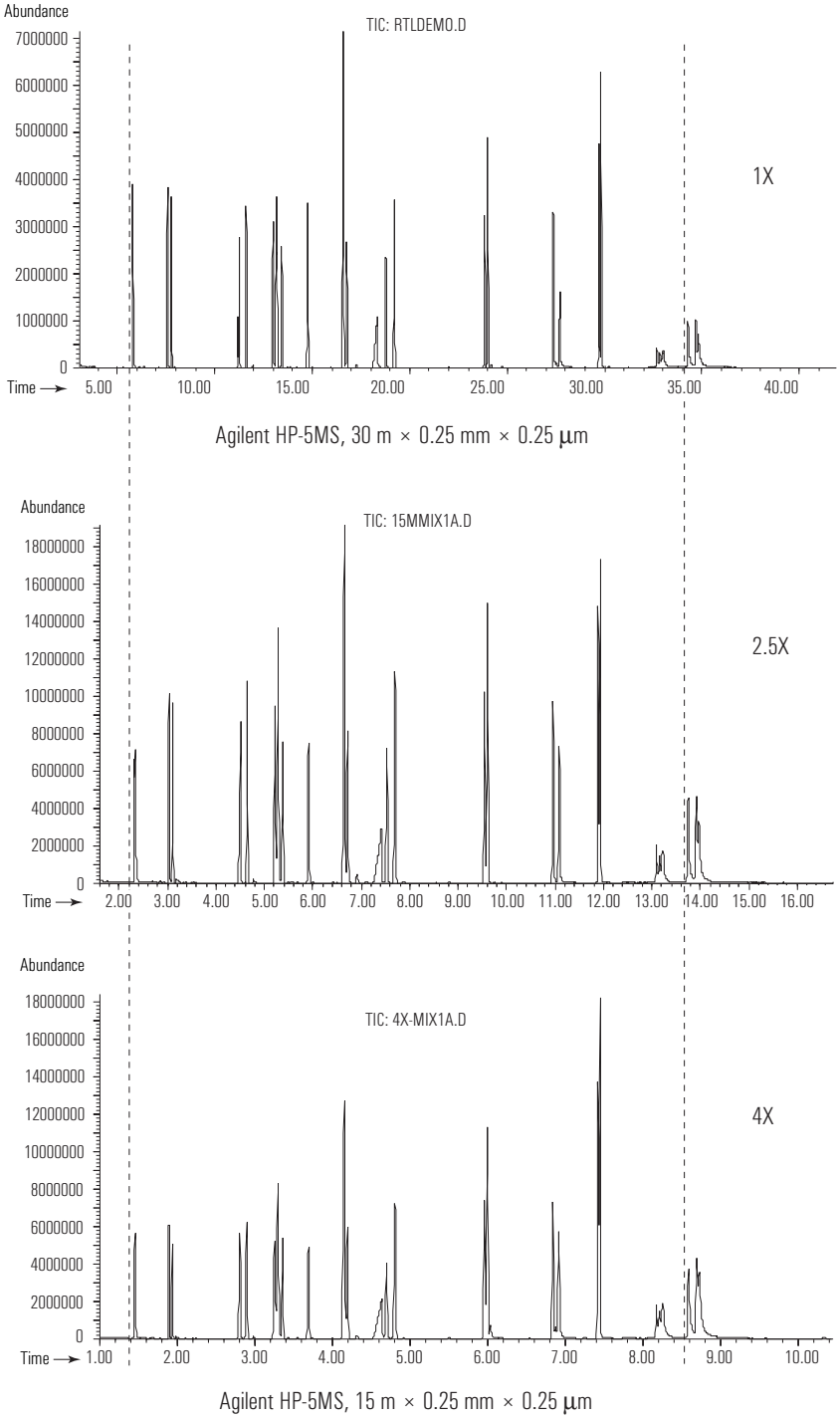

Figure 2. The TICs of the 2.5X and 4X speedups. The standard analysis (1X) was 42 minutes long.

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5980-1057E

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Fast Dual-Column GC/ECD Analysis of Chlorinated Pesticides—EPA Methods 608 and 8080

Application Note
228-305

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Abstract

Dual-column analysis with HP-35 and HP PAS-1701 columns was used to analyze chlorinated pesticides targeted in EPA Methods 608 and 8080 for wastewater and solid wastes. GC parameters were optimized using the Agilent 5890 Series II gas chromatograph (GC) with electronic pressure control (EPC), a dual injector, and a dual electron capture detector (ECD) system. The analysis of 18 pesticides was completed in 12 minutes.

Introduction

Currently, many testing laboratories use dual-column/dual-ECD GC systems to analyze the chlorinated pesticides specified in EPA Methods 608 and 8080[1,2]. For this application, EPC was used with an HP-35 column (35% phenyl, 65% methyl polysiloxane phase) as the primary column and the HP PAS-1701 column for confirmation.

The unique selectivity of the HP-35 column for this set of chlorinated pesticides permitted focus on the optimization of oven temperature for the HP PAS-1701 column. Individual EPC ports for each injector permitted individual regulation of column flow for both the HP-35 and the HP PAS-1701.

Experimental

EPA Method 608 and 8080 targeted pesticides were separated using 30 m x 0.53 mm x 1.0 µm HP-35 and HP PAS-1701 columns (part no. 19095G-123 and 19094U-023, respectively). Analyses were performed on an HP 5890 Series II GC with EPC, dual split/splitless inlets, and dual ECDs. An Agilent 7673 automatic liquid sampler was used to process the simultaneous splitless injections. A deactivated single-tapered glass liner with a small plug of glass wool (part no. 5181-3316) and a Merlin Microseal septum (part no. 5181-8816) were used with each split/splitless inlet. Instrumentation and GC conditions are listed in **Table 1**.

A test mix containing 18 pesticides (50 ppb per component) and two surrogates was prepared from the dilution of certified standard mixes with pesticide-grade hexane (Burdick & Jackson). Pesticides in the test mix are listed in **Table 2**.

Table 1. Experimental Conditions

Instrument Requirement	
Gas Chromatograph	Agilent Technologies 5890 Series II with EPC
Injection Ports	Dual split/splitless inlets
Column	HP-35, 30 m x 0.53 mm x 1.0 µm (Part no. 19095G-123)
	HP PAS-1701, 30 m x 0.53 mm x 1.0 µm (Part no. 19095S-123)
Detector	Dual ECD
Sample Introduction	7673 automatic sampler with dual injectors
Data Collection	3365 ChemStation and HP Vectra 486/33T PC
Experimental Conditions	
Injection	Splitless 1 µl, purge delay, 0.75 min, inlet temperature of 250°C
Carrier gas	(A) HP-35, pressure program: 8.6 psi (1 min) at 0.5 psi/min to 12 psi and at 3.0 psi/min to 25 psi (0 min)
	(B) HP-1701, helium, 10 ml/min constant flow
Oven	160°C (1 min) to 280°C at 10°C/min and to 300°C (2 min) at 25°C/min
Detector	ECD (300°C), 120 ml/min N_2 makeup, 6 ml/min anode purge

Results and Discussion

In a dual-column/dual-ECD system, samples introduced in a single injection can be split between two columns using a Y-connector and detected by different ECDs. However, when using a Y-connector without EPC, the split sample flow to each column cannot be optimized, and equal and consistent sample splits cannot be presumed. The only variable that can be optimized, in dual-column ECD analysis using a Y-connector is the oven temperature program, which can be optimally balanced for the two dissimilar columns. Using dual-column GC/ECD without EPC, it would typically require 45 to 60 minutes to obtain baseline separations for EPA Method 608 and 8080 targeted pesticides (see **Figure 1**).

A typical run from an environmental testing laboratory for a test mix containing 18 targeted pesticides and two surrogates is shown in **Figure 1**. A Yconnector was used to split samples for both columns, DB-608 and DB-1701, and good baseline separations were obtained for most analytes. This dual-column run was completed in 45 to 53 minutes using the following oven temperature program: 150°C (1 minute) to 260°C (18.34 minute) at 3°C/minute, then to 275°C (5 minutes) at 25°C/minute. Clearly this oven temperature program was optimized to separate critical pairs, such as DDE/dieldrin, DDD/endosulfan II, endosulfan sulfate/mehtoxychlor, and methosychlor/endrin ketone for both columns.

Table 2. Chlorinated Pesticides.

Peak No.	Pesticides
1	Tatrachloro-m-xylene (SS1)
2	alpha-BHC
3	Lindane
4	beta-BHC
5	Heptachlor
6	delta-BHC
7	Aldrin
8	Heptachlor epoxide
9	Endosulfan I
10	4,4′-DDE
11	Dieldrin
12	Endrin
13	4,4′-DDD
14	Endosulfan II
15	4,4′-DDT
16	Endrin aldehyde
17	Endosulfan sulfate
18	Methoxychlor
19	Endrin ketone
20	Decachlorobiphenyl (SS2)

Figure 2 shows chromatograms of the same pesticide test mix using the HP-35 and HP PAS-1701 columns and EPC. The oven program, 160°C (1 minute) to 280°C at 10°C/minute and to 300°C (2 minutes) at 25°C/minute, was optimized to separate the critical pairs, endosulfan

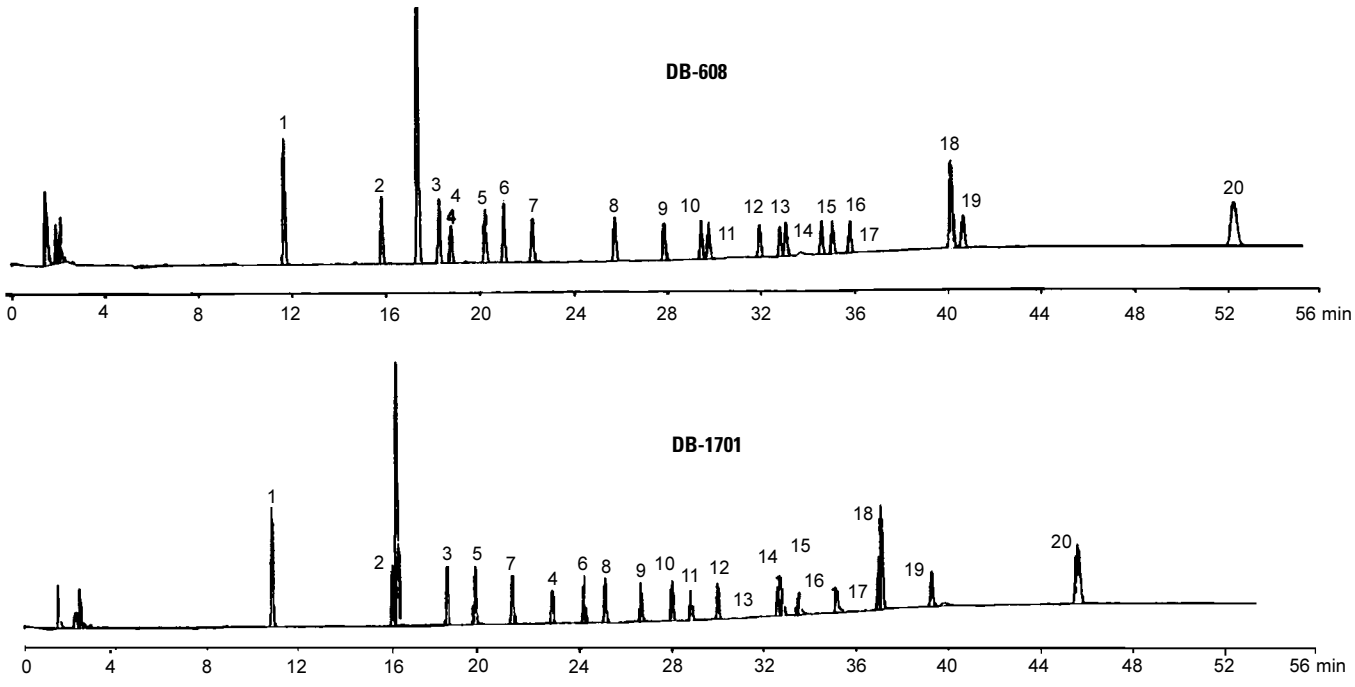

Figure 1. Typical chromatograms of a pesticides standard mix using DB-608 and DB-1701 columns under GC conditions used in environmental testing laboratories. (See Table 2 for peak identification.)

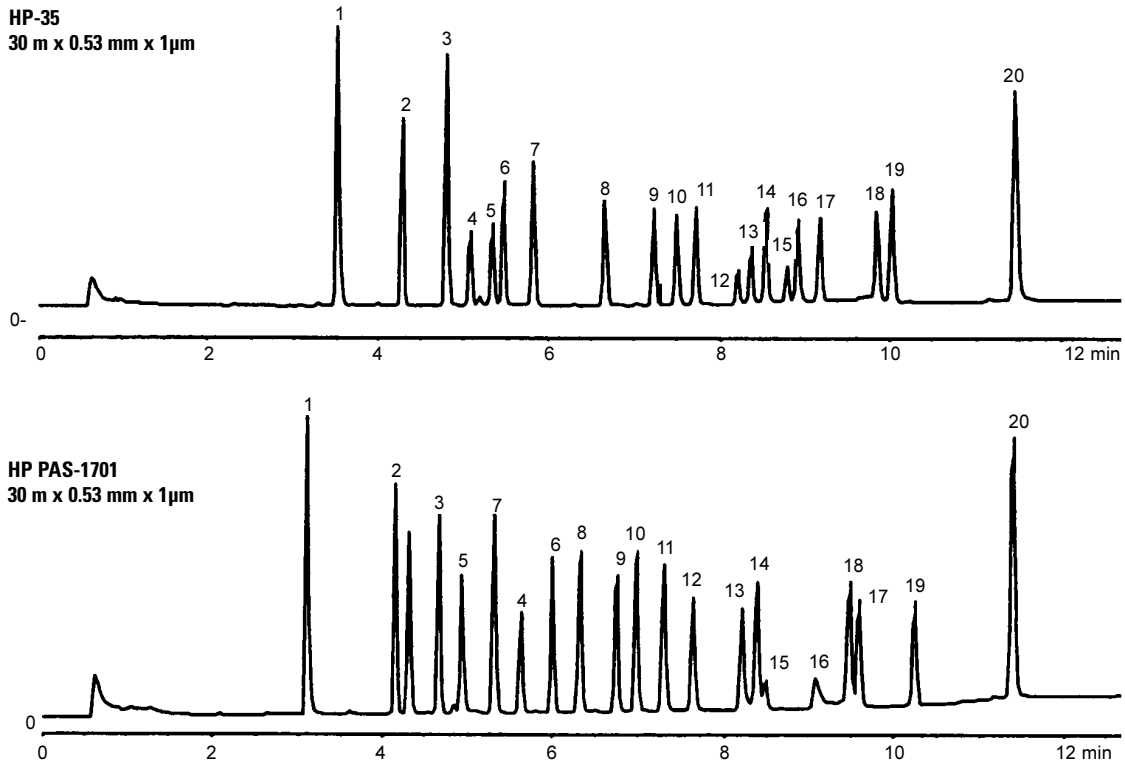

Figure 2. Chromatograms of a pesticides standard mix using HP-35 and HP PAS-1701 columns under the GC conditions listed in Table 1. (See Table 2 for peak identification.)

II/DDT and methoxychlor/endosulfan sulfate, for the HP PAS-1701 column. In this run, EPC provided a constant 10 ml/minute helium flow to the HP PAS-1701 column throughout the entire run.

For the HP-35 column, the following pressure program was used: 8.6 psi (hold 1 minute) at 0.5 psi/minute to 12 psi and at 3.0 psi/minute to 25 psi (hold for constant flow for the remaineder of the run). This pressure program actually provided a 10 ml/minute constant flow to elute most of the pesticides and an increased flow (up to 20 ml/minute) near the end of the run to elute the last analyte, surrogate decachlorobiphenyl and other high-boiling materials from the column.

GC parameters optimized for dual-column/dual-injector/dual-ECD analysis of chlorinated pesticides reduced analysis time to less than 12 minutes. In addition to speed, all EPA Methods 608 and 8080 targeted pesticides and surrogates were well resolved with good sharp peaks for accurate quantitation.

Conclusion

The use of EPC permitted individual column flow control to each ECD. The unique selectivity of the HP-35 column for chlorinated pesticides permitted focus on the optimization of oven temperature for the HP PAS-1701 column. Run time was 11.5 minutes with good baseline separations for all 20 target pesticides and surrogates. The result was a reduction in sample turnaround time from 54 to 11.5 minutes for a 400% increase in productivity. This is more than a twofold improvement in productivity when compared with conventional methods currently used at many environmental testing laboratories with DB-608 and DB-1701 columns.

Acknowledgement

The author wishes to thank Ms. Joann Faulkner and her colleagues at the Pace Laboratory in Petaluma, California, for providing chromatograms and pesticide standards.

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Printed in USA 04/00

Printed on recycled paper
Publication Number 5963-6734E

Validation Analysis of EPA CLP Target Organochlorine Pesticides with the Agilent 6890 Series GC and Micro-ECD

Application

Gas Chromatography

February 1998

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Abstract

Generating environmental data for organochlorine pesticides in various matrices can be time-consuming for laboratories and engineering firms. To keep a gas chromatograph/electron capture detector (GC/ECD) system operating within control limits, precious analytical time must be spent on tasks such as recalibration, reinjection of samples, detector cleaning, and reintegration of chromatographic peaks. These tasks take time away from running billable samples and adversely affect laboratory throughput.

The Agilent 6890 Series Micro-ECD used in this study shows improved performance in several key areas: increased linear working range (greater than 4 orders of magnitude for some components), increased sensitivity (organochlorine pesticides at sub-ppb levels), increased stability, and increased resistance to contamination.

Introduction

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are found worldwide in the environment. Because many of these pesticides are suspected to be carcinogenic and/or endocrine hormone disrupters[1,2], determination of their presence in water, air, soil, and food is required by governmental agencies such as the U.S. EPA, the FDA, and the World Health Organization.

The U.S. EPA provides several comprehensive guidelines[3,4] and regulations[5,6] for analysis of OCPs and PCBs by gas chromatography with electron capture detectors (GC/ECD). These include EPA method 8081 for wastewater/solid wastes, EPA methods 505 and 508 for drinking water/water supplies, EPA method 608 for municipal and industrial discharges, and the Contract Laboratory Program (CLP) method for waste/clean-up sites. Most contract laboratories competing for the large number of potential CLP samples find that competition is strong and profit margins very low compared with other environmental methods.

CLP methods have very specific performance criteria that can be very time-consuming for laboratories to meet consistently. To keep a GC/ECD system operating within control limits, precious analytical time must be spent on tasks such as recalibration, reinjection of samples, detector cleaning, reintegration of chromatographic peaks, etc. Spending too much time with any of these tasks takes time away from running billable samples, and adversely affects the throughput and profitability of the laboratory.

In this study, the 6890 Series Micro-ECD greatly reduced the time required to meet CLP quality control criteria for CLP analysis of OCPs and PCBs. Validated results show four key improvements: increased linear working range (greater than 4 orders of magnitude), increased sensitivity (detecting OCPs at sub-ppb level), more stable calibration, and increased resistance to contamination (more robust, fast detector recovery and reduced maintenance).

Experimental

Water and soil samples were extracted after spiking with surrogates tetrachloro-m-xylene (TCX) and decachlorobiphenyl (DBC). Extracts of OCPs were analyzed in accordance with EPA CLP method OLM03.1[6]. Typically, a 1-L volume of water sample was extracted with methylene chloride by liquid-liquid extraction or a 30-g aliquot of soil/sediment sample extracted with 1:1 acetone/methylene chloride by sonication. These extracts were concentrated and solvent-exchanged into a 10-mL volume of hexane.

Working standards for checking linearity and CLP QA/QC criteria were prepared from certified standards (available commercially) in hexane, as described in the CLP method[5].

All analyses were performed using a 6890 Series GC with an automatic liquid sampler, a single split/splitless inlet, a pair of primary and confirmatory columns, and two 6890 Micro-ECDs. Instrument conditions are listed in table 1.

A sample extract or working standard (1 µL) was injected into the 6890 Series GC in the splitless mode. A guard column (equivalent to a 5-m retention gap, part no. 19095-60610) was used. It was connected to a "Y" glass butt connector that split the sample equally between the pair of columns.

Column A (an equivalent of the Agilent HP-608 column) was used as the primary analytical column, and column B (an equivalent of the Agilent HP-1701 column) was used as the confirmatory column, in accordance with the CLP method.

In the case of poor chromatography or a failing control limit for inlet degradation, routine maintenance was performed. This involved changing the inlet septum, installing a new inlet liner, and clipping a short piece of the retention gap. Columns were routinely conditioned to remove late-eluting column contaminants. When CLP criteria could not be met after routine maintenance, columns were replaced with new columns of the same type.

Table 1. Experimental Conditions

Sampler	Agilent 7673, 10-µL syringe, 1-µL injection
Inlet	Split/splitless; 200 °C, pulsed splitless mode (28 psi for 1 min)
Carrier	Helium, 16.8 psi (150 °C); 3.5 mL/min constant flow (each column)
Column	(1) 30 m, 0.53 mm id, 0.8-µm film DB-608, an equivalent of Agilent HP-608 (part no. 19095S-023)
	(2) 30 m, 0.53 mm id, 1.0-µm film RTX-1701, an equivalent of Agilent HP PAS-1701 (part no. 19095S-123)
Oven	150 °C (0.5 min); 5 °C/min to 280 °C (5–15 min).
Detector	330 °C; makeup gas: nitrogen, constant column and makeup flow (60 mL/min)

Results and Discussion

Sensitivity

Figures 1 and 2 show chromatograms of CLP target organochlorine pesticides on column A using the GC conditions listed in table 1. All 20 OCPs in the midpoint calibration standards (mix A and mix B) were baseline resolved with both the primary analytical column (column A) and the confirmation column (column B, shown in figure 3). The amount of individual OCPs in the midpoint calibration standard was 20–40 pg on-column (methoxychlor was 200 pg). Table 2 lists the concentration of midpoint calibration standards, peak identification, and the Contract Required Quantitation Limits (CRQLs)[6] for all CLP target OCPs.

Figures 1 and 2 also show good responses for dilute OCPs (0.25–0.5 pg on-column, 1/20th of the concentration of those for CRQLs). Quantitation at this level was easy with the micro-ECD; most OCPs exhibited a signal-to-noise ratio greater than 10 (see the lower chromatograms in figures 1 and 2). These results, confirmed by column B and the second micro-ECD (see figure 3), show that the 6890 Series Micro-ECD can easily detect low levels of OCPs (lower than 1/20 of those required by CLP). This is in good agreement with Channel and Chang[7], who reported detection of OCPs as low as 0.050 pg on-column. However, detection of this low level is not necessary because the CRQLs[5] range from 5 to 10 pg (methoxychlor at 50 pg) on-column (see table 2).

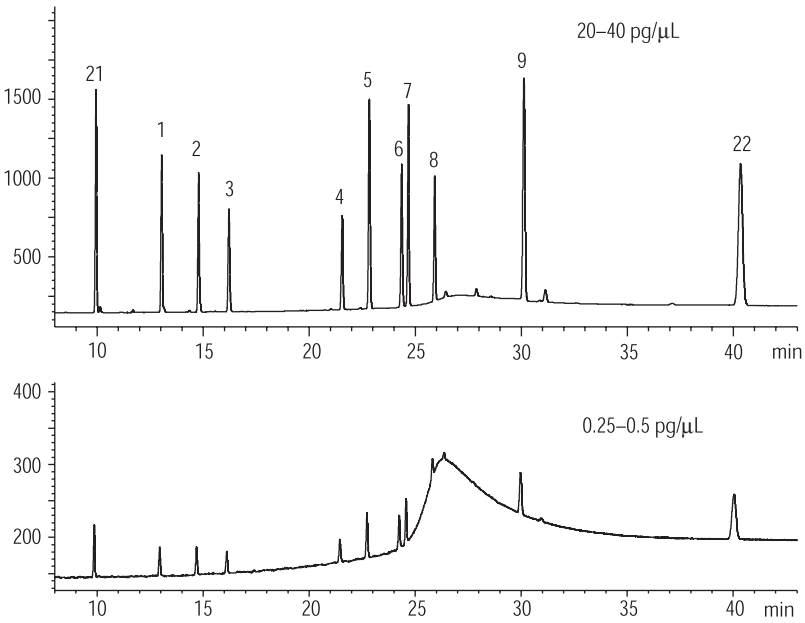

Figure 1. Pesticides in CLP calibration mix A on the primary column (A). 20 pg/µL (upper chromatogram) and 0.25 ng/µL (lower chromatogram) for methoxychlor (peak 9).

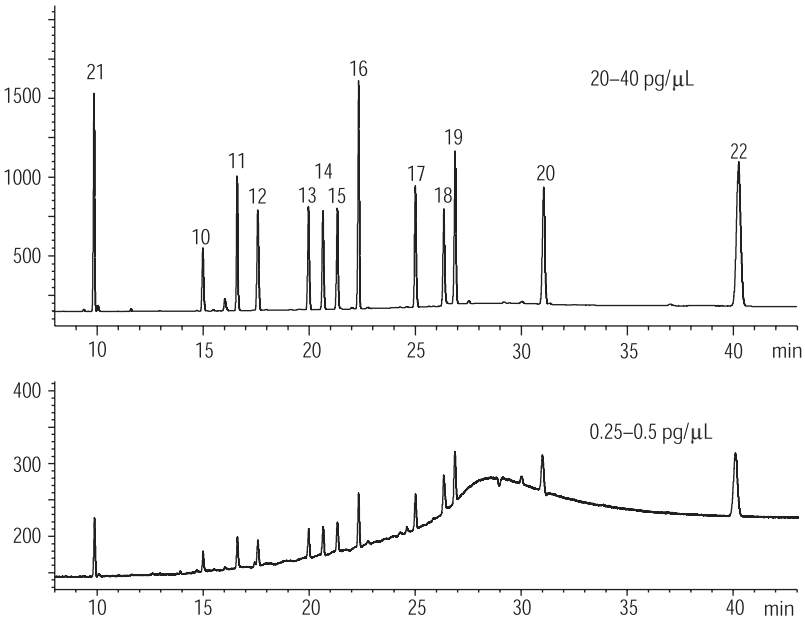

Figure 2. Pesticides in CLP calibration mix B on the primary column (A).

To ensure reliable results, three-point initial calibrations were routinely performed in accordance with CLP requirements using standards of 5, 20, and 80 pg/µL for lindane. Table 2 lists typical response factors and percent relative standard deviation (% RSD) for all CLP target OCPs. Typical % RSDs ranged from 2 percent for beta-BHC to 14 percent for endrin aldehyde, easily meeting the CLP criterion of 20.0 percent or less over the CLP calibration range.

Micro-ECD Linearity

Although classical electron capture detectors can provide sensitive detection, they are notorious for nonlinear response toward OCPs. For example, linearity is problematic for isomers of BHCs, particularly at the high concentration level. On the other hand, linearity as well as low response is problematic for methoxychlor, particularly at the low concentration level. These problems were not encountered using the 6890 Series GC system with micro-ECDs.

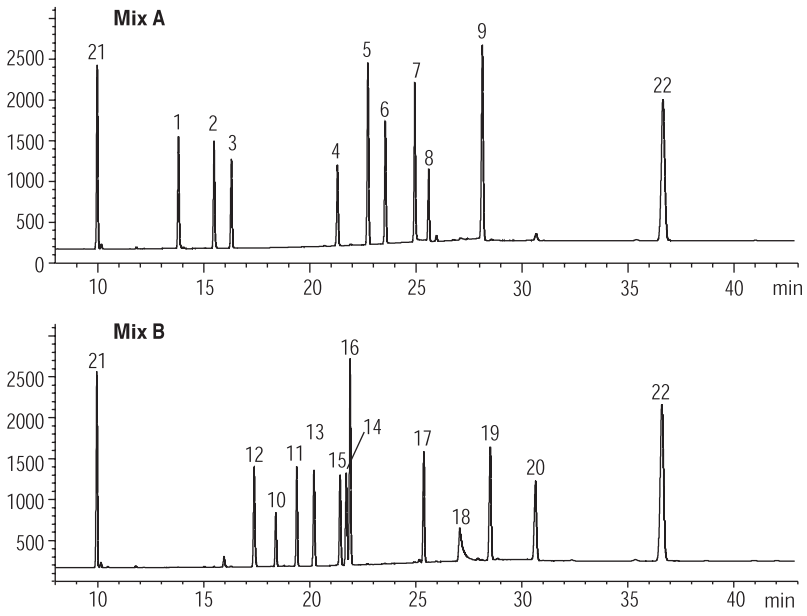

Figure 3. Pesticides on the confirmatory column (B). 20 to 40 pg/µL each, 200 pg/µL for methoxychlor.

Table 2. CLP Target Organochlorine Pesticides and Responses

	Peak	Pesticides	Mid-Level Standard pg/µL (4X)	CRQLs (on column) pg	Response Factors*	
					Average (peak height)	% Relative Standard Deviation
Mix A	1	alpha-BHC	20	5	23052	10.90
	2	gamma-BHC(lindane)	20	5	21729	6.51
	3	Heptachlor	20	5	17661	3.40
	4	Endosulfan I	20	5	15536	2.68
	5	Dieldrin	40	10	16204	4.83
	6	Endrin	40	10	10515	4.54
	7	4,4'-DDD	40	10	14334	5.06
	8	4,4'-DDT	40	10	12418	7.65
	9	Methoxychlor	200	50	4652	5.55
	21	TCX	20	50	16567	2.13
	22	DCB	40	10	5752	14.73
Mix B	10	beta-BHC	20	5	16190	2.40
	11	delta-BHC	20	5	10586	5.40
	12	Aldrin	20	5	20609	11.58
	13	Heptachlor epoxide	20	5	16482	7.01
	14	alpha-Chlordane	20	5	15929	5.20
	15	gamma-Chlordane	20	5	16527	5.69
	16	4,4'-DDE	40	10	15913	5.29
	17	Endosulfan II	40	10	16791	9.52
	18	Endrin aldehyde	40	10	8453	14.20
	19	Endosulfan sulfate	40	10	8926	5.59
	20	Endrin ketone	40	10	2144	3.39
	21	TCX	20	5	10114	3.01
	22	DCB	40	10	5667	14.97

* Typical three-point calibration from column A (concentrations: 1X, 4X, and 16X)

Linearity of the micro-ECD was determined by analyzing a series of dilutions of OCPs at concentrations ranging from 0.1 pg/µL to 3.2 ng/µL for lindane (see the 15-level calibration in table 3). For most OCPs, correlation coefficients were better than 0.99 over a concentration range greater than 5 orders of magnitude (0.1 to 3.2 pg/µL for lindane).

Table 3. Linearity Study

Pesticides		15-Point Calibration		10-Point Calibration			
		Concentration pg/µL	Correlation Coefficients	Concentration pg/µL	Response Factors		Correlation Coefficients
					Average	% Relative Standard Deviation	
Mix A	alpha-BHC	0.1 to 32,000	0.995	1 to 1,600	52,557	19.3	0.998
	Lindane	0.1 to 32,000	0.997	1 to 1,600	46,635	17.3	0.997
	Heptachlor	0.1 to 32,000	0.997	1 to 1,600	35,712	18.0	0.997
	Endosulfanl	0.1 to 32,000	0.997	1 to 1,600	31,858	13.9	0.998
	Dieldrin	0.2 to 64,000	0.995	2 to 3,200	35,718	19.0	0.995
	Endrin	0.2 to 64,000	0.992	2 to 3,200	24,849	19.5	0.996
	4,4'-DDD	0.2 to 64,000	0.995	2 to 3,200	33,903	17.3	0.996
	4,4'-DDT	0.2 to 64,000	0.992	2 to 1,600	20,618	18.2	0.993
	Methoxychlor	1 to 320,000	0.990	10 to 4,000	8,199	16.1	0.998
	TCX	0.1 to 32,000	0.997	1 to 1,600	72,423	10.8	0.998
	DCB	0.2 to 64,000	0.996	2 to 3,200	23,956	17.2	0.998
Mix B	beta-BHC	0.1 to 32,000	0.995	1 to 1,600	21,388	11.6	0.998
	delta-BHC	0.1 to 32,000	0.993	1 to 1,600	47,532	17.0	0.997
	Aldrin	0.1 to 32,000	0.994	1 to 1,600	35,851	14.3	0.997
	Heptachlor epoxide	0.1 to 32,000	0.994	1 to 1,600	36,234	11.9	0.998
	alpha-Chlordane	0.1 to 32,000	0.995	1 to 1,600	34,958	12.2	0.997
	gamma-Chlordane	0.1 to 32,000	0.995	1 to 1,600	35,250	11.3	0.997
	4,4'-DDE	0.2 to 64,000	0.989	2 to 3,200	40,065	18.6	0.996
	Endosulfan II	0.2 to 64,000	0.991	2 to 1,600	24,212	16.4	0.997
	Endrin aldehyde	0.2 to 64,000	0.990	2 to 3,200	18,628	16.6	0.995
	Endosulfan sulfate	0.2 to 64,000	0.992	2 to 3,200	27,644	14.7	0.996
	Endrin ketone	0.2 to 64,000	0.990	2 to 3,200	20,803	13.6	0.996

For a smaller concentration range (3 orders of magnitude), correlation improved and % RSDs of calibration factors for most OCPs were within 20 percent as required by CLP (see the 10-point calibration in table 3). Figure 4 shows a linear curve for lindane (1 to 1,600 pg/µL), typical of most OCPs in this concentration range. Figure 4 also shows the linear curve for methoxychlor (10 to 4000 pg/µL), a pesticide that typically responds poorly to classical ECD. This concentration range, typically from 1 to 1,600 or from 2 to 3,200 pg/µL for most OCPs, represents a 100-fold improvement over that required by CLP (CLP specifies 5 to 80 pg/µL for lindane). This wider linearity range allows more analyses for samples without requiring rework (dilution/concentration and re-analysis). If dilution of samples is required, the higher linearity of the detector results in more accurate estimations of correct dilution factors to bring sample concentrations within the CLP range.

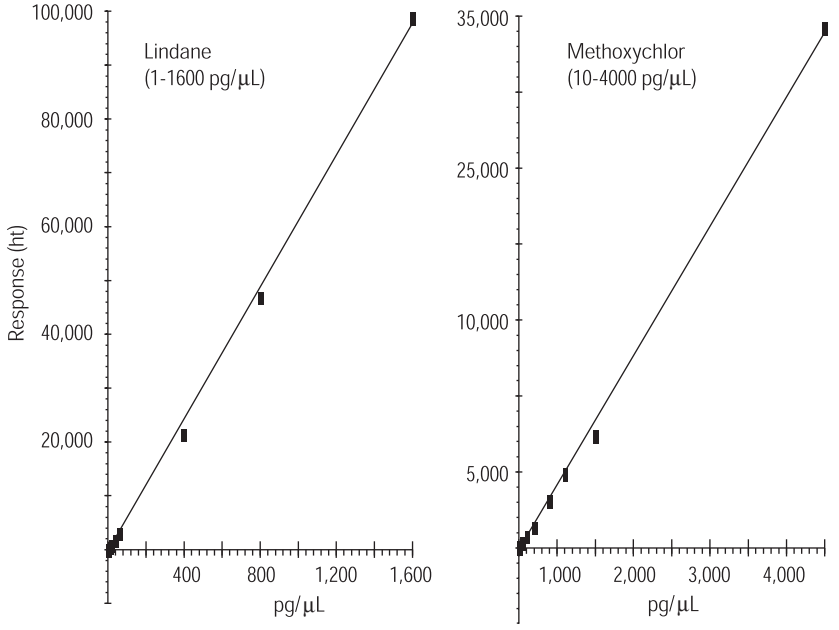

Figure 4. Linear calibration curves for lindane and methoxychlor over extended ranges.

Calibration Stability and System Robustness

The 6890 Series GC system with 6890 Micro-ECDs was regularly calibrated in accordance with CLP requirements. Analyses of blanks, continuous calibration using the midlevel standards, and performance evaluation mix were performed for each 12 hours of operation or every 10 to 20 samples. If results of these analyses failed to meet CLP breakdown, retention time, and response criteria, routine maintenance (such as changing inlet septum and liner or clipping a few inches off the guard column) was performed. If necessary, the instrument was recalibrated (using a three-point initial calibration). No cleaning or baking of the micro-ECD was required, even though a wide variety of samples was analyzed, including some dirty soil extracts[8].

At a minimum, CLP requires that system stability be monitored by analyzing midpoint calibration standards every 12 hours. In this study, system (or calibration) stability was based on verification of the calibration factors and retention times of target OCPs to match those from the initial calibration run within specific limits. The difference in calibration response (RPD—relative percent difference) between the later midpoint calibration run and the initial calibration run must be less than ± 25 percent (upper and lower RPD control limits).

Figure 5 is a continuous calibration verification (CCV) control chart of RPD for lindane and methoxychlor on column A over a 6 month period, typical of most OCPs on both column A and column B.

Throughout this study, the system was within RPD control limits and other calibration verification criteria for several days at a time without performing any re-calibration. When any OCP failed to meet CLP calibration verification criteria (that is, when an OCP was outside the RPD control limits of the CCV), nonintrusive system maintenance was conducted and a new initial calibration was performed. These steps were also done when the instrument was switched for 1 or 2 weeks to analyze a different type of sample, requiring a different GC method. When the instrument was switched back to the original CLP analysis of OCPs and PCBs, the instrument still met calibration verification criteria (within the RPD control limits). This represents a significant improvement over previous designs that usually required full recalibration after switching between methods and indicates that using the micro-ECD saved time and improved laboratory productivity.

Over a period of 6 months, the 6890 Series GC/dual micro-ECD system was in continuous operation

and performed several different methods. For example, the system was used for 2 to 3 weeks to analyze pesticides and aroclors by the CLP method and solid waste method (EPA method 8081). The system was then switched to a drinking water method[8] for a few weeks and later returned to the CLP method for OCPs. In other instances, the system was switched to analyze herbicides (EPA method 8150), then to drinking water (EPA method 504), and back again to the CLP method or method 8081 for OCPs and aroclors. In each case, the stabilization of the micro-ECDs was fast, requiring only a few injections of hexane blanks prior to running the CCV calibration standards.

Throughout this study (which included continuous operations over 6 months), even though routine column and inlet maintenance was needed (columns were replaced once during the course of the study), no micro-ECD maintenance was needed.

Conclusion

The improved performance of the Agilent 6890 Series GC/dual micro-ECD system met all CLP criteria for the analysis of OCPs over a period of 6 months. System validation was performed throughout this period for a wide variety of samples and analyses of different EPA methods. The 6890 Series GC with micro-ECDs easily met and maintained CLP criteria during the study. In addition, the micro-ECD showed improved sensitivity, greater dynamic and linear operating ranges, and more stable response. Moreover, it required minimal maintenance, and showed rapid recovery after switching between methods. Use of the Agilent 6890 Micro-ECD has a high potential to save time, improve quality of data, and increase laboratory productivity.

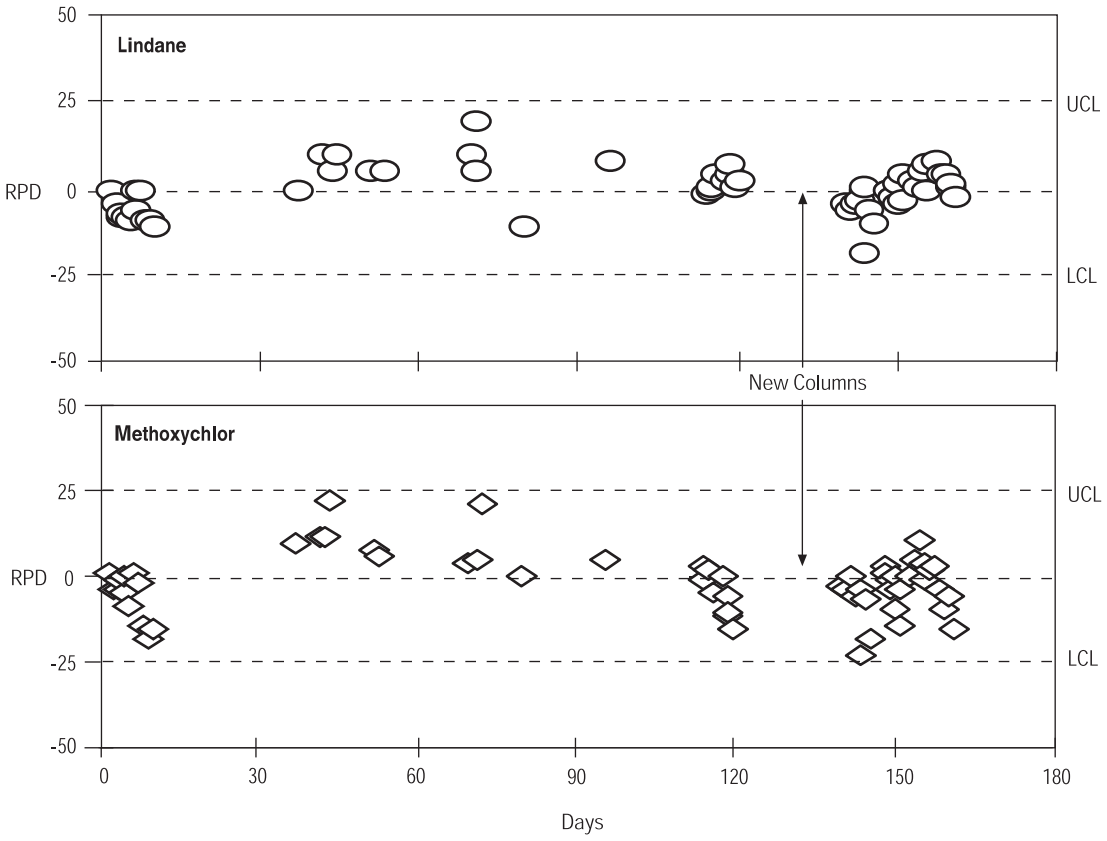

Figure 5. CCV control chart demonstrating stability of response and performance during the study.

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Printed in the USA 4/2000
5966-3742E

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Innovating the HP Way

Trace Level Pesticide Analysis by GC/MS Using Large-Volume Injection

Application

Gas Chromatography

September 1997

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Abstract

Large-volume injection (LVI) using the Agilent programmable temperature vaporizing (PTV) inlet can improve gas chromatography system detection limits by one to two orders of magnitude over standard methods that call for 1- or 2-µL injections. An Agilent 6890 Series gas chromatograph (GC), configured with a PTV inlet, a 6890 Series automatic liquid sampler (ALS), and an Agilent 5973 mass selective detector (MSD), was used for the analysis of pesticides in standards and several food extracts. By making 100-µL injections, several pesticides could be identified by scanning gas chromatography/mass spectrometry (GC/MS) at the 100 ppt (100 ng/L) level. The PTV inlet tolerated dirty food extracts very well; more than 1,500 µL of such samples were injected into a single PTV liner. This application note includes recommendations for doing LVI using the PTV/6890/5973 GC/MSD system.

Introduction

More than 700 pesticides are registered for use in the world[1] , and many more continue to persist in the environment, even though they are no longer being applied. For the protection of human health and the environment, pesticide residues are routinely monitored in food, water, soil, and tissue samples. "Acceptable" residue limits have been set for various foods and environmental samples by agencies such as the United States Environmental Protection Agency (U.S. EPA), the Codex Alimentarius Commission[2] , and many other governmental organizations around the world. A great many methods have been developed to screen for pesticides in food[3-7] and the environment[8-10] to ensure that risks associated with pesticide use are minimized.

Recently, concern has increased that certain pesticides and other synthetic chemicals may be acting as pseudo hormones which disrupt the normal function of the endocrine system in wildlife and humans. Birth defects, behavioral changes, breast cancer, lowered sperm counts, and reduced intelligence are among the many disorders that have been blamed on these "endocrine disrupting" compounds, though much research must be done to verify these assertions. In 1996, Colborn, Domanoski, and Myers[11] brought these issues into the public spotlight with the publication of their book *Our Stolen Future*. Recently, the United States Congress passed legislation calling for increased testing of suspected endocrine disrupters and monitoring their levels in food[12] and water[13] supplies. Because the endocrine system can be exquisitely sensitive to extremely low hormone concentrations, there is a need to measure concentrations of suspected endocrine disrupters (many of which are pesticides) at very low levels. Initiatives such as the Pesticide Data Program, developed by the United States Department of Agriculture[14] , seek to

determine the lowest measurable pesticide levels in various foods to develop a total exposure model. Clearly, there is pressure to push pesticide detection limits to even lower levels than are routinely achieved today. Most residue measurements are made by gas chromatography using a variety of element-selective or mass spectral detectors (GC/MS). Therefore, to achieve lower detection limits, it is necessary to improve the detection limits of these GC methods.

In GC, there are primarily four ways to improve method detection limits: 1) increase the concentration of analytes in a sample, usually by reducing the volume of an extract; 2) increase the sensitivity of the detector; 3) increase the selectivity of the detector to reduce chemical background "noise" or 4) increase the volume of sample injected. Because GC/ MS can be highly selective and extremely sensitive, it is often the method of choice for pesticide analysis and/or confirmation. However, for the reasons discussed above, there are occasions when even greater sensitivity is required. This application note describes a method for increasing GC/MS system detection limits by making large-volume injections (LVI) using Agilent's new programmable temperature vaporizing (PTV) inlet. Because this LVI technique is detector-independent, it is applicable to other GC configurations that may be used for pesticide residue analysis.

Experimental

Pesticide Standard Solution

Stock solutions of 14 pesticides were prepared at 1 mg/mL by adding 10 mg each of trifluralin, hexachlorobenzene, pentachloronitrobenzene, dichloran, chlorothalonil, chlorpyrifosmethyl, chlorpyrifos, endosulfan I, p,p'-DDE, propargite, iprodione, methoxychlor, and fenvalerate (mix of isomers I and II) to individual 20mL vials and diluting with 10.0 mL of acetone. Permethrin was obtained as a mixture of permethrin I and permethrin II comprising 32 percent and 27 percent of the sample, respectively, so 16.95 mg of this mixture was diluted with 10 mL of acetone giving a solution in which the combined permethrins represented 1 mg/mL. A stock mixture was prepared by adding 4 mL of the permethrin and fenvalerate solutions and 1 mL of each of the other stock solutions to a 100-mL volumetric flask and diluting to volume with acetone. The resultant solution contained 40 ng/µL each of the combined permethrin and fenvalerate isomers and 10 ng/µL each of the other 12. This sample was diluted further with acetone to prepare standards that were analyzed by LVI. All these pesticides were obtained in neat form from Chem Service (West Chester, PA USA).

Extracts

Fruit and vegetable extracts were obtained from the Florida Department of Agriculture and Consumer Services (Tallahassee, FL USA). Commodities were extracted using a version of the Luke procedure[15-17] that gave a final sample representing 1.75 g of the commodity per mL of extract.

Table 1. Instrumentation and Conditions Used for Pesticide Samples

GC/MS System	
Gas chromatograph	6890 Series GC
Automatic liquid sampler	6890 Series ALS
Mass spectral detector	5973 Series MSD
Programmable temperature vaporizing inlet	PTV with CO_2 cooling
Computer for data acquisition and analysis	HP Vectra XU 6/200
Software	G1701AA Version A.03.00 running Microsoft® Windows™ 95
Column	30 m x 0.25 mm x 0.25 µm Agilent HP-5MS
Instrumental Conditions	
GC Parameters	
Carrier gas	Helium
Inlet liner	Prototype deactivated borosilicate with fritted glass on interior walls (part no. 5183-2041)
Syringe size	50 µL
Injection volume	100 µL (Inject 10 µL 10 times)
Injection delay	12 sec
Inlet temperature program	40 °C (4.2 min), 200 °C/min to 320 °C (2 min)
Vent flow	400 mL/min Vent pressure 0.0 psi for 4.00 min
Purge flow to split vent	50.0 mL/min at 6.50 min
Column head pressure	0 psi (4 min) then 17.3 psi (constant pressure)
Oven temperature program	50 °C (6.13 min), 30 °C/min to 150 °C (2 min), 3 °C/min to 205 °C (0 min), 10 °C/min to 250 °C (20 min)
MSD Parameters	
Acquisition mode	Scan (35-550 amu)
Temperatures	Transfer line = 280 °C, MS quad = 150 °C, MS source = 230 °C

Instrumentation

Table 1 lists the instrumentation and chromatographic conditions used for LVI and GC/MS analysis of pesticide samples.

Brief PTV Tutorial

Before focusing on the PTV/GC/ MS analysis of pesticides, it is important to understand how the PTV inlet operates in the solvent vent mode for large-volume injections.

The PTV Inlet

The PTV inlet has the same basic functions as the split/ splitless inlet except that it is temperature programmable from -60 °C (using CO_2 cooling) or -160 °C (using liquid N_2 cooling) to 450 °C at rates up to 720 °C/min. However, the PTV's design has been optimized for its main uses-LVI and cold split/splitless injection. Although hot split and splitless injections may be made with or without a pressure pulse, care must be taken not to exceed the small internal volume of the PTV inlet. In practice, it is best to choose the Agilent split/splitless inlet for hot injections and the PTV inlet for LVI and cold split/ splitless techniques.

Most GC pesticide methods call for injecting 1-2 µL; splitless injection is used because it is compatible with dirty extracts of food, soil, or water. Pulsed splitless injection allows one to make injections of up to 5 µL using standard equipment[18]. Enormous gains in system sensitivity can be realized by using the PTV inlet in the "solvent vent" mode, which is compatible with injections of 5-1,000 µL. These large injections may be made manually or automatically using either a standard 6890 Series ALS in the multiple injection mode or by using a controlled speed injector available from Gerstel[19]. Because the injection process may take several minutes, manual injections are usually impractical and good precision may be hard to achieve.

The 6890 Series ALS is designed to make one or more injections of up to 25 µL into the PTV inlet. After the desired number of injections has been made, the inlet is heated and the chromatography begins. Though the system controls allow up to 99 injections, a reasonable upper limit is about 10, making 250 µL the typical injection volume limit for this system. For even larger injections, the controlled speed injector[19] should be used. For all of the analyses described below, 100 µL were injected by making 10 sequential injections of 10 µL each.

How the PTV Works in the Solvent Vent Mode

Figure 1 shows a diagram of the PTV inlet. For large-volume injections, three steps are required. These are: 1) injection and solvent elimination; 2) splitless sample transfer to the GC column; and 3) chromatographic separation and, if desired, a simultaneous inlet bake-out step. The steps are described more completely below.

Injection and Solvent Elimination (Step 1)

During injection, the column head pressure is set to 0 psi to eliminate or, in the case of GC/MS, reduce the flow through the column. When mass spectral detection is used, there is still

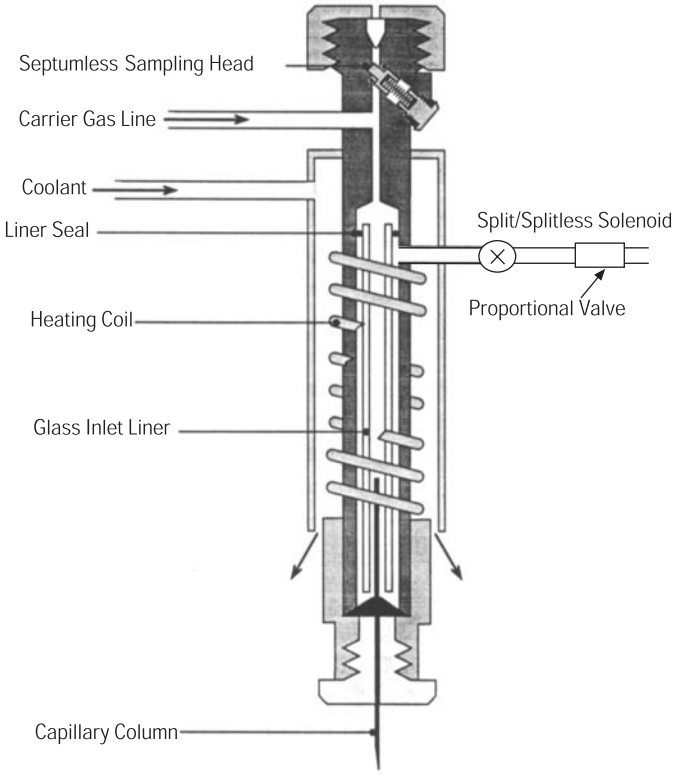


Figure 1. The PTV inlet shown with the septumless head. The inlet is also available with a septum head that may be equipped with a standard septum or a Merlin Microseal. (Figure reproduced with permission of Gerstel GMBH.)

some flow because the column outlet is under vacuum. At the same time, a steady stream of carrier gas passes through the inlet and out through the split vent. This flow is typically between 100 and 500 mL/min. The sample is injected into the cool liner where it remains as a liquid, dispersed over the liner walls or any packing material that may be in the liner. The steady flow of carrier gas through the liner causes the solvent (and any volatile fraction of the sample) to evaporate and be swept with the carrier gas out through the split vent. This is analogous to "blowing down" a sample with a stream of inert gas, except that this takes place inside the PTV inlet. When most of the solvent has evaporated, the next injection is made and the evaporation process repeats, accumulating more sample in the inlet. To recover an analyte completely, its boiling point should be at least 100 °C greater than that of the solvent; most pesticides fall into this category.

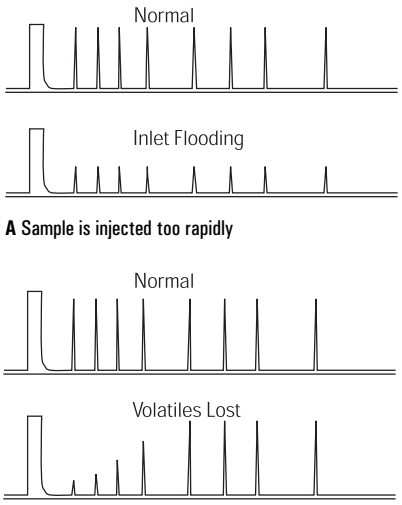


Figure 2. Chromatograms A and B illustrate the result of poor timing of multiple injections.

The timing of these multiple injections can be important. If the sample is introduced too rapidly, the liner may become flooded and liquid will be forced out through the split vent. Chromatographically, this shows up as reduced area counts for all analytes (see figure 2A). If there is too much time between injections, all of the solvent may evaporate and more of the volatile analyte fraction may be lost too. This results in poor recovery of volatiles but 100 percent recovery of the less volatile compounds (see figure 2B). Set-points such as inlet temperature, vent flow, and injection delay times can affect recovery of volatiles. Note that for 100 percent recovery, an analyte should have a boiling point at least 100 °C greater than the solvent. One can adjust the delay between injections by entering the desired value in the ChemStation software. Some experimentation is usually necessary when setting this delay for a new method. It will be dependent upon such factors as the solvent type, injection volume, vent flow, and inlet temperature.

Splitless Sample Transfer to the GC Column (Step 2)

Once the desired number of injections has been made, the column head pressure is restored and the vent flow is tur ned off. At this point, the inlet temperature is programmed up to a value that is sufficient to transfer all of the desired analytes to the GC column. This step is similar to a splitless injection, except that instead of flash vaporization, the sample is transferred as the inlet temperature is programmed up. For the most gentle treatment of labile analytes, slow ramp rates may be used. This allows analytes to be flushed into the column at the minimum temperature needed for volatilization. When sample decomposition is not a problem, the inlet may be heated as fast as 720 °C/min.

Chromatographic Separation (Step 3)

During sample transfer, the oven temperature is usually held between 30 °C below and 20 °C above the solvent's atmospheric boiling point, depending on whether the solvent effect is needed to focus the more volatile fraction of the analytes. Again, some experimentation is necessary to optimize peak shapes. After the sample has been transferred in step 2, the oven temperature is programmed up and chromatography begins.

After the inlet has reached its maximum temperature and sufficient time has elapsed to transfer the sample to the column, a purge flow of 30-50 mL/min is restored to the split vent. If desired, one can set a very large split flow for a few minutes and bake out the inlet at a higher temperature to remove nonvolatile impurities. To conserve carrier gas, gas saver should be turned on at the end of this bake-out step.

Entering PTV Inlet Parameters into the Agilent ChemStation

When preparing the PTV portion of a GC method, one should first decide on the sample size and how many injections are required. In this work, ten 10-µL injections were made for a total of 100 µL. When entering parameters into the ChemStation screen, the Injector icon is first selected (figure 3) under the "GC edit parameters" menu. Next, the Configure button is pressed to enter the syringe size and enable multiple injections. From the main injector screen, the injection volume (10 µL) and number of injections are entered[10] . For this work, a 12-second delay was chosen between injections to allow for solvent evaporation.

The estimated total injection time is listed on the Inlets screen (figure 4). This is helpful when setting the inlet and oven parameters. First, the vent flow rate (400 mL/min for these analyses) is chosen, which sets the vent pressure to 0 psi until the injection sequence is done and solvent from the last injection has largely evaporated (4.00 min in figure 4). This is done by entering these values in the following fields:

Vent Flow 400 mL/min
Vent pressure 0.0 psi until 4.00 min

Next, the purge flow and elapsed time are set by entering values in the following field:

Purge Flow to Split Vent
50.0 mL/min @ 6.50 min

Note that as an aid in setting up the method, the "estimated total injection time" is shown just above the previous data entry fields.

In this example, the normal column head pressure was restored and the vent flow was turned off at 4.00 min. This prepares the inlet for the splitless transfer of the sample to the column. The vent flow remained off until it was set to 50 mL/min at 6.5 min. Thus, there is a 2.5-min period for inlet temperature programming and splitless sample transfer to the column. In this example, the inlet was held at 40 ° C for 4.2 min, enough time to make 10 injections, turn off the purge flow, and restore the column head pressure; the PTV was then programmed to 320 ° C at 200 ° C/min (figure 4).

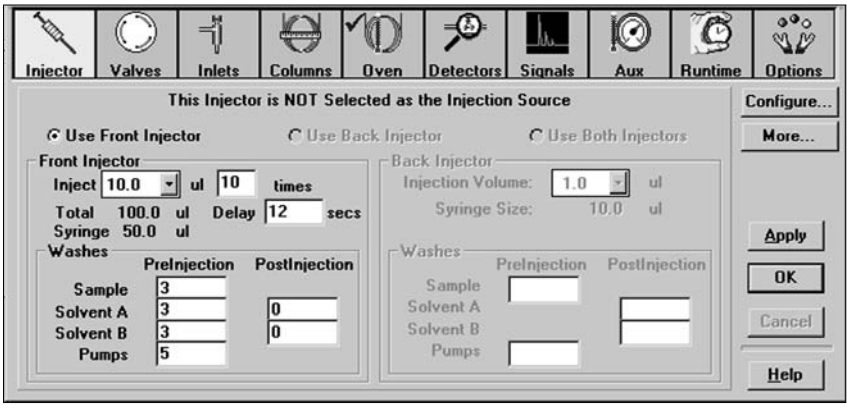


Figure 3. The injector screen from Agilent GC and GC/MS ChemStation software showing the setpoints available for multiple injections. To configure the sampler for multiple injections, set the syringe size, and choose slow injection, click on the Configure button.

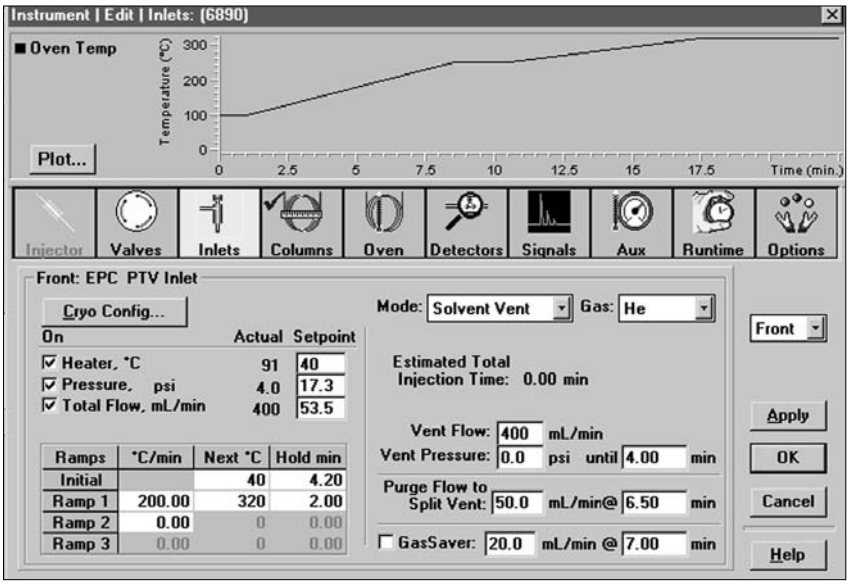


Figure 4. The inlets screen from Agilent GC and GC/MS ChemStation software showing the setpoints available for operation of the PTV inlet in the solvent vent mode.

Although not done for these analyses, the inlet could be baked out by setting the "purge flow to split vent" to a large value (perhaps 500 mL/min) at the end of the splitless time (6.50 min) and at the same time, program the inlet to a higher temperature. After the bake-out period, the inlet temperature is programmed downward and gas saver is turned on.

Normally, the GC oven is held at its starting temperature until the splitless injection is complete (6.50 min in this case) at which time oven temperature programming is begun. For this work, the oven temperature program was begun at 6.13 min so that the pesticide retention times would match a retention time data base that was in use. Figure 5 diagrams the PTV and GC oven setpoints used for this work.

PTV Inlet Liner Considerations

The correct liner choice is critical to the success of any pesticide analysis by PTV injection. The liner must be thoroughly deactivated or many labile pesticides may decompose or adsorb in the inlet. In general, any liner containing glass wool will be unsatisfactory for the analysis of labile pesticides, whether or not the glass wool is deactivated. At this time, two PTV liners are suggested for pesticide analysis:

- Part no. 5183-2037 is a deactivated, open multibaffled liner with no internal packing that may be used for single or multiple injections of 5 µL or less. This liner gives very good recovery for pesticides, even extremely difficult ones such as acephate and methamidophos.
- Part no. 5183-2041 is a deactivated liner with an internal coating of sintered glass to give it more surface area and is, therefore, suitable for single or multiple 25-µL injections. This liner gives better than 70 percent recovery for most pesticides, although tests have shown that acephate and methamidophos cannot be analyzed using this liner, and that recoveries of guthion are often less than 50 percent. A prototype version of this liner was used for all of the work described in this application note.

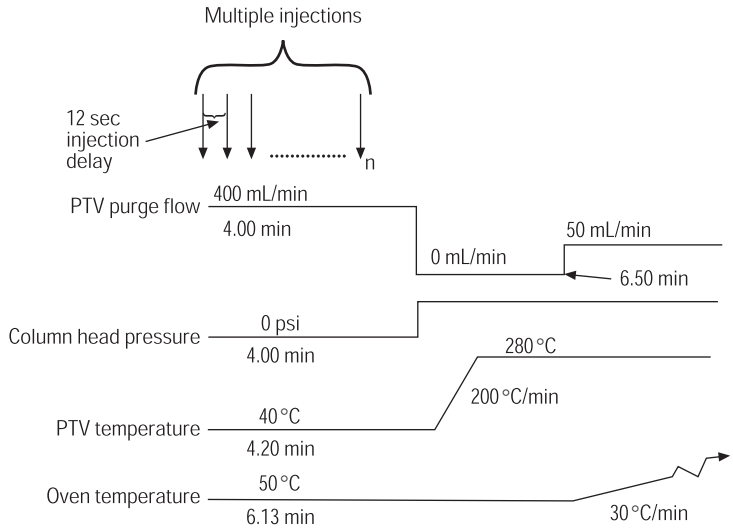
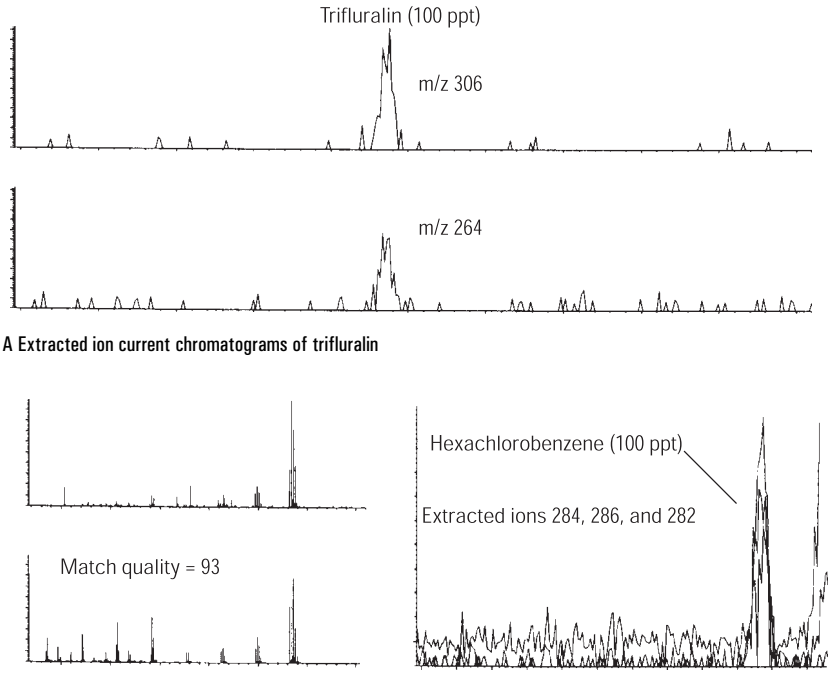


Figure 5. Illustration of the GC and sampler setpoints used for 100-µL injections of pesticide samples. Note that normally, the GC oven hold period would have been at least 6.5 min for this method. A value of 6.13 min pesticide retention times to a data base.

Results and Discussion

When compared to a typical 2-µL splitless injection, 100-µL PTV injections can often result in a 50-fold improvement in system detection limits. Selective detectors such as the MSD can help the analyst to realize the full measure of this sensitivity improvement by excluding background that may be introduced from solvent impurities, vial cap extract, and indigenous compounds coextracted with the analytes. In this application, it was possible to see most of the pesticides in the 14-component mixture at 100 ppt in the scan mode (400 ppt for the isomer mixes of permethrin and fenvalerate). Figure 6 shows extracted ion chromatograms for trifluralin and hexachlorobenzene (HCB) at 100 ppt. Library searching gave a match quality of 93 for the HCB peak. Fenvalerate isomers I and II were found in the solution in a ratio of about 78:22. Figure 7 shows extracted ion chromatograms for fenvalerate I at a concentration of 311 ppt.



A Extracted ion current chromatograms of trifluralin

B Extracted ion current chromatogram of HCB with its mass spectrum and library match

Figure 6. Scanning GC/MS results for a pesticide standard containing Trifluralin and Hexachlorobenzene at 100 ppt. (Ten 10-µL injections were made using the PTV inlet.)

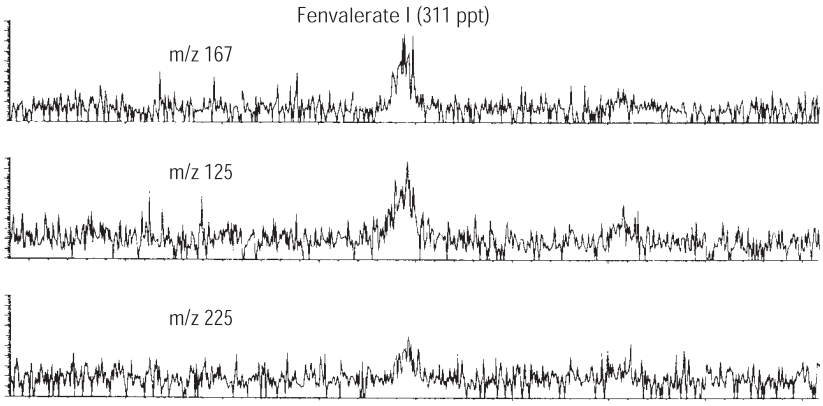


Figure 7. Extracted ion current chromatograms of Fenvalerate I at a concentration of 311 ppt in a pesticide standard. (Ten 10-µL injections were made using the PTV inlet.)

Analysis of a bell pepper extract revealed several pesticide residues. As seen in figure 8, chlorpyrifos and the endosulfans were easily detected. The Florida Department of Agriculture determined the concentration of chlorpyrifos, alpha-endosulfan, betaendosulfan, and endosulfansulfate to be 0.210, 0.011, 0.018, and 0.013 ppm, respectively. It is important to note that these compounds could be detected with very high selectivity by extracting high mass ions that are characteristic of these pesticides but not of the matrix. Using LVI, there is ample signal from these less abundant ions for good quantitation. With normal injection volumes, selectivity may have to be compromised and the most abundant ions extracted in a pesticide spectrum to gain sensitivity.

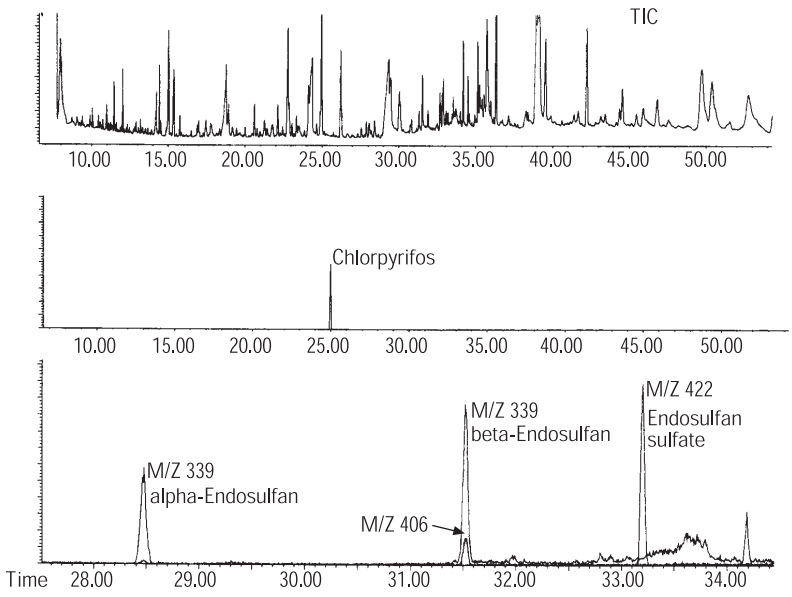


Figure 8. GC/MS Analysis of a bell pepper extract. (Ten 10-µL injections were made using the PTV inlet.) Using LVI, there was sufficient signal to use high mass ions with smaller abundances to achieve greater selectivity.

Phosmet, captan, and propoxur were all easily detected in a pear sample. The total ion current chromatogram (TIC) is shown in figure 9 along with spectrum obtained for captan juxtaposed with the library spectrum. Figure 10 shows the propoxur peak along with 2,4,6-tribromoanisole and 2,4,6-tribromophenol, two other compounds that were surprising to find in a pear sample. Though the origin of these brominated compounds is not known, a recent paper by Hoffmann and Sponholz 20 suggests that tribromophenol is used to treat storage palettes for the prevention of fire and mold growth, and that the anisole is formed from the phenol microbiologically. Perhaps these pears were shipped in containers that had been similarly treated.

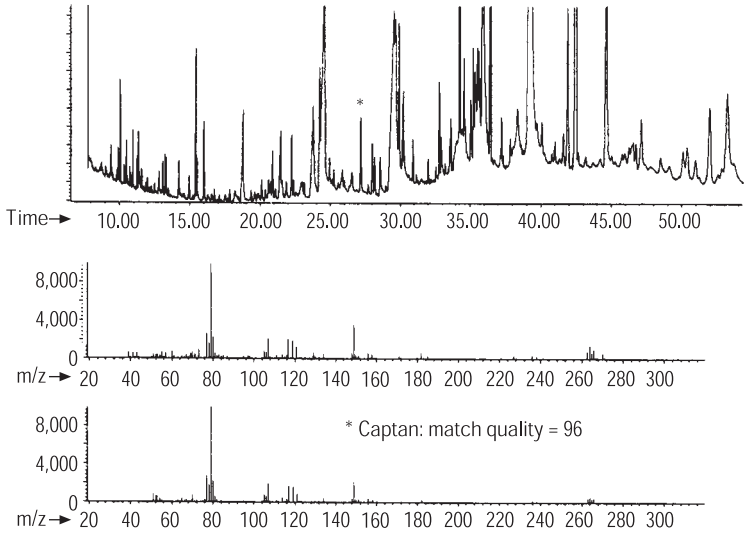


Figure 9. TIC of a pear extract resulting from a 100-µL Injection (10 x 10 µL). Captan was easily detected, and its spectrum gave a library match quality of 96.

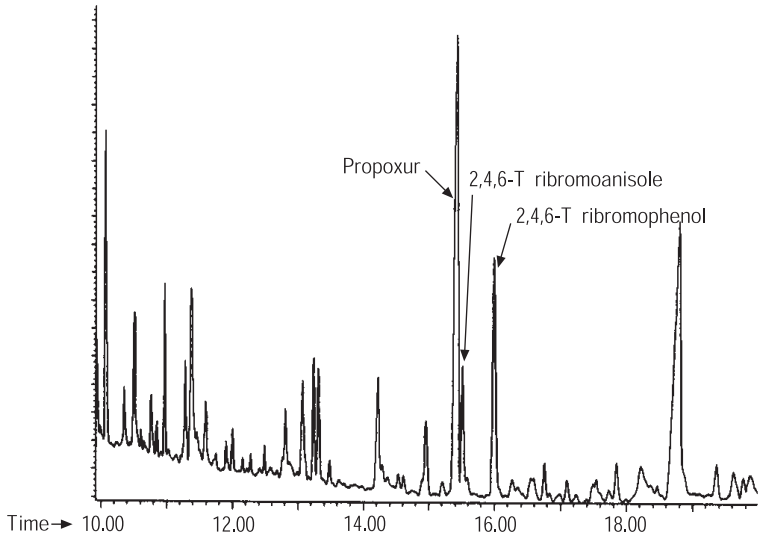


Figure 10. TIC of a pear extract resulting from a 100-µL Injection (10 x 10 µL). Propoxur and two brominated phenolics were easily identified.

A single sintered glass coated liner of the type described above (part no. 5183-2041) was used for about ten 50- and ten 100-µL injections (ca. 1,500 µL total) of vegetable extracts before it was replaced. All of the extracts were rather dirty, and an inlet bake-out step was not used. Although the liner looked somewhat discolored for about 2 cm where injections were made, it still performed well at the time it was replaced.

Conclusion

Using the PTV inlet in the solvent vent mode, it is relatively simple to increase system detection limits by one or two orders of magnitude. When combined with the Agilent 6890 Series automatic liquid sampler, multiple injections of up to 25 µL each into the inlet can be made, allowing the solvent to vent while pesticides and other less volatile analytes accumulate. After the desired sample volume has been introduced (typically 5-250 µL), the solvent vent is closed and the sample is transferred to the column in a temperature-programmed splitless injection. By making 100-µL injections into a PTV-equipped Agilent 6890 Series GC coupled to the Agilent 5973 MSD, it was possible to see several pesticides at the 100 ng/L level (100 ppt) in the scan mode. With such low detection limits, less abundant ions can be used to identify and quantitate pesticides at low ppb levels, thereby gaining in selectivity as well.

When performing LVI, there are several parameters to adjust and some method development time is usually required. However, the method described herein worked well and can be duplicated for the PTV/GC/MS analysis of pesticides in food.

Acknowledgment

The author wishes to thank Ms. Joanne Cook of the Florida Department of Agriculture and Consumer Services for supplying the food extracts used in these experiments and Dr. Bill Wilson (Agilent Technologies) for supplying liner deactivation test results.

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Printed in the USA 4/2000
5966-1214E

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The Analysis of Chlorinated Pesticides and PCBs Using the HP-608 Capillary Column

Application Note
228-236

Authors
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Abstract

Chlorinated pesticides and PCBs targeted in EPA Methods 608, 8080, 8081, and CLP pesticides for wastewater and solid wastes are analyzed under optimum conditions at a constant flow of 2.4 ml/min. The merits of splitless and on-column injection techniques using the Agilent 5890 Series II GC with electronic pressure control (EPC) are compared.

Key Words: chlorinated pesticides, PCBs, on-column injection, splitless injection, HP-608 capillary column, EPA 608, EPA 8080/8081, CLP pesticides, electronic pressure control.

Introduction

Chlorinated pesticides and PCBs have been banned in the U.S. for several years. However, because of their persistence in the environment, EPA methods 8080/8081 and CLP pesticides target 16 to 20 chlorinated organic pesticides in the evaluation of solid waste. This includes pesticides, their degradation products, technical grades of chlordane, toxaphene, and PCBs in solid waste.[1,2] EPA Method 608 targets similar pesticides in industrial and wastewater discharges.[3] EPA Methods 608 and 8080 prescribe packed-column analysis, whereas Methods 8081 and CLP pesticides prescribe capillary column analysis.

These EPA Methods allow laboratories to substitute columns of their choice provided that performance data such as chromatographic resolution, analyte breakdown, and MDLs (minimum detectable levels) are equal to or better than those provided with the EPA methods.

The HP-608 is a wide bore (530 µm-id) capillary column specially designed for the analysis of organic pesticides. GC/ECD separations of chlorinated pesticides and PCBs were done using the HP-608 column with both on-column and splitless inlet sample introductions. In both cases, the HP-608 provided superior chromatographic resolution, excellent reproducibility, and minimal analyte breakdown for the analysis of pesticides and PCBs.

Experimental

A 30 m x 530 µm x 0.5 µm HP-608 column (part no. 19095S-023) was used under constant carrier gas flow using the 5890 Series II GC with EPC equipped with a split/splitless inlet and a cool on-column inlet. Equipment included the 7673 automatic sampler with tray and the electron capture detector (ECD).

Samples were introduced in both the on-column and splitless modes. The Merlin™ Microseal septum (part no. 5181-8816) was used in the split/splitless inlet to replace the conventional inlet septum. A deactivated tapered glass liner (part no. 5181-3316) was used for all splitless injection runs. GC conditions were controlled using the HP 3365

Table 1. Experimental Conditions

Instrument Requirements	
Gas chromatograph:	Agilent 5890 Series II with EPC
Injection ports:	Split/splitless inlet with temperature and pressure programmable features On-column inlet with temperature and pressure programmable features
Column:	HP-608, 30 m x 530 µm x 0.5 µm (Part number 19095S-023)
Detector:	ECD
Sample introduction:	7673 splitless fast injection On-column injection
Data collection:	3365 ChemStation and HP Vectra 486/133T
Experimental Conditions	
Column:	HP-608, 30 m x 530 µm x 0.5 µm (Part number 19095S-023)
Carrier gas:	He, 20 cm/sec, 2.2 psi at 80°C with EPC under constant flow of 2.4 ml/min
Oven:	First ramp: 80°C (hold 1 min) to 190°C at 30°C/min Second ramp: 190°C to 280°C (hold 1 min) at 6°C/min Third ramp: 280°C to 300°C (hold 2 min) at 20°C/min
Injection:	Splitless: 1 µl, inlet temperature of 250°C On-column: 1 µl oven track for inlet temperature program
Detector:	ECD (330°C), 65 ml/min N_2 makeup, 6 ml/min anode purge
Sample:	Pesticides and PCB standard solutions in isooctane

ChemStation. Data was managed with a HP Vectra PC (486/33T). Instrument parameters and experimental conditions are listed in **Table 1**.

Pesticide solutions containing 16 to 22 components were prepared from the dilution of certified standards (part no. 8500-5873 and 8500-5876, mixes A and B: level 2) with isooctane (pesticide residue grade from Burdick & Jackson). Pesticide standards (part no. 5062-3589), including four vials of 16 EPA-608 pesticides and two vials of two component inlet check solutions (endrin/DDT concentrations are 50 ppb/100 ppb), were used without further dilution. These pesticide compounds are listed in **Table 2**.

Figure 1. Chromatograms of the 16 chlorinated pesticides under optimum GC conditions, 100 pg of each pesticide injected. Peak identification in Table 2.

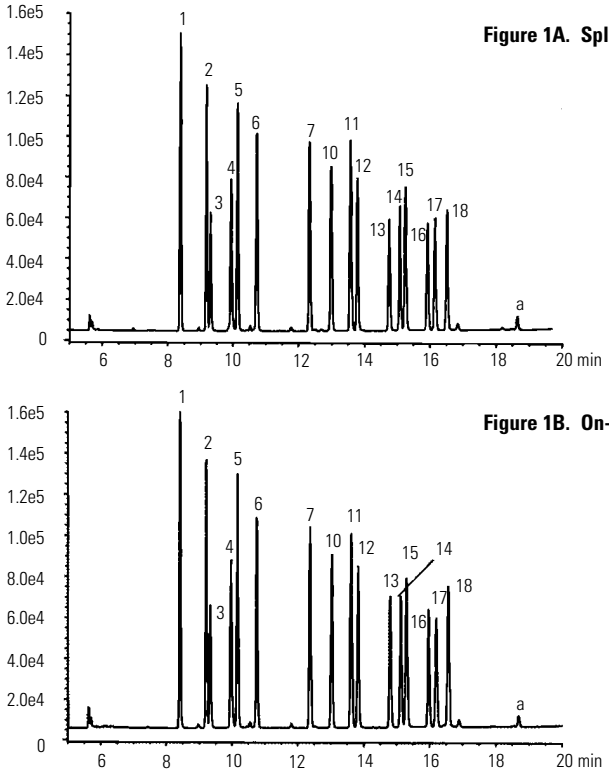


Table 2. Chlorinated Pesticides

Peak No.	EPA-608	Compound Name EPA-8080/8081	EPA-CLP Pesticides
1	alpha-BHC	alpha-BHC	alpha-BHC
2	Lindane	Lindane	Lindane
3	beta-BHC	beta-BHC	beta-BHC
4	Heptachlor	Heptachlor	Heptachlor
5	delta-BHC	delta-BHC	delta-BHC
6	Aldrin	Aldrin	Aldrin
7	Heptachlor epoxide	Heptachlor epoxide	Heptachlor epoxide
8		Chlordane-gamma	Chlordane-gamma
9			Chlordane-alpha
10	Endosulfan I	Endosulfan I	Endosulfan I
11	4,4′-DDE	4,4′-DDE	4,4′-DDE
12	Dieldrin	Dieldrin	Dieldrin
13	Endrin	Endrin	Endrin
14	4,4′-DDD	4,4′-DDD	4,4′-DDD
15	Endosulfan II	Endosulfan II	Endosulfan II
16	4,4′-DDT	4,4′-DDT	4,4′-DDT
17	Endrin aldehyde	Endrin aldehyde	Endrin aldehyde
18	Endosulfan sulfate	Endosulfan sulfate	Endosulfan sulfate
19		Methoxychlor	Methoxychlor
20	a-Degradation product		Endrin ketone
SS1			Tetrachloro-m-xylene
SS2			Decachlorobiphenyl

Results and Discussion

Splitless Analysis

Figure 1A shows the analysis of a standard solution containing the 16 EPA-608 targeted pesticides at a constant column flow of 2.4 ml/minute. One microliter of sample (100 pg of each component) was introduced in splitless mode at 250°C under the conditions[4] listed in **Table 1**. All 16 components were well resolved in sharp symmetric peaks, and the analysis was completed in less than 17 minutes. The 30-m HP-608 (530 µm id) column possesses sufficient efficiency to completely resolve the complex pesticides mix, including chlorinated compounds with similar or isomeric structures. The absence of coeluting peaks on the HP-608 column permitted fast and accurate identification and quantitation.

Low-Temperature On-Column Analysis

Figure 1B shows the same pesticides standard mix using the cool on-column injection technique. On-column injection of 1 µl of sample at 80°C resulted in little sample degradation, minimal byproducts, and good sensitivity (see **Table 3**). Common to both **Figures 1A** and **1B** is the absence of tailing peaks, including the endrin aldehyde peak (peak 17), indicating the HP-608 column surface is very inert.

Reproducibility

Reproducibility for the analysis of chlorinated pesticides using HP-608 columns with the HP GC/ECD system was excellent (see **Table 3**). The RSD (relative standard deviation) in absolute area counts for all 16 EPA targeted pesticides was less than 2% for on-column runs (two sets of six replicate injections). Similarly, the peak area counts reproducibility for all splitless injection runs (three sets of six replicate injections) was in the 1% to 2% RSD range using the same standard sample.

The standard deviation of retention times was within 0.003–0.005 minutes and 0.002 minutes for on-column and splitless runs, respectively. In comparison, the standard deviation of retention times for EPA Method 8081 analysis (**Table 10**, reference 1) using wide-bore capillary columns ranged from 0.007 minutes to 0.013 minutes for the same set of pesticides. This clearly demonstrates that chromatographic reproducibility obtained using the HP-608 capillary column is better than that obtained using the capillary columns stipulated in EPA Method 8081.

Table 3. Reproducibility of Pesticide Analysis

	Retention Times, min			Area Counts		
Pesticides	**Mean**	**Std Dev**	**% RSD**	**Mean**	**Std Dev**	**% RSD**
A. On-column injection (100 pg each component)						
alpha-BHC	8.423	0.004	0.047	431643	7497	1.74
Lindane	9.225	0.004	0.046	393514	6496	1.65
beta-BHC	9.352	0.004	0.046	208287	3428	1.65
Heptachlor	9.984	0.004	0.042	310294	5430	1.75
delta-BHC	10.181	0.005	0.044	390027	7428	1.90
Aldrin	10.760	0.004	0.039	359246	6996	1.95
Heptachlor epoxide	12.385	0.003	0.028	359586	5740	1.60
Endosulfan I	13.036	0.004	0.031	321622	5478	1.70
4,4′-DDE	13.623	0.004	0.026	341930	7070	2.07
Dieldrin	13.838	0.004	0.027	336042	4832	1.44
Endrin	14.814	0.004	0.025	268560	5298	1.97
4,4′-DDD	15.135	0.004	0.024	254389	3017	1.19
Endosulfan II	15.311	0.004	0.025	297580	4326	1.45
4,4′-DDT	15.975	0.003	0.021	259369	3881	1.50
Endrin aldehyde	16.208	0.004	0.022	205588	1876	0.91
Endosulfan sulfate	16.570	0.003	0.021	281397	4143	1.47
a, Degradation product	18.690	0.003	0.017	3416	97	2.83
B. Splitless injection (100 pg each component)						
alpha-BHC	8.351	0.002	0.020	376446	7222	1.92
Lindane	9.146	0.002	0.020	317405	6592	2.08
beta-BHC	9.273	0.002	0.018	165105	3129	1.90
Heptachlor	9.898	0.002	0.018	207924	4637	2.23
delta-BHC	10.097	0.001	0.013	301779	6113	2.03
Aldrin	10.671	0.002	0.015	308689	6422	2.08
Heptachlor epoxide	12.289	0.001	0.011	289985	6216	2.14
Endosulfan I	12.938	0.002	0.014	253489	5496	2.17
4,4′-DDE	13.527	0.001	0.011	313249	6102	1.95
Dieldrin	13.735	0.002	0.014	209054	3925	1.88
Endrin	14.710	0.002	0.013	160235	3104	1.94
4,4′-DDD	15.034	0.002	0.013	168113	3094	1.84
Endosulfan II	15.207	0.002	0.015	228810	4868	2.13
4,4′-DDT	15.874	0.002	0.012	168810	2129	1.26
Endrin aldehyde	16.103	0.002	0.010	148655	3687	2.48
Endosulfan sulfate	16.467	0.002	0.013	190284	3003	1.58
a, Degradation product	18.584	0.002	0.012	21513	1747	8.12

Comparison of Sample Introduction Techniques

For all on-column injection runs, degradation was negligible due to the low initial column temperature (80°C) and the direct introduction of a liquid sample plug into an inert column. As a result, inlet-related sample discrimination, alteration, and degradation were eliminated, while the advantages of solvent focusing and stationary phase focusing were maximized. Routine analysis of the inlet check solution (specified by the EPA methods) showed that the average degradation was less than 3% for endrin and 1% for DDT.

As demonstrated by the clean baseline in **Figure 1A**, little sample degradation occurred at an inlet temperature of 250°C. However, a small endrin ketone peak (RT of 18.69 minutes) appeared on the chromatograms from the GC runs with both on-column and splitless injection shown in **Figures 1A** and **1B**. A closer look (**Table 3**), shows that the area counts for endrin ketone (peak a, a byproduct of endrin degradation) measured 5 times larger in the splitless runs than for the on-column runs (average absolute area counts of 3,400 versus 21,000). The GC runs of the inlet check standard (after 200 repeated splitless injections), showed a 7% endrin degradation and 10% DDT degradation. These values were well below the EPA requirement of 15% degradation for both endrin and DDT.

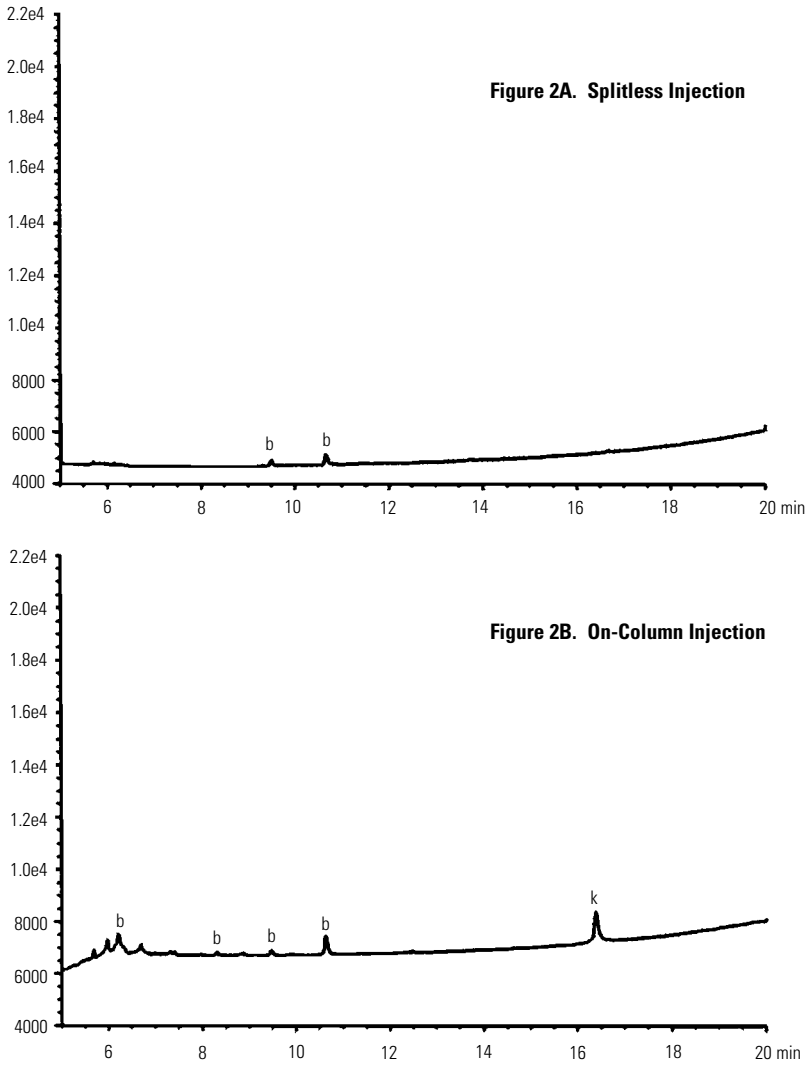
Use of the Merlin™ Microseal[5] and the deactivated glass liner also contributed directly to the low degradation rate in the splitless mode. The Microseal is designed to provide a good inlet seal without using a conventional septum. By eliminating the introduction of particulates into the inlet liner from conventional septum, useful life for the inlet liner is extended, down time (to change a liner and a conventional septum) is reduced, and laboratory throughput is increased.

The use of splitless injection technique may also prevent interference from extraneous and high boiling materials in dirty samples. This is demonstrated in **Figures 2A** and **2B**. **Figure 2** shows the analysis of isooctane solvent (pesticide-residue grade) using both splitless (**Figure 2A**) and on-column injection (**Figure 2B**). The late-eluting peak (peak k) , at 16.69 minutes retention time in the on-column run, does not appear in the chromato-gram of the splitless run (**Figure 2A**).

This peak, possibly a high boiling contaminant in isooctane, appears again in **Figure 3B**. **Figures 3A** and **3B** show analyses of a 10-ppb pesticide standard using splitless injection and on-column injection, respectively. The peak (peak k) eluting just before endosulfan sulfate (peak 18) may cause a higher value for the determination of trace endosulfan sulfate in the sample.

Both area counts and peak heights for the splitless runs were smaller than those for the on-column injection runs (see **Table 3**). For example, the average counts of lindane from the splitless runs were approximately 80% of those from the on-column injections (**Table 3**). Therefore, on-column injection is a good choice for clean samples and trace analyses demanding high sensitivity and low detection limits (large area counts).

Figure 2. Chromatograms of isooctane under optimum GC conditions, 1 µl injected. (b,k=solvent contaminants)



Analysis of PCBs and EPA Methods 8080, 8081, and CLP Pesticides

For wastewater and solid waste samples, the EPA recommends splitless injection for the determination of pesticides and PCBs. Using splitless injection under optimum 5890 Series II GC conditions, all 17 pesticides targeted by EPA Method 8080B are resolved as shown in **Figure 4**.

Among the 20 components targeted by EPA Methods 8081 and CLP pesticides, all but alpha-chlordane and endosulfan I (they are partially separated) are well resolved by the HP-608 column (**Figure 5**). Since the HP-608 column can effectively separate the complex mix of these pesticides, it is a good column choice for the determination of PCBs and multiple-peak response pesticides such as chlordane and toxaphene. **Figure 6** shows a comparison of chromatograms for technical grade chlordane and toxaphene, while **Figure 7** is a comparison of chromatograms for seven PCBs, all analyzed under the same GC conditions using the HP-608 capillary column.

Figure 3. Chromatograms of dilute pesticides mix under optimum GC conditions; 10 pg of each pesticide injected. (Peak ID, see Table 2)

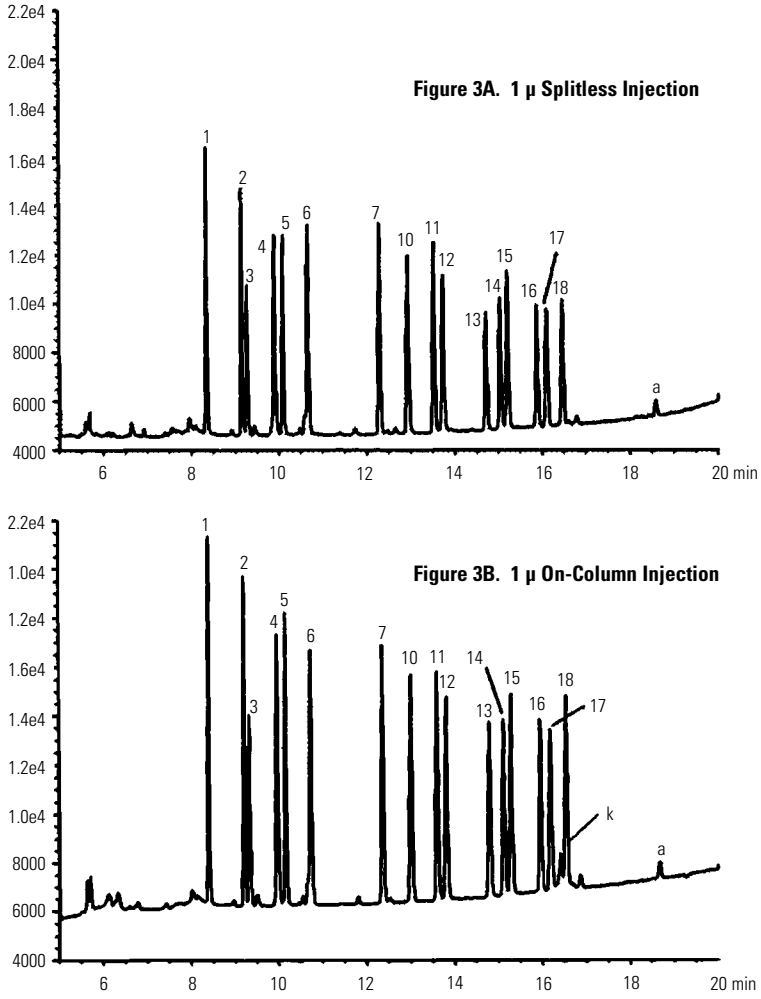
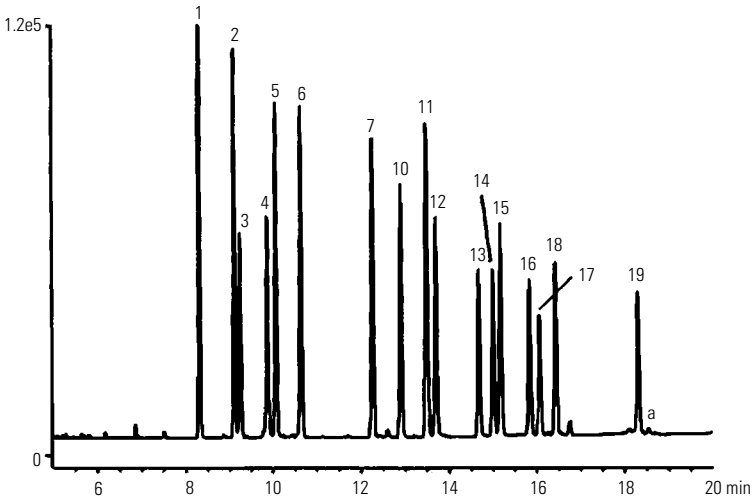


Figure 4. Chromatograms of the EPA-Method 8080 pesticides under optimum GC conditions. Splitless injection of 100–200 pg per component. (Peak ID, see Table 2)



Conclusion

Under optimal conditions, the HP-608 column separates 16 EPA-608 pesticides in 17 minutes and 20 EPA-CLP pesticides (and EPA-8081 pesticides) in 19 minutes (22 minutes including the surrogate, decachlorobiphenyl). Both splitless and on-column injections yield little sample degradation and provide excellent reproducibility of retention times and area responses. On-column injection is more suitable for clean samples and trace analysis, while splitless injection is better used for wastewater and waste samples.

Figure 5. Chromatogram of pesticides targeted in EPA-method 8081 and CLP pesticides under optimum GC conditions. Splitless injection of 50–100 pg per component. (Peak ID, see Table 2)

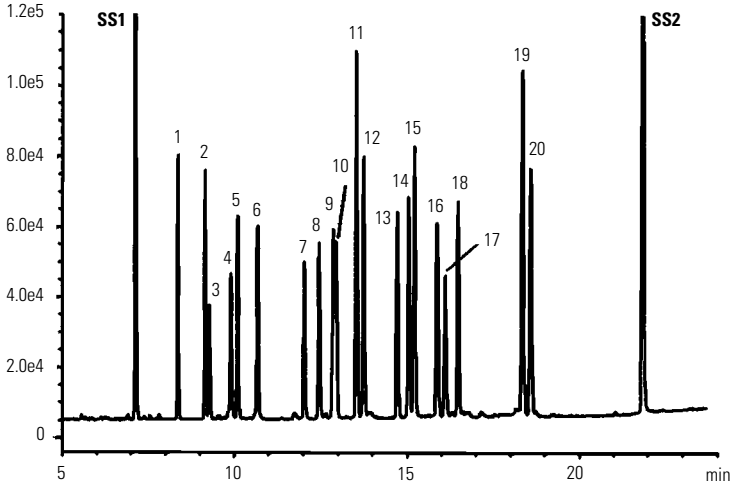


Figure 6. Chromatogram of technical grade toxaphene and chlordane under optimum GC conditions. Splitless injection of 1 µl 2.5 ppm mix

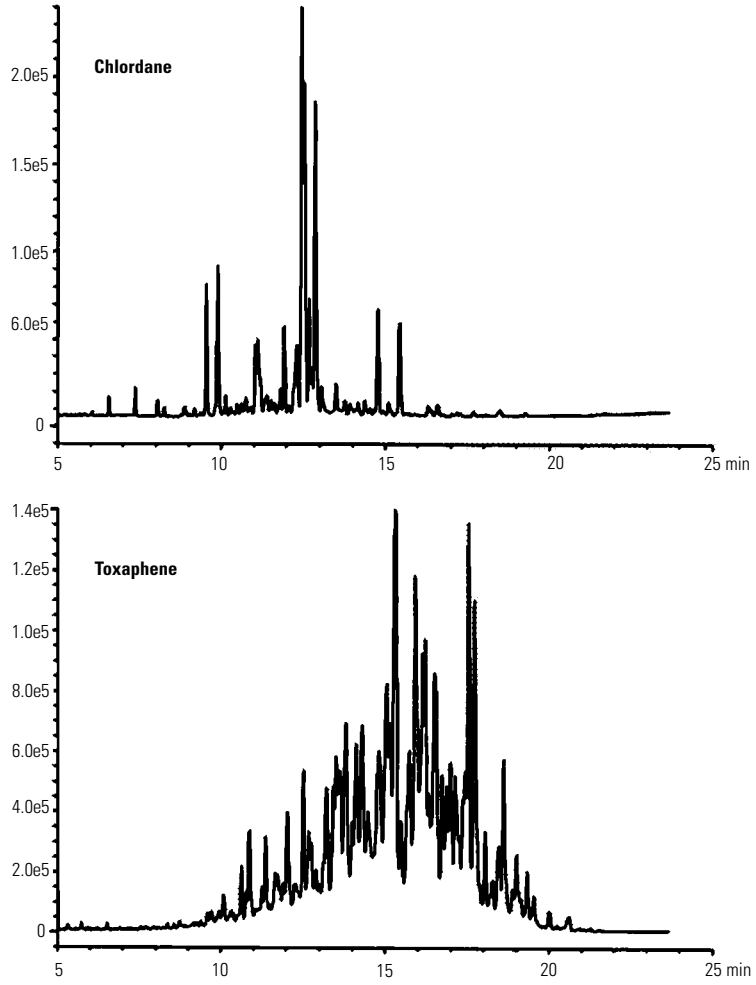
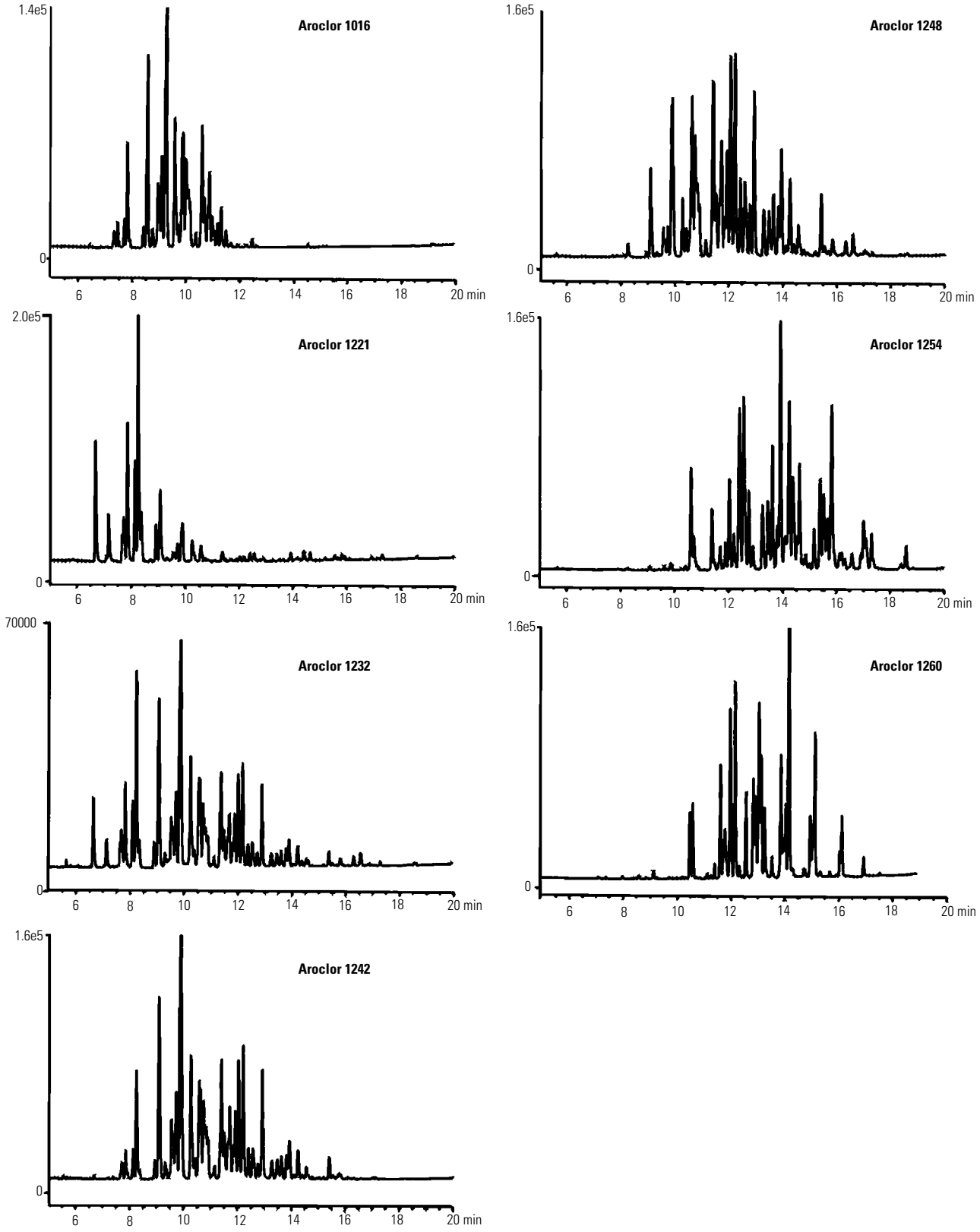


Figure 7. A comparison of seven PCBs under optimum GC conditions. Splitless injection of 1 µl 2.5 ppm each



Acknowledgment

The authors wish to thank Dr. D. Pautler for his many helpful discussions.

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5091-7567E

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Analysis of Organochlorine Pesticides and PCB Congeners with the Agilent 6890 Micro-ECD

Application

Gas Chromatography

June 1997

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Abstract

A new electron capture detector (ECD) for the Agilent 6890 Series gas chromatograph (GC) was used to analyze polychlorinated biphenyl congeners and organochlorine pesticides. The linearity of the 6890 Micro-ECD in the calibration range of 2 to 400 ppb was evaluated. The micro-ECD easily meets the linearity requirements of U.S. EPA contract laboratory programs for pesticides. Its limit of detection for these compounds goes down to less than 50 ppt. The micro-ECD also exhibits good reproducibility.

Key Words

Organochlorine pesticides, PCB congeners, 6890 GC, micro-ECD; pesticide analysis, ECD.

Introduction

The electron capture detector (ECD) is the detector of choice in many Contract Laboratory Programs (CLP)[1] and EPA methods for pesticide analysis because of its sensitivity and selectivity for halogenated compounds. However, there are drawbacks to the ECD design. The ECD is inherently nonlinear[2], with a limited linear range. The limited linear range means that dilution and reanalysis are frequently required for samples that are outside the calibration range.

Also, the typical ECD is designed to be compatible with both packed and capillary columns. This results in a flow cell that is larger than that required for capillary columns alone, which reduces detector sensitivity.

To address these problems, a new ECD was developed for the 6890 Series gas chromatograph (GC). The 6890 Micro-ECD has a smaller flow cell optimized for capillary columns and was redesigned to improve the linear operating range.

This application note examines the linearity, reproducibility, and limit of detection of the new ECD with mixtures of polychlorobiphenyl (PCB) congeners and organochlorine pesticides (OCPs).

Experimental

All experiments were performed on an 6890 Series GC with electronic pneumatics control (EPC) and the 6890 Micro-ECD. Table 1 shows the experimental conditions for PCB congeners and OCPs.

Table 1. Experimental Conditions for PCB Congener and OCP Analysis.

System Conditions	PCB Congener Analysis	OCP Analysis
Oven	80 °C (2 min); 30 °C/min to 200 °C; 10 °C/min to 320 °C (5 min).	80 °C (2 min); 25 °C/min to 190 °C; 5 °C/min to 280 °C; 25 °C/min to 300 °C (2 min).
Inlet	Split/splitless; 300 °C	Split/splitless; 250 °C
Carrier	Helium, 16.8 psi (80 °C); 1.3-mL/min constant flow	Helium, 23.9 psi (80 °C); 2.2-mL/min constant flow
Sampler	Agilent 7673, 10-µL syringe, 1-µL splitless injection	7673, 10-µL syringe, 1-µL splitless injection
Column	30-m, 250-µm id, 0.25-µm film HP-5MS (part no. 19091S-433)	30-m, 250-µm id, 0.25-µm film HP-5MS (part no. 19091S-433)
Detector	330 °C; makeup gas: nitrogen, constant column and makeup flow	330 °C; makeup gas: nitrogen, constant column and makeup flow

The solutions were prepared by making appropriate dilutions of a stock solution with isooctane. For PCB congeners, the stock solution was an EPA PCB congener calibration check solution (from Ultra Scientific Company, part number RPC-EPA-1). For OCPs, the solution was an OCP calibration check solution (part number 8500-5876).

Results and Discussion

Linearity and Response Factors

A series of dilutions of the PCB mixture from 2 ppb to 200 ppb and of the OCP mixture from 2 ppb to 400 ppb was injected into the 6890 Micro-ECD system. The linearity was determined by calculating the correlation coefficient from the resulting calibration curve.

Figures 1 and 2 present typical chromatograms of OCPs and PCBs at 20 or 40 ppb and 50 ppb, respectively. Figure 3 is a calibration curve of decachlorobiphenyl, typical of other PCB congeners. Figure 4 shows the calibration curve of 4, 4' DDE, typical of OCPs. The correlation coefficient,

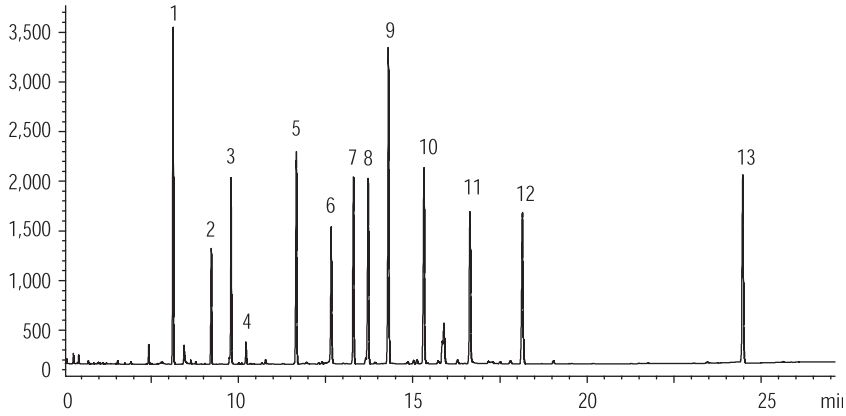

Figure 1. Typical chromatogram of OCPs at 20 or 40 ppb.
See table 1 for conditions. See table 5 for peak identification.

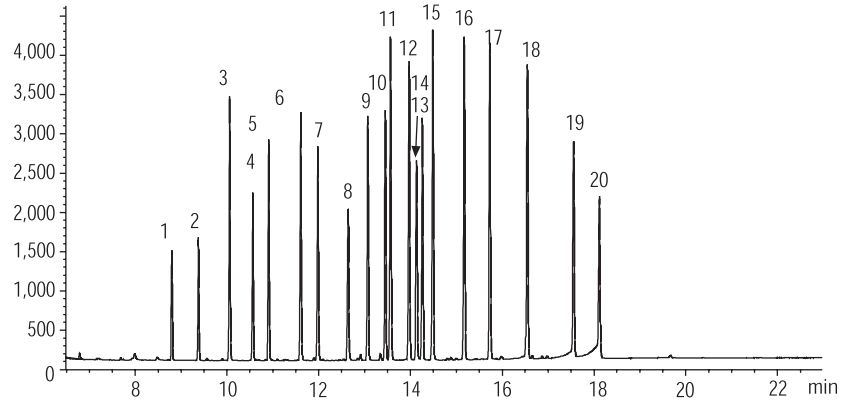

Figure 2. Typical chromatogram of PCB congeners at 50 ppb.
See table 1 for conditions. See table 4 for peak identification.

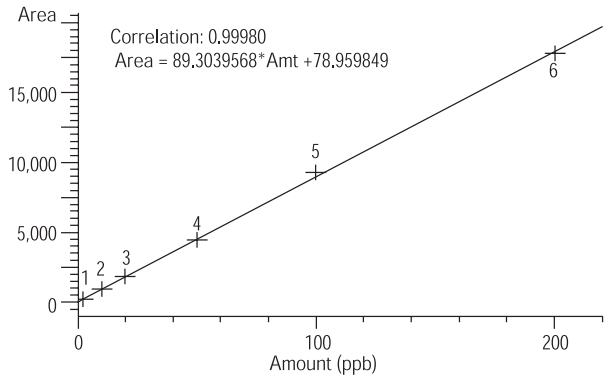

Figure 3. Typical linearity of PCB congener analysis: decachlorobiphenyl from 2-200 ppb.

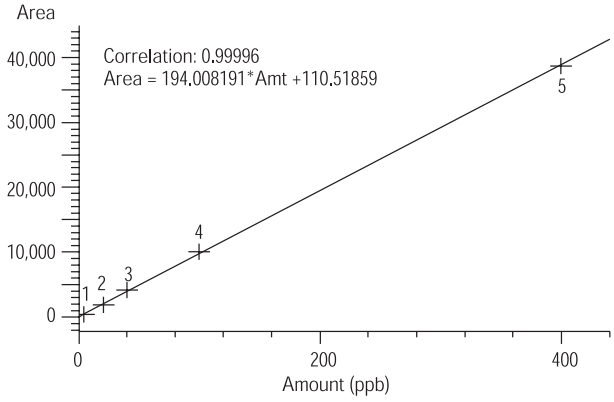

Figure 4. Typical linearity of OCP analysis: 4,4' DDE from 4 to 400 ppb.

average response factors, and percent relative standard deviation (%RSD) for the response factors for each analyte are shown in tables 2 and 3.

All correlation coefficients were at least 0.9996. In these experiments, the 6890 Micro-ECD is linear over this range. The typical range required by CLP methods is 5-80 ppb[1], so the 6890 Micro-ECD exceeds the range by almost twofold.

In addition, the CLP method requires the percent RSD of the response factors for most components to be less than 20 percent for a three-point calibration curve (5 to 80 ppb). As shown in tables 2 and 3, the percent RSD of the response factors ranged from 0.55 percent to 12. 5 percent for the PCB congeners and from 2.8 percent to 10 percent for the OCPs over a concentration range of two orders of magnitude (2 to 400 ppb). Furthermore, the average response factor of each analyte was so consistent and reproducible that the internal standard technique can be used to quantitate all OCPs and PCB congeners.

Table 2. PCB Congener Analysis: Linearity of the 6890 Micro-ECD 2 ppb to 200 ppb.
See table 1 for conditions.

Peak	Name	Average Response Factor	%RSD of Response Factor	Correlation (%)
1	2,4-Dichlorobiphenyl	2e-2	12.5	99.97
2	2,2′,5-Trichlorobiphenyl	2e-2	11.1	99.97
3	2,4,4′-Trichlorobiphenyl	8.5e-3	7.5	99.99
4	2,2′,5,5′-Tetrachlorobiphenyl	1.3e-2	10.2	99.97
5	2,2′,3,5-Tetrachlorobiphenyl	1e-2	9.4	99.98
6	2,3,4,4′-Tetrachlorobiphenyl	8e-3	6.7	99.99
7	2,2′,4,5,5′-Pentachlorobiphenyl	9e-3	8.8	99.98
8	3,3′,4,4′-Tetrachlorobiphenyl	1.2e-2	12.6	99.97
9	2,3,4,4′,5-Pentachlorobiphenyl	8e-3	5.5	99.99
10	2,2′,4,4′,5,5′-Hexachlorobiphenyl	8e-3	8.1	99.98
11	2,3,3′,4,4′-Pentachlorobiphenyl	6e-3	1.9	99.99
12	2,2′,3,4,4′,5-Hexachlorobiphenyl	6.5e-3	3.8	99.99
13	3,3′,4,4′,5-Pentachlorobiphenyl	9e-3	6.5	99.99
14	2,2′,3,4,5,5′,6-Heptachlorobiphenyl	8e-3	5.7	99.99
15	2,2′,3,3′,4,4′-Hexachlorobiphenyl	5.6e-3	1.8	99.99
16	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	5.8e-3	1.0	99.99
17	2,2′,3,3′,4,4′,5-Heptachlorobiphenyl	5.8e-3	0.57	99.99
18	2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl	6e-3	0.78	99.99
19	2,2′,3,3′,4,4′,5,5′,6-Nonachlorobiphenyl	8e-3	3.1	99.96
20	Decachlorobiphenyl	1e-2	9.5	99.98

Table 3. OCP Analysis: Linearity of the 6890 Micro-ECD *2 or 4* ppb to *200 or 400* ppb.
See table 1 for conditions.

Peak	Name	Average Response Factor	% RSD of Response Factor	Correlation (%)
1	2,4,5,6-Tetra-m-xylene	4.2e-3	5.3	99.97
2	beta-BHC	1.1e-2	7.1	99.99
3	delta-BHC	6.4e-3	4.7	99.99
4	Aldrin	4.7e-3	9.5	99.97
5	Heptachlor epoxide	4.7e-3	5.4	99.99
6	gamma-Chlordane	6.6e-3	6.6	99.99
7	alpha-Chlordane	5e-3	4.3	99.98
8	4,4' DDE	5e-3	2.8	99.99
9	Endosulfan II	2.9e-3	4.4	99.98
10	Endrin aldehyde	4.5e-3	5.9	99.94
11	Endosulfan sulfate	5.1e-3	5.3	99.97
12	Endrin ketone	4.7e-3	9.0	99.89
13	Decachlorobiphenyl	3.7e-3	9.9	99.96

Reproducibility

The reproducibility of the 6890 Micro-ECD was established by analyzing each mixture using identical conditions five times. Each analyte in the PCB congener mixture was injected at a concentration of 50 ppb, and the analytes in the OCP mixture were 20 or 40 ppb. The results are shown in tables 4 and 5. The highest %RSD for any analyte is 3.69 percent for aldrin, which is well below the CLP maximum allowable RSD of 15 percent.[1]

Table 4. PCB Congener Analysis: Reproducibility of the 6890 Micro-ECD 50 ppb; N=5.
See table 1 for conditions.

Peak	Name	Average Area	RSD (%)
1	2,4-Dichlorobiphenyl	2229	1.26
2	2,2′,5-Trichlorobiphenyl	2547	1.29
3	2,4,4′-Trichlorobiphenyl	5687	1.41
4	2,2′,5,5′-Tetrachlorobiphenyl	3721	1.43
5	2,2′,3,5-Tetrachlorobiphenyl	4941	1.46
6	2,3,4,4′-Tetrachlorobiphenyl	5943	1.40
7	2,2′,4,5,5′-Pentachlorobiphenyl	5089	1.47
8	3,3′,4,4′-Tetrachlorobiphenyl	3822	1.72
9	2,3,4,4′,5-Pentachlorobiphenyl	6203	1.62
10	2,2′,4,4′,5,5′-Hexachlorobiphenyl	6189	1.44
11	2,3,3′,4,4′-Pentachlorobiphenyl	8375	1.68
12	2,2′,3,4,4′,5-Hexachlorobiphenyl	7538	1.56
13	3,3′,4,4′,5-Pentachlorobiphenyl	5092	2.02
14	2,2′,3,4,5,5′,6-Heptachlorobiphenyl	6224	1.69
15	2,2′,3,3′,4,4′-Hexachlorobiphenyl	8921	1.67
16	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	8527	1.82
17	2,2′,3,3′,4,4′,5-Heptachlorobiphenyl	8625	1.91
18	2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl	8338	2.13
19	2,2′,3,3′,4,4′,5,5′,6-Nonachlorobiphenyl	6097	2.55
20	Decachlorobiphenyl	4622	2.85

Table 5. OCP Analysis: Reproducibility of the 6890 Micro-ECD; N=5.
See table 1 for conditions.

Peak	Name	Concentration (ppb)	Average Area	RSD (%)
1	2,4,5,6-Tetra-m-xylene	20	4785	0.7
2	beta-BHC	20	1802	0.81
3	delta-BHC	20	3251	1.50
4	Aldrin	20	402	3.69
5	Heptachlor epoxide	20	4316	1.58
6	gamma-Chlordane	20	2958	1.23
7	alpha-Chlordane	20	4219	1.06
8	4,4' DDE	40	4103	1.76
9	Endosulfan II	40	7176	1.27
10	Endrin aldehyde	40	4719	0.85
11	Endosulfan sulfate	40	4040	3.04
12	Endrin ketone	40	4386	2.52
13	Decachlorobiphenyl	40	5369	0.85

Detection Limit

To establish the lower limit of detection for the 6890 Micro-ECD with PCBs and OCPs, 1-µL injections were made at gradually decreasing concentrations. Figures 5 and 6 show chromatograms with analyte concentrations of 50 to 100 ppt.

All the analyte peaks for both the PCB congener and OCP mixtures are still easy to quantitate, and in fact smaller concentrations can be reliably analyzed. Aldrin, which has the lowest response of the OCPs, still exhibits an adequate signal-to-noise ratio at the 50 ppt level under these analysis conditions.

Conclusion

The Agilent 6890 Micro-ECD response was linear over the concentration range of 2 to 200 ppb, produced reproducible results, and exhibited excellent sensitivity for mixtures of PCB congeners and OCPs.

References

1. U.S. EPA Contract Laboratory Program, "Statement of Work for Organic Analysis," OLM03.1, August 1994.

2. R. Buffington and M.K. Wilson, eds., *Detectors for Gas Chromatography*, Agilent Technologies, Inc., Part Number 5958-9433, 1987.

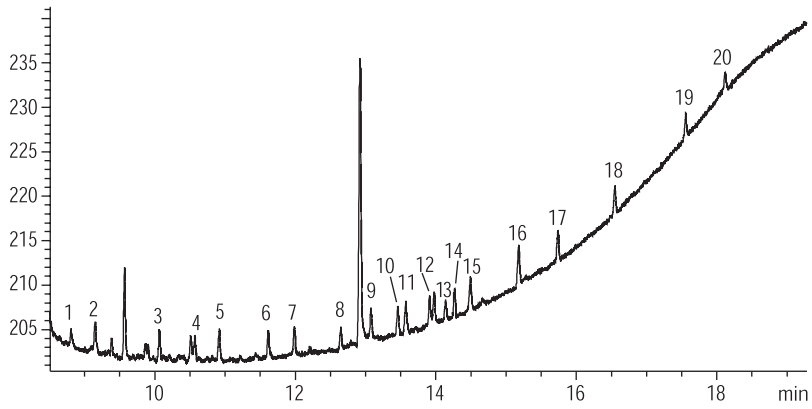

Figure 5. PCB congener mixture at 50 ppt each.
See table 1 for conditions. See table 4 for peak identification.

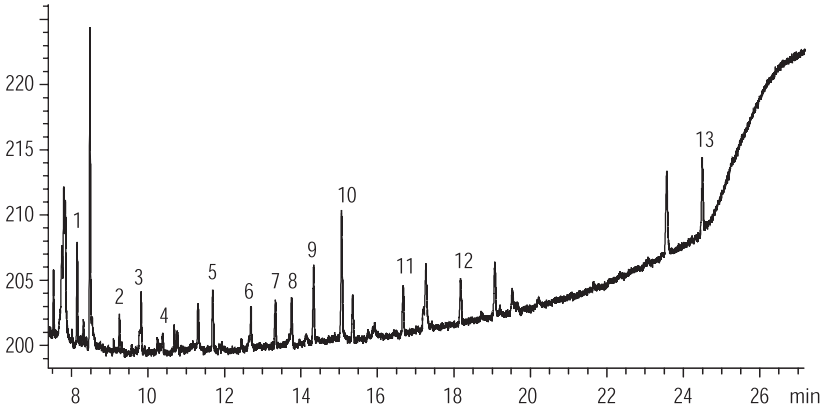

Figure 6. OCP Mixture at 50 to 100 ppt.
See table 1 for conditions. See table 5 for peak identification.

Printed in the USA 3/2000
5965-8556E

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Analysis of Sulfur and Phosphorus Compounds with a Flame Photometric Detector on the Agilent 6890 Series Gas Chromatograph

Application

Gas Chromatography

February 1997

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Abstract

A gas chromatograph (GC) with a flame photometric detector (FPD) is frequently employed in analyzing complex samples for specific compounds. The wavelength filter of the FPD can be set to select for many elements, but it is most commonly used to detect sulfur and phosphorus. This application note discusses the uses of the FPD in gas chromatography, demonstrates the linearity and method detection limits (MDL) of the 6890 Series GC with an FPD, and gives examples of analyses of organophosphorus pesticides using the 6890 GC with an FPD.

Key Words

Gas chromatography, flame photometric detector, FPD, sulfur analysis, phosphate analysis, pesticides, organophosphorus pesticides, EPA method 1618, EPA method 622.

Introduction

The flame photometric detector is one of the most widely used selective detectors in gas chromatography. The FPD consists of a reducing flame that produces chemiluminescent species. These species emit characteristic light that is optically filtered for the desired wavelength; the wavelength selection determines which compound is detected. The filtered light is measured by a photomultiplier and transduced into a signal. A second photomultiplier can be added, which allows simultaneous detection of a second signal.

FPD filters can be selected for many different compounds, but the most common uses are for the selective detection of sulfur and phosphorus compounds in complex mixtures. The selectivity of classical FPDs is typically (as a ratio by weight to carbon) 10^5 for sulfur and 10^6 for phosphorus. The FPD operates over a dynamic range of 1×10^3 for sulfur and 1×10^4 for phosphorus.[1]

Gas chromatography with an FPD can be used to detect sulfur compounds in crude oil and sulfur contaminants in natural gas.

In food analysis it is used to detect off-flavors resulting from the liberation of volatile sulfur compounds. It is also used to simultaneously detect sulfur and phosphorus in chemical warfare agents. In the environmental area, the FPD is used for detection of organophosphorus pesticides and herbicides. Several EPA methods for pesticide detection, including EPA methods 1618[2] and 622[3], specify the use of an FPD.

A schematic of a single FPD for the 6890 Series GC is shown in figure 1. A dual wavelength version is available that has a second photomultiplier mounted perpendicular to the first for simultaneous detection of a second wavelength. The 6890 GC is available with either a single or dual FPD.

The sensitivity of any FPD is affected by detector temperature, flame chemistry, and filter wavelength.

- **Detector temperature**. To protect the photomultiplier, the maximum temperature limit for the 6890 FPD is 250 °C. Photomultiplier tube (PMT) noise increases with setpoint temperature, so the detector temperature should be as low as possible. Generally, the temperature should be set about 25 °C above the highest temperature reached in the oven program. To prevent water condensation and clouding of the window, the minimum operating temperature is 120 °C.[4]

- **Flame chemistry**. FPD sensitivity is highly dependent on detector gas flows. On the 6890 GC, the gas flows are electronically controlled. This allows rapid and precise optimization of flow rates. Sulfur and phosphorus modes have different optimum flow requirements, so the ability to easily set and reset flows increases the quality of results and saves time.
- **Filter wavelength**. For the FPD, filters of specific wavelength are physically installed in the detector. A 394-nm filter is used for sulfur detection, and 526-nm filter for phosphorus detection.

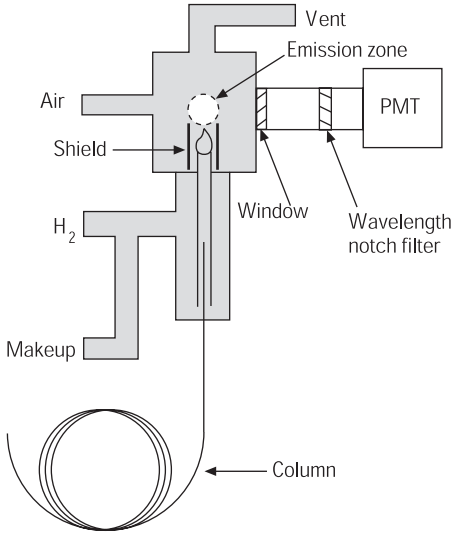


Figure 1. Single photomultiplier tube FPD for 6890 Series GC

Experimental

All experiments were performed on a 6890 Series GC with electronic pneumatics control (EPC) and an Agilent 7673 automatic liquid sampler (ALS). An Agilent 1707A ChemStation was used for instrument control and data acquisition. Chromatography conditions are shown with the individual chromatograms in figures 2, 3, and 4.

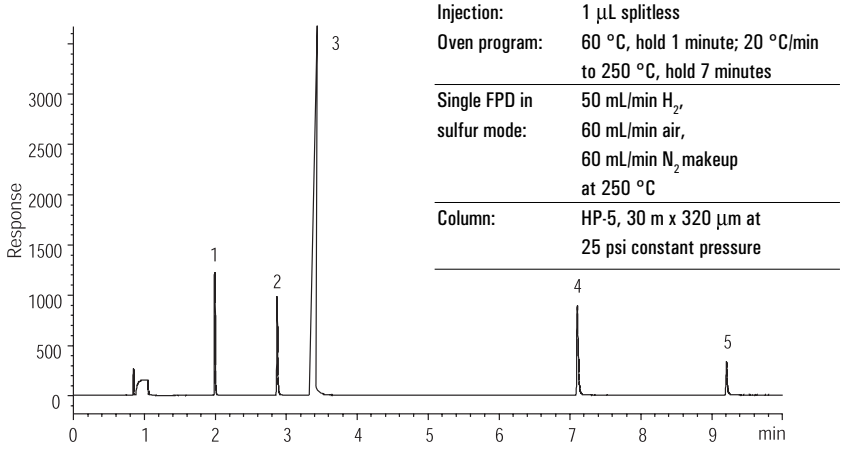
Table 1. MDL and Linearity over 10^2 Range Sulfur Mix on the FPD

Peak Number	Compound Name	MDL pgS/sec n = 11	Linearity r^2 n = 15
1	2,5-dimethylthiophene	26.22	0.9986
2	sec-butylsulfide	20.10	0.9983
3	1,4-butanedithiol	22.27	0.9972
4	dodecanethiol	16.90	0.9985
5	octyl sulfide	16.14	0.9979

Results and Discussion

Linearity and MDL

In sulfur mode, the response of the FPD is proportional to analyte concentration squared. The calculated MDL and r^2 values from linearity experiments for a single photomultiplier in sulfur mode are listed in table 1, and the chromatogram for a 20–40 ppb sample from the experiment is shown in figure 2. The square of the concentration was used to calculate regression statistics. When using a ChemStation for data analysis, a quadratic calibration fit is used for sulfur.



Conditions

Injection:	1 µL splitless
Oven program:	60 °C, hold 1 minute; 20 °C/min to 250 °C, hold 7 minutes
Single FPD in sulfur mode:	50 mL/min H_2, 60 mL/min air, 60 mL/min N_2 makeup at 250 °C
Column:	HP-5, 30 m x 320 µm at 25 psi constant pressure

Figure 2. 1 µL of 1.5 ppm sulfur standard, FPD in sulfur mode (The peaks are identified in table 1.)

The response of the FPD is linear in phosphorus mode. Table 2 shows the r^2 values for an organo-phosphorus pesticide mixture and the MDL calculated from the study. Figure 3 shows the chromatogram. A standard linear curve fit is used for phosphorus when using a ChemStation for data analysis.

Analysis of EPA Method 1618

Figure 4 shows the chromatogram obtained from the analysis of organophosphorus pesticides according to EPA method 1618. The injected concentration of each compound was 1–2 ppm.

Table 2. MDL and Linearity over 10^3 Range for Organophosphorus Pesticides on the FPD

Peak Number	Compound Name	MDL pgPesticide/sec n = 11	Linearity r^2 n = 15
1	phorate	1.85	0.9996
2	demeton	1.13	> 0.9998
3	disulfoton	1.31	> 0.9999
4	diazinon	1.74	> 0.9999
5	malathion	1.74	> 0.9999
6	fenthion	1.75	> 0.9999
7	parathion	1.84	> 0.9999
8	trichloronate	2.27	> 0.9999
9	tokuthion	2.51	> 0.9999
10	fensulfothion	—	> 0.9999
11	ethion	1.29	> 0.9999
12	sulprofos	2.36	> 0.9999
13	guthion	1.24	> 0.9999
14	coumaphos	2.08	> 0.9999

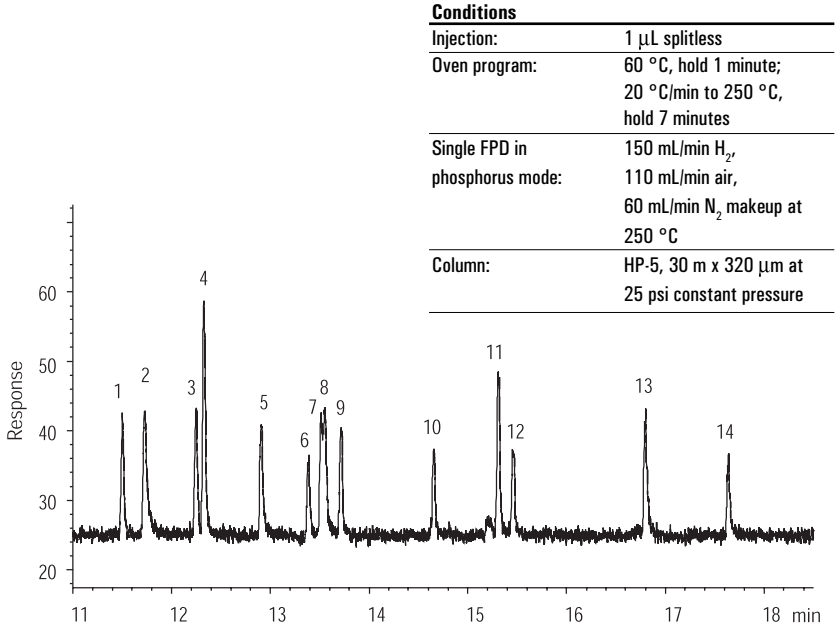


Figure 3. 1 µL Splitless injection of 20–40 ppb organophosphorus pesticide standard, FPD in phosphorus mode (The peaks are identified in table 2.)

Conditions	
Injection:	1 µL cool on-column
Oven program:	60 °C, hold 0.5 minute; 25 °C/min to 110 °C, 3 °C/min to 250 °C, hold 10 minutes
Single FPD in phosphorus mode:	75 mL/min H_2, 100 mL/min air, 60 mL/min N_2 makeup at 250 °C
Column:	1.5 µm HP-1, 30 m x 530 µm, 7 mL/min He constant flow

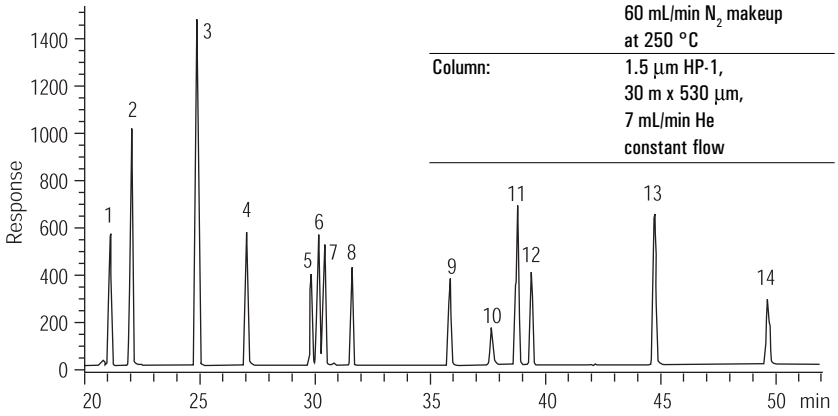


Figure 4. Analysis of organophosphorus pesticides according to EPA method 1618, 1 µL on-column injection of 1–2 ppm standard, FPD in phosphorus mode (The peaks are identified in table 2.)

Conclusions

The Agilent 6890 Series GC with an FPD can be used for the sensitive, and selective measurement of sulfur- and phosphorus-containing compounds in complex mixtures. The electronic pneumatics control on the Agilent 6890 GC ensures rapid and accurate gas flow control, provides for easier method setup and documentation, and simplifies optimization.

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2. EPA Method 1618, *USEPA Method 1618: Organo-halide Pesticides, Organo-phosphorus Pesticides, and Phenoxy-Acid Herbicides by Wide-Bore Capillary Column Gas Chromatography with Selective Detectors*, Revision A, 1989, Office of Water Regulations and Standards.
3. EPA Method 622, *USEPA Method 622: The Determination of Organophosphorus Pesticides in Municipal and Industrial Wastewater*, USEPA Report #600/4-82-008, NTIS #82-156027.
4. *Operating Manual*, *Flame Photometric Detector*, Agilent Technologies, Part Number G1535-90100.

Printed in the USA 3/2000
5965-7442E

A Method Used to Screen for 567 Pesticides and Suspected Endocrine Disrupters

Application

Gas Chromatography

May 1998

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Abstract

A gas chromatographic (GC) method has been developed that can be used to screen for 567 pesticides and suspected endocrine disrupters. In principle, it can be used to screen for any GC-amenable pesticide, metabolite, or endocrine disrupter. The method relies on a technique called retention time locking (RTL). RTL is a procedure that allows the chromatographer to reproduce analyte retention times independent of GC system, column length, or detector so long as columns with the same stationary phase, nominal phase ratio, and diameter are used. Because RTL increases retention time precision and predictability, raw retention times can be used as a more reliable indicator of compound identity. The chromatographer first locks the GC method so that all retention times match those listed in a 567-compound pesticide and endocrine disrupter retention time table. After analyzing a sample by GC with atomic emission detection (GC-AED), the analyst enters a peak's retention time and known elemental content (presence or absence of heteroatoms) into a dialog box. If element-selective detectors are used, detector response can be entered in addition to or in place of GC-AED data. The software then searches the pesticide table for those compounds that elute at the correct retention time and have the right elemental content or detector response. Most often, the software finds just one compound that meets these criteria, and rarely does it find more than three. Confirmation is performed by GC with mass spectral detection (GC-MS) or by calculation of elemental ratios using GC-AED data. With retention time locking, pesticides have the same retention time on all GC systems; this makes GC-MS confirmation much easier because the analyte's retention time is already known.

Key Words

Pesticides, endocrine disrupters, gas chromatography, retention time locking, RTL

Introduction

The Pesticide Manual[1] lists 759 compounds and biological agents that are used currently as active ingredients in various pesticide formulations. Many compounds, though no longer used, still persist in the environment. For the protection of human health and the environment, acceptable limits in food and water have been set by governmental bureaus such as the United States Environmental Protection Agency (USEPA) and the Codex Alimentarius Commission.[2] Numerous methods have been developed to screen for pesticide contamination in food[3–7] and the environment[8–10] to ensure that these standards are met.

Certain pesticides and other synthetic chemicals have been suspected of behaving as pseudo hormones, disrupting normal functions of the endocrine system in wildlife and humans. Maladies such as birth defects, behavioral changes, breast cancer, lowered sperm counts, and reduced intelligence have been blamed on exposure to endocrine disrupters.[11] The 1996 publication of *Our Stolen Future*, a book by Colborn,

Dumanoski, and Myers,[11] brought these concerns to the attention of the public. Recently passed legislation in the U.S. calls for more testing of suspected endocrine disrupters and monitoring of them in food[12] and water[13] supplies. To facilitate more research into the endocrine disrupter issue, methods are needed to detect suspected compounds at trace levels.

Because so many pesticides are in use, it is usually impractical to screen for large numbers of them individually and, therefore, multiresidue methods are preferred. Most laboratories that analyze for pesticides in food or the environment screen for only a few dozen compounds because it is often very difficult to screen for more. Recently however, methods have been developed using gas chromatography with mass spectral detection (GC-MS), that can screen for more than 200[5] or even 300[6] pesticide residues.

Still, there is no universal method to analyze for all GC-amenable pesticides. While GC-MS methods are gaining in popularity, there are still some limitations. When methods employ selected ion monitoring (SIM) or tandem mass spectrometry (MS-MS), method development is more tedious and any shift in GC retention times requires that individual analyte retention time windows be shifted accordingly. These methods are only capable of detecting compounds on the target list; there are still hundreds of pesticides, metabolites, and suspected endocrine disrupters that could be missed. On the other hand, methods based on scanning GC-MS alone may require more sample cleanup to avoid interferences from co-extracted indigenous compounds. Typically, these methods do not screen for many pesticide metabolites, endocrine disrupters, or other environmental contaminants. A method that could be used to screen for endocrine disrupters and almost all of the volatile pesticides and metabolites would offer a better means of monitoring the food supply and the environment.

This paper describes a universal method that, in principle, could be used to screen for any pesticide, metabolite, or endocrine disrupter that can elute from a gas chromatograph. The screening procedure relies on a new gas chromatographic technique called retention time locking (RTL)[14–16] with database searching based on retention time and elemental content or detector response. This technique is used to narrow an analyte's identity to a few possibilities. Confirmation is performed by GC-MS or by calculation of a compound's elemental ratio using GC with atomic emission detection (GC-AED).

Experimental

Standards and Extracts

Pesticide standards used to develop the retention time table were obtained from Chem Service (West Chester, PA, USA), Promochem Ltd (Welwyn Garden City, Hertfordshire, England), Dr. Ehrenstorfer (Augsburg, Germany), Hayashi Pure Chemical Industries, Ltd (Osaka, Japan), Wako Pure Chemical Industries, Ltd (Osaka, Japan), and GL Sciences Inc (Tokyo, Japan).

Fruit and vegetable extracts were obtained from the Florida Department of Agriculture and Consumer Services (Tallahassee, FL, USA). Samples were extracted with acetonitrile followed by solid-phase extraction (SPE) using a C-18 cartridge. Extracts intended for analysis by halogen-selective detectors were also subjected to floracil SPE.

Pesticide Retention Time Table

The table containing GC and GC-MS retention times for 567 pesticides, metabolites, and suspected endocrine disrupters was obtained from Agilent Technologies, Wilmington, DE, USA (G2081AA).

Instrumentation

Table 1 lists the instrumentation and chromatographic conditions used for GC-AED screening and GC-MS confirmation.

Software for Method Translation

Software for use in translating the normal GC method to one that runs three times faster was obtained from Agilent Technologies,Wilmington, DE, USA.[17]

Results and Discussion

Retention Time Locking

Key to the development of this method is a new concept in gas chromatography called retention time locking (RTL).[14–16] Agilent RTL software allows the chromatographer to match analyte retention times from run to run, independent of the GC system, detector, or manufacturing variations in column dimensions. The only requirement is that the columns used have the same stationary phase and the same nominal diameter and phase ratio. For example, with RTL it is possible to match analyte retention times on a GC-AED and a GC-MS even though the MS operates under vacuum and the AED operates at 1.5 psi above ambient pressure. The

procedure also compensates for differences in GC column length resulting from variations in manufacturing or from column cutting required during routine maintenance.

RTL is accomplished by adjusting the GC column head pressure until a given analyte, such as an internal standard, has the desired retention time. When this is done, all other analytes in the chromatogram will have the correct retention times as well. Software has been developed that can be used to determine the column head pressure that will lock the retention times correctly after one or two "scouting" runs.

With RTL, it is possible to measure pesticide retention times using a given GC method, and then reproduce those retention times in subsequent runs on the same or different instruments. With this increased retention time precision and predictability, retention times become a far more useful indicator of analyte identity. For many years, relative retention times[3,6] or retention indices[18,19] have been used to identify compounds. These techniques were developed to compensate for the fact that retention times were not predictable from day to day, column to column, or instrument to instrument. With the increased retention time precision of the Agilent 6890 GC and RTL, it seemed that raw retention times could be used for compound identification instead of retention indices. The chromatographer could simply scan a table of pesticide retention times, eliminating all possibilities but those with close elution times under the same locked GC conditions.

Table 1. Instrumentation and Conditions of Analysis

Agilent GC-AED System	
Gas chromatograph	6890
Automatic sampler	6890 Series automatic sampler
Atomic emission detector	G2350A atomic emission detector
Computer for data acquisition and analysis	HP Vectra XM Series 4 5/150
Software	G2360AA GC-AED software running on Microsoft® Windows™ 3.11
Column	30 m × 0.25 mm × 0.25 µm HP-5MS (part no. 19091S-433)
GC inlet	Split/splitless, 250 °C or 260 °C
Inlet liner	Single-tapered deactivated (part no. 5181-3316) with 2-cm deactivated glass wool plug centered ~3 cm from the top
Injection volumes	3–5 µL splitless when running method at 3× speed; 2–3 µL splitless at 1× speed
Inlet pressure (splitless)*	87.5 psi constant pressure for method at 3× speed; 27.6 psi constant pressure for 1× speed
Inlet pressure program (pulsed splitless)*	60 psi (2.01 min), 10 psi/min to 27.9 psi (hold)
Oven temperature program	70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C/min to 200 °C (0 min), 8 °C/min to 280 °C (10 min)
AED transfer line temperature	290 °C
AED cavity temperature	320 °C
AED elements and wavelengths (nm)	Group 1: Cl 479, Br 478 Group 2: C 193, S 181, N 174 Group 3: P 178 Group 4: F 690 (optional)
Agilent GC-MS System	
Gas chromatograph	6890
Automatic sampler	6890 Series automatic sampler
Mass selective detector	5973 MSD
Computer for data acquisition and analysis	HP Vectra XU 6/200
Software	G1701AA Version A.03.00 running on Microsoft® Windows® 95
Column	30 m × 0.25 mm × 0.25 µm HP-5MS (part no. 19091S-433)
Inlet	Split/splitless, 250 °C
Inlet liner	Single-tapered deactivated with small amount of glass wool at the bottom (part no. 5062-3587)
Injection volume	2 µL
Inlet pressure*	15.5 psi (constant pressure)
Oven temperature program	Same as GC-AED
MSD parameters	
Acquisition mode	Scan (35–550 amu)
EM voltage	200 rel
Solvent delay	3.20 min
Threshold	150
Scans/sec	2.86
Temperatures	Transfer line = 280 °C, MS quad = 150 °C, MS source = 230 °C

*The column head pressures shown are typical values. Exact values were determined as part of the retention time locking procedure.

Pesticides almost always contain heteroatoms and often have several in a single molecule. The most frequently encountered heteroatoms are O, P, S, N, Cl, Br, and F. GC with atomic emission detection (GC-AED) has been shown to be a useful tool for pesticide screening because it is selective for all of the elements found in these compounds.[20–22] Thus, GC-AED screening provides valuable information about the elemental content of an unknown molecule. By including this elemental information along with the retention time, it should be possible to narrow pesticide "hits" to just a few possibilities.

To implement this screening procedure, a table of pesticide and endocrine disrupters retention times had to be created using a suitable method under locked conditions.

GC Method for Pesticide Screening

First, a GC method was needed that could elute hundreds of pesticides and endocrine disrupters in a reasonable time with adequate separation. However, the goal was not to separate every possible analyte in a single GC run. Because the intention was to build a table of locked retention times using this method, it had to reproduce these retention times under a variety of conditions. For example, the method needed to accommodate a variety of injection techniques including splitless, pulsed splitless,[23,24] cold splitless using a PTV inlet, and on-column injection which is occasionally used for the more labile pesticides.

The method also needed to perform well with samples dissolved in common solvents such as acetone and methylene chloride. Because a retention gap (or guard column) is sometimes added to protect the analytical column, the method had to be tested to see if it could still be locked with a retention gap installed.

The column chosen for the method was a 30 m × 0.25 mm × 0.25 µm HP-5MS because the same column could be used with any GC-detector combination. In particular, this column was chosen for its low bleed at high temperatures and because its optimum column flow is compatible with GC-MS. The 5% phenyl methyl silicone phase in this column has been widely used for pesticides.

Method translation software[17,25,26] can be used to increase the speed of a method while retaining the same relative retention times. This can be done by translating the method to a column having the same phase ratio but a smaller id or by increasing the flow rate and oven temperature program while using the same column. The final goal was to design a method that could run at three times the normal speed on the 30-m × 0.25-mm × 0.25-µm HP-5MS column or be translated to a 100-µm id column.

After several weeks of method development, the GC oven temperature program shown in figure 1a was chosen because it met all of the development criteria. Chlorpyrifos-methyl ($C_7H_7Cl_3NO_3PS$) was chosen as the locking standard. It is an ideal choice because chlorpyrifos-methyl elutes near the middle of the chromatogram (16.596 minutes), has good peak shape, and can be seen by most element-selective detectors. Because GC-AED requires three runs to generate element-selective chromatograms for C, Br, Cl, N, S, and P, the method was translated to run three times faster using software for method translation.[17,25,26] The faster oven temperature program used by this method requires 6890 GC systems that are configured for fast oven temperature ramping. The method translation software can be used to speed up the method by any desired factor; even 120-V 6890 GCs can run the method two times faster. However, the original method must be used for GC-MS because of the restriction in flow rates into the MSD. Figure 1b lists the threefold (3×) faster GC method.

Pesticide Retention Time Table

Once developed, this method was employed to create a table of locked retention times for the 567 pesticides, metabolites, and suspected endocrine disrupters. Increasing international food trade requires the analysis of pesticides that may be used in the supplying country but not in the recipient country. The goal was to create a table that included pesticides used around the world so pesticide standards were obtained from sources in Europe, Japan, and the USA.

A list of suspected endocrine disrupters was compiled from various lists published on the World Wide Web.[27–31] Many of these compounds are, in fact, pesticides. Most of the GC-amenable endocrine disrupters were analyzed and their retention times appear in the table. However, the 209 polychlorinated biphenyl congeners were not included because their inclusion might actually complicate the identification of organochlorine pesticides.

Standards, diluted to 10 ppm in acetone, were first analyzed by GC-MS using the oven temperature program shown in figure 1a and instrumental conditions listed in table 1. Compound identities were verified by matching their spectra to library entries,[32] by comparison with a published spectral compendium,[33] or by matching spectra to a list of charac-

teristic ions.[6] When reference spectral information was not available, the pesticides were verified by spectral interpretation. Samples were then analyzed on two different 6890 GC-FID instruments under the same locked conditions (chlorpyrifos-methyl retention time = 16.596 minutes). The GC-MS retention time and the average of the two GC-FID retention times were tabulated for each compound along with its molecular formula, molecular weight, and CAS number. In addition to these fields, there are four user-definable columns in table 2 that can be used to add such things as mass spectral information, internal catalog numbers, or comments. Table 2 lists a small portion of the database. It must be noted that all retention time values were created using constant column head pressure. This is because GC-MS retention times are very close to those obtained with other detectors when constant pressure is used. In this mode, GC-MS and GC-FID retention times match within ± 0.1 minute except for three compounds that elute at the very end of the chromatogram. Even in this case, the differences are no more than 0.2 minute. The discrepancy between GC-MS and GC-FID retention times is larger in the constant flow mode.

Pesticide Screening Method

Figure 2 diagrams the pesticide screening method. First, RTL was used to match GC-AED and GC-MS analyte retention times to those listed in the pesticide table. Software for RTL[14–16] was used to determine the

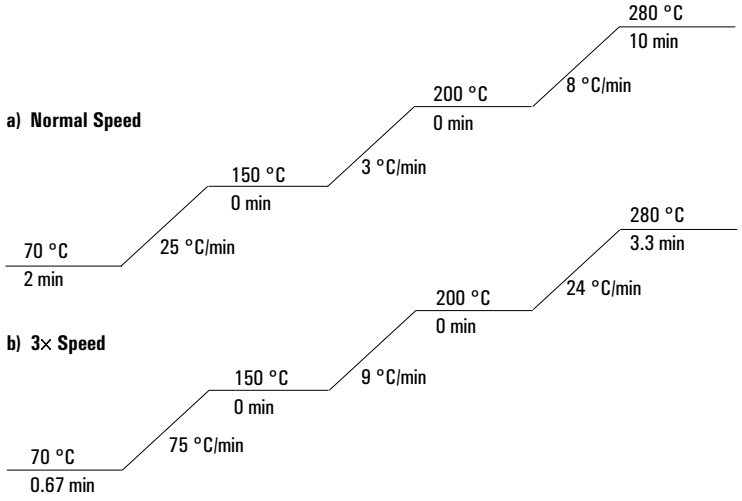

Figure 1. a) GC oven temperature program for the Agilent pesticide method at normal speed. When using this method, chlorpyrifos-methyl must be locked to 16.596 minutes. This method is used by GC-MS and can be used by any other GC system. b) GC oven temperature program for the Agilent pesticide method translated to run three times faster. This method may be used with 6890 GCs configured with any detector except an MSD so long as the GC is configured for fast oven temperature ramping. Chlorpyrifos-methyl must be locked to 5.532 minutes.

Table 2. Small Portion of the Pesticide and Endocrine Disrupter Retention Time Table That Contains 567 Entries. The retention times shown here are for the pesticide method run at normal speed as shown in figure 1a. Chlorpyrifos-methyl was locked to 16.596 minutes (± 0.015 minute for the collection of the tabulated retention time values. The table includes four additional columns for user-defined information.

FID RT	Name	CAS No.	Molecular Formula	MW	MSD RT
16.542	Acetochlor	34256-82-1	C:14,H:20,Cl:1,N:1,O:2,	269.77	16.542
16.549	Fuberidazole	3878-19-1	C:12,H:8,N:2,O:1,	196.21	16.549
16.583	Methyl parathion	298-00-0	C:8,H:10,N:1,O:5,P:1,S:1,	263.20	16.594
16.596	Chlorpyrifos methyl	5598-13-0	C:7,H:7,Cl:3,N:1,O:3,P:1,S:1,	322.53	16.593
16.637	Vinclozolin	50471-44-8	C:12,H:9,Cl:2,N:1,O:3,	286.11	16.630
16.650	Plifenat	21757-82-4	C:10,H:7,Cl:5,O:2,	336.43	16.641
16.689	Terbucarb	001918-11-2	C:17,H:27,N:1,O:2,	277.41	16.686
16.730	Chloranocryl	2164-09-2	C:10,H:9,Cl:2,N:1,O:1,	230.09	16.736
16.752	3-Hydroxycarbofuran	16655-82-6	C:12,H:15,N:1,O:4,	237.26	16.741
16.773	Heptachlor	76-44-8	C:10,H:5,Cl:7,	373.32	16.796
16.800	Carbaryl	63-25-2	C:12,H:11,N:1,O:2,	201.22	16.806

column head pressure needed to produce a retention time of 16.596 minutes for chlorpyrifos-methyl. When analyzing samples by GC-AED, the method was usually run at 3× speed and chlorpyrifos-methyl was locked to 5.532 minutes.

Figure 3 shows the RTL software screen that is used to develop the retention time calibration. To accomplish this for the pesticide method, one should install the 30 m × 0.25 mm × 0.25 µm HP-5MS column (part no. 19091S-433) and set the column head pressure to one of the appropriate nominal values as shown below, making sure to use the constant pressure mode.

- 26 psi for atmospheric pressure detectors run at normal speed (eg, NPD, FPD)
- 16 psi for GC-MSD operated at normal speed
- 27.5 psi for GC-AED operated at normal speed
- 88 psi for GC-AED operated at 3× speed

To prepare a calibration table similar to the one shown in figure 3, the chromatographer must make five analyses of chlorpyrifos-methyl at the following column head pressures: the nominal pressure, the nominal pressure + 20%, the nominal pressure + 10%, the nominal pressure – 10%, and the nominal pressure – 20%. Because of the first run affect, it is usually wise to make one or two blank runs before performing the five calibration runs. The five pressures and the chlorpyrifos-methyl retention times are entered into the table provided by the RTL software. This calibration table stays with the method and can be used to lock, or re-lock, the GC

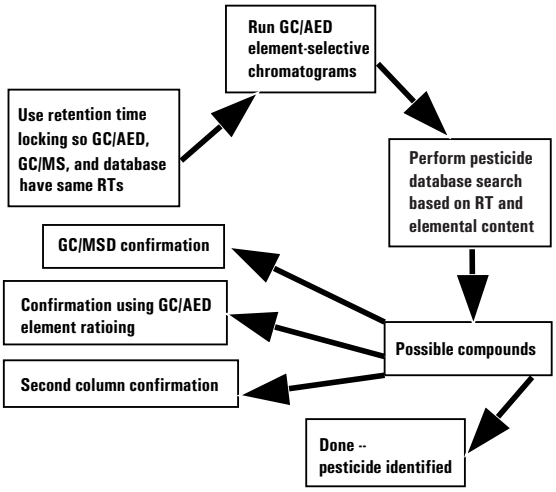

Figure 2. Diagram of the screening method that uses retention time locking and retention time table searching to identify pesticides and suspected endocrine disrupters.

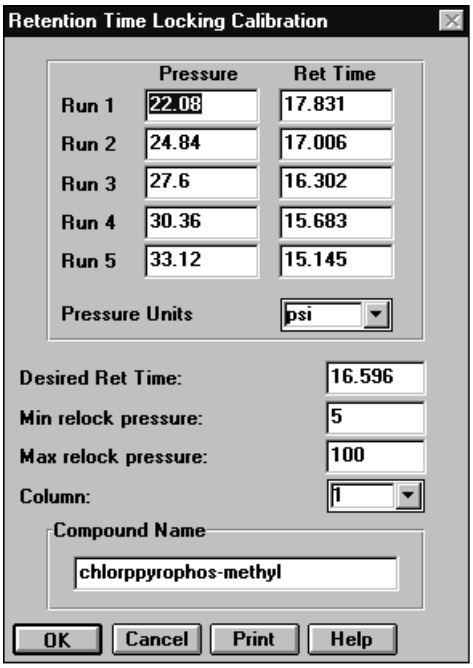

Figure 3. RTL software screen showing typical retention time locking calibration data for the pesticide method run at normal speed using a GC detector that operates at atmospheric pressure.

method as long as that method is used. That is, the five calibration runs only need to be made once for a given method.

The software screen for locking the GC method is shown in figure 4. To lock the method, one enters the retention time of chlorpyrifos-methyl and clicks on the "Calc new pressure" button. The RTL software calculates the pressure needed to lock the chlorpyrifos-methyl peak at the desired retention time. By clicking on the "Update current 6890 Method" button, this value is entered automatically into the method.

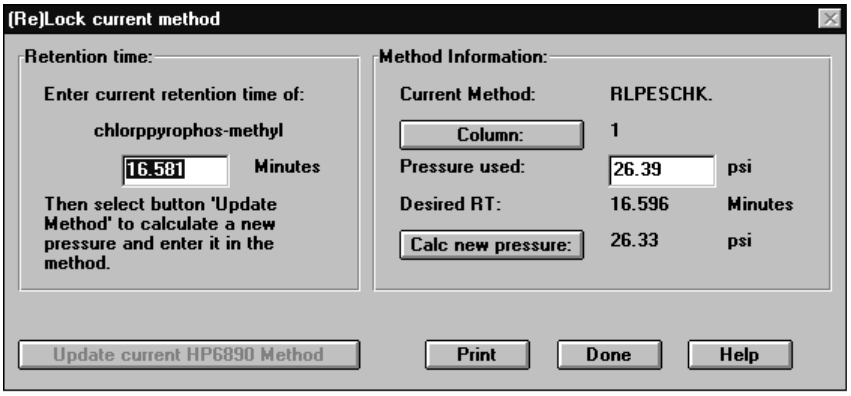

Figure 4. RTL software screen used to calculate the column head pressure needed to lock or re-lock a method. In this case, the chlorpyrifos-methyl retention time was 16.581 minutes and the pressure needed to re-lock the method was calculated to be 26.33 psi. By clicking on the "Update current 6890 Method," button, the new pressure is entered automatically into the GC method.

One can use Agilent's software for method translation[17] to convert the method to other speeds (eg, 1.9×) and determine the nominal column head pressure required. If this is done, the pesticide table must be exported to a spreadsheet program where the analyte retention times can be divided by the appropriate factor (1.9 in this case). This new table can then be imported back into the ChemStation for use with the new method.

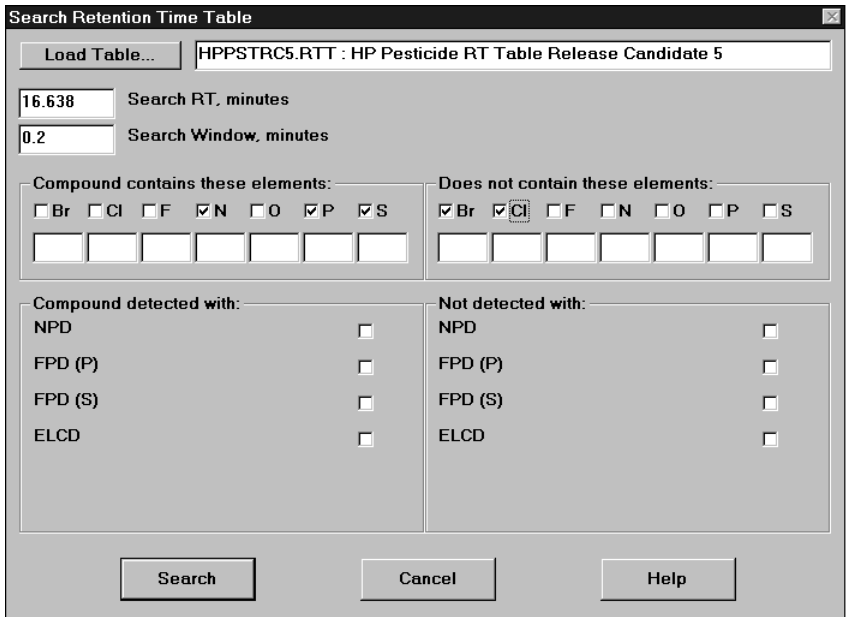

Figure 5. RTL software screen used to search a retention time table on the basis of retention time and known elemental content. In this case, the software will search the Agilent pesticide table at 16.638 ± 0.1 minutes for compounds that contain N, P, and S but do not contain Br or Cl. If element-selective detectors (such as the NPD) are used, this information can be provided to the search routine. Up to six different element-selective detectors can be configured as shown for NPD, FPD (P), FPD (S), and ELCD.

After locking the method to the table, GC-AED element-selective chromatograms were obtained for C, Cl, Br, N, S, P, and sometimes F. From the GC-AED chromatograms, it was usually possible to determine which heteroatoms were present or absent in the suspected pesticide peak. RTL software was then used to search the database by retention time and elemental content. Figure 5 shows the RTL software screen used for retention time table searching. One can enter the elements known to be present or not present in the GC-AED peak of interest. Up to six other element-selective detectors can be configured for use in the search algorithm. When the presence or absence of a heteroatom is uncertain, nothing is added to the search routine for that element.

One must choose a search time window wide enough to include the correct analyte, but narrow enough to eliminate as many extraneous "hits" as possible. Experience has shown that the normal speed method requires a search window of 0.2 to 0.3 minute. The 3× speed method can use a search window of 0.1 minute. If the heteroatom content is known for a peak, retention time table searching

with these search windows most often finds just one pesticide and rarely finds more than three possibilities.

Confirmation is usually done by GC-MS under locked conditions so that all GC-MS retention times match the values listed in the pesticide retention time table. This was found to be of enormous benefit. Prior to GC-MS confirmation, the analyst already knows which pesticides to look for and their expected retention times. Alternatively, when there is adequate signal to quantitate the analyte in multiple AED element-selective chromatograms, it is often possible to confirm a pesticide's identity simply by calculating its heteroatom ratio. GC-AED software for element ratioing facilitates this procedure.

Analysis of a Green Onion Extract

Numerous samples of fruit and vegetable extracts have been analyzed using this methodology. The results for a green onion extract illustrate the versatility and potential of this method.

Green onion extracts are usually very dirty and contain a large number of co-extracted sulfur compounds that can obscure sulfur-containing pesticides. The onion chromatograms shown in figure 6 were run under locked conditions at 2× speed in Tallahassee, Florida, by the Department of Food and Agriculture using a 5890 SERIES II/5921A GC-AED system. Retention time searching indicated that folpet was present in the sample, but it could not be confirmed at the time. The same sample was sent to the Agilent Technologies Little Falls Site in Wilmington, DE, where it was analyzed by scanning GC-MS using an 6890/5973 system. As shown in figure 7, folpet was easily confirmed at the expected retention time. In addition, the pesticides trichlorophenol, chlorothalonil, propoxur, and prochloraz were identified. Searching the Cl peak at about 6 minutes gave no pesticide hits. However, GC-MS suggested the presence of a trichloronaphthalene isomer at the corresponding retention time in the GC-MS chromatogram (about 12 minutes because the GC-MS was operated at normal speed). Though not a pesticide, trichloronaphthalene is considered to be a hazardous compound that should not be in food.

The same green onion sample was then analyzed by the newer model GC-AED system (6890/ G2350A) at 3× speed (figure 8). Several more pesticides were identified by searching the pesticide/ endocrine disrupter table using a 0.1-minute retention time window. Table 3 lists the pesticide hits that were obtained for each retention time search using the available GC-AED data. Sulfur was not included in any of the searches because onion extracts have such a high sulfur background.

Confirmation by GC-MS was much easier because the GC-MS retention time for each pesticide hit was printed out with the RT search report. Thus, the retention times and probable identities of each pesticide were already known before the GC-MS analysis was run. As is shown in figure 7 for folpet, one can simply extract the ions characteristic for each pesticide hit and look in the extracted ion chromatogram at the expected retention time.

Quantitative Analysis

The Agilent pesticide screening method is a qualitative tool to identify any of the 567 pesticides and endocrine disrupters listed in the retention time table. This, of course, is the first step in any pesticide screening method. Quantitative analysis can be performed in one of two ways.

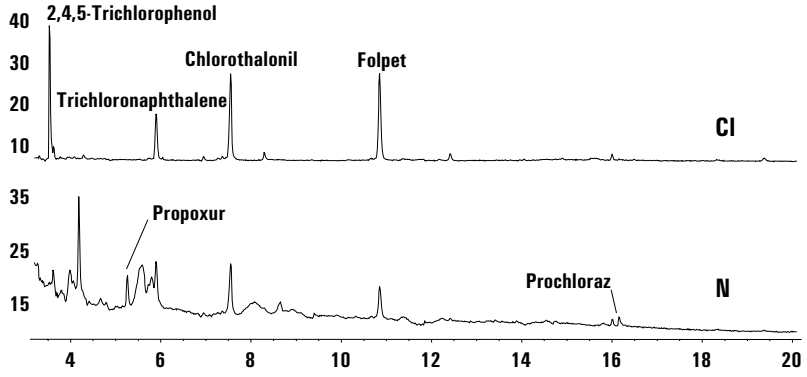

Figure 6. Cl- and N-selective chromatograms of a green onion extract from an 5890/5921A GC-AED system. The analysis was performed at 2× speed under locked conditions in Tallahassee, Florida, by the Department of Agriculture and Consumer Services. In addition to folpet, trichlorophenol, propoxur, and prochloraz were identified by retention time table searching and confirmed by GC-MS at their expected retention times. There were no hits for the Cl peak at about 6 minutes, which was identified by GC-MS as a trichloronaphthalene isomer.

The traditional method is to inject standards into the GC, GC-AED, or GC-MS system to determine response factors from which quantitative results are calculated by the ChemStation software. However, because the GC-AED elemental response is almost independent of molecular structure, compound-independent calibration (CIC) can be used to quantitate all of the pesticides and endocrine disrupters that are found. For example, one could spike chlorpyrifos-methyl ($C_7H_7Cl_3NO_3PS$) at a known concentration into each pesticide extract and obtain element-specific calibration curves for Cl, N, P, and S. These curves could then be used to calibrate for any other compound containing one or more of these elements. Because the GC-AED is quite stable, external standard CIC often works just as well. The GC-AED software facilitates CIC. Unfortunately, this procedure determines the amount of a compound that reaches the AED and does not compensate for losses due to decomposition or adsorption in the inlet or column.

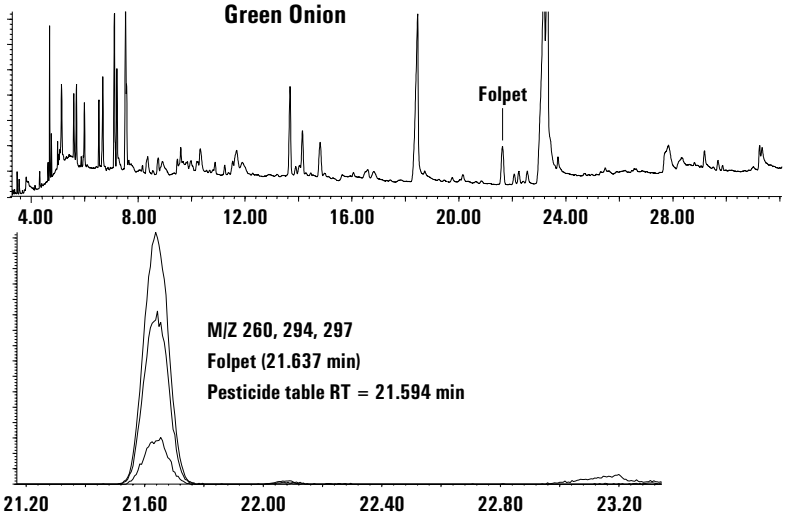

Figure 7. Confirmation of folpet in a green onion extract. The tabulated GC-MS retention time is 21.594 minutes, and folpet was detected in this sample at 21.637 minutes by simply extracting its characteristic ions.

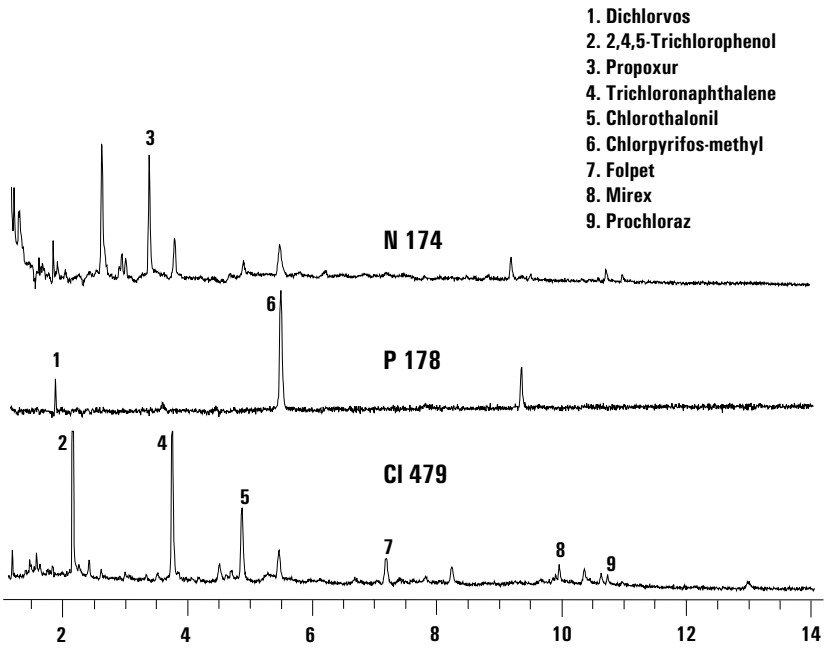

Figure 8. Element-selective chromatograms obtained for the same green onion extract shown in figure 6. These chromatograms were obtained at 3× speed using an 6890/G2350A GC-AED system.

Conclusions

Most screening procedures in use today are capable of finding only a fraction of the pesticides that are registered around the world. This new method has the capability of screening for virtually any volatile pesticide, metabolite, or endocrine disrupter. Although confirmation is usually required, GC-MS analysis is made much easier and more reliable because the pesticide's retention time and probable identity are already known.

While GC-AED is an ideal tool for element-selective pesticide screening,[20–22] many laboratories rely on a combination of other selective detectors. It is still possible to apply this method if each GC system runs the Agilent pesticide method under the same locked conditions. Any combination of GC-AED and/or element-selective detector response data can be entered into the RTL searching software.

When combined with RTL and retention time searching, GC-AED and GC-MS provide the most comprehensive and reliable screening method available for pesticides, metabolites, and suspected endocrine disrupters. Unlike most target compound methods in use today, this procedure has a good chance of finding and identifying unexpected or unknown pesticides, even in complex food extracts. RTL software makes it easy to add more compounds to the method, simply by determining their retention times under the same locked conditions.

Retention time locking with database searching could easily be applied to similar types of analyses. For example, one might use the procedure to identify polychlorinated biphenyls, polynuclear aromatics, drugs of abuse, or flavor and fragrance compounds.

Table 3. Green onion pesticide "hits" obtained by searching the 567-compound pesticide/endocrine disrupter RT table using a 0.1-minute RT window and element-selective GC-AED data. Compounds confirmed by GC-MS are shown for comparison. The GC/MS (figure 7) and GC-AED (figure 8) chromatograms were obtained at normal and 3X speeds, respectively. Sulfur peaks were not used to narrow the search because of the high background of sulfur-containing compounds in the onion extract.

GC-AED RT	RT Search Hits	Confirmed by GC-MS
1.933	Dichlorvos	Dichlorvos
2.281	2,4,6-Trichlorophenol 2,4,5-Trichlorophenol	2,4,5-Trichlorophenol
3.440	Fenobucarb Propoxur 4,6-Dinitro-o-cresol	Propoxur
3.854	No pesticide hits	Trichloronaphthalene isomer
4.955	Terbacil Chlorothalonil	Chlorothalonil
5.538	Chlorpyrifos-methyl	Chlorpyrifos-methyl
7.232	Folpet Chlorbenside	Folpet
9.965	Mirex	Mirex
10.588	Prochloraz	Prochloraz

Acknowledgments

The authors wish to thank the following people for their contributions to the development of this method: Joanne Cook and Marc Engel (Florida Department of Agriculture and Consumer Services) for supplying a green onion chromatogram, for contributing fruit and vegetable extracts, and for beta testing the method; Matthew Klee and Leonid Blumberg for many useful discussions; and James Green and Takeshi Otsuka for their help in developing the retention time table.

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Printed in the USA 3/2000
5967-5860E

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Analysis of EDB and DBCP in Water with the Agilent 6890 Series Gas Chromatograph and Agilent 6890 Micro-Electron Capture Detector — EPA Method 504

Application

Gas Chromatography

August 1997

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Abstract

The pesticides 1,2-ethylene dibromide (EDB) and 1,2-dibromo-3-chloropropane (DMCP) were analyzed by dual-column gas chromatography with dual micro-electron capture detectors (Agilent 6890 micro-ECDs) after micro-extraction with hexane in accordance with U.S. EPA method 504.

Stability, sensitivity, and linearity of the micro-ECD were significantly better than the classical ECD. Relative standard deviation (% RSD) for the entire method was less than 7% over a concentration range greater than two orders of magnitude with method detection limits of 0.003 µg/L or lower.

Key Words

Micro-ECD, 6890 GC, EPA Drinking Water Method 504, ethylene dibromide, 1,2-dibromo-3-chloropropane, GC/ECD analysis

Introduction

Ethylene dibromide (EDB) and 1,2-dibromo-3-chloropropane (DBCP) are volatile pesticides and suspect carcinogens. The U.S. EPA regulates maximum contaminant levels (MCLs) for these compounds in drinking water supplies at very low levels (EDB at 0.05 µg/L and DBCP at 0.2 µg/L). Both EDB and DBCP can be determined by performing a micro-extraction with hexane and analyzing the extract by gas chromatography using an electron capture detector (ECD), as described in EPA Method 504.[1]

EPA method 504 reported method detection limits (MDLs) of 0.01 µg/L for both pesticides.[1,2] Results using an Agilent 6890 GC with the micro-ECD show that these analytes can be determined down to 0.01 µg/L with MDLs of less than 0.003 µg/L. The micro-ECD had a stable baseline and was linear from 0.010 to 1.14 µg/L.

Table 1. Experimental Conditions

Sampler	Agilent 7673, 10-µL syringe, 2-µL splitless injection
Inlet	Split/splitless; 200 °C, pulsed splitless mode (20 psi for 1 min)
Carrier	Helium, 6 psi (40 °C); 3.5 mL/min constant flow (each column)
Column	(A) 30 m, 0.53-mm id, 0.8-µm film DB-608, an equivalent of HP-608 (part number 19095S-023)
	(B) 30 m, 0.53-mm id, 1.0-µm film RTX-1701, an equivalent of HP-PAS 1701 (part number 19095S-123)
Oven	40 °C (4 min); 10 °C/min to 240 °C
Detector	330 °C; Makeup gas: nitrogen, constant column and makeup flow (60 mL/min)

Experimental

Samples and standards were prepared as described in EPA drinking water method 504.[1] All analyses were performed using a 6890 Series GC with a single split/splitless inlet and dual micro-ECDs. Instrument conditions are listed in table 1.

A water sample (35 mL) was extracted with 2 mL of hexane. From that extract, 2 µL were injected into the 6890 Series GC in the splitless mode. A "Y" connector was used to split the sample equally between two polar but dissimilar columns. Column A (an equivalent of the HP-608 column), which provided separation of EDB and DBCP without interference from trihalomethanes, was used as the primary analytical column. Column B (an equivalent of the HP-1701 column) was used as the confirmation column. These columns were previously installed and used in the GC system to analyze pesticides and arochlors according to U.S. EPA CLP and 8080/8081 methods.

Results and Discussion

A common problem in determining EDB and DBCP in drinking water by gas chromatography/electron capture detection (GC/ECD) is interference from chlorination disinfection byproducts such as trihalogenated methanes. For example, dibromochloromethane (DBCM), commonly found in drinking water supplies in relatively high concentrations, can elute very close to EDB and thus can be misidentified as EDB.

Using the optimized GC conditions listed in table 1, EDB was clearly separated from significant levels of DBCM on both columns. Typical chromatograms of a hexane extract of a calibration standard are shown in figure 1. Both EDB and DBCP are well separated from possible interference, including DBCM and dibromomethane (DBM).

Micro-ECD Linearity

Linearity of the 6890 micro-ECD was determined by preparing standards from 0.005 to 1.14 µg/L in reagent water. The standards were extracted according to EPA method 504 and analyzed by gas chromatography. Typical average response factors (based on peak heights), relative standard deviations (% RSD) of response factors (RFs), and correlation coefficients of the linear curves are listed in table 2.

Figure 2 shows linear calibration curves for EDB and DBCP with correlation coefficients better than 0.999 (see table 2). The % RSD of RFs was 4% to 7%, over a concentration range greater than two orders of magnitude (0.005 to 1.14 µg/L). This easily met method 504 requirements for 20% RSD for a similar concentration range. The micro-ECD continued to meet these requirements over a period of 2 to 3 months with little or no maintenance required except for routine septum and liner changes.

MDLs, Precision, and Accuracy

Method detection limits (MDL) were calculated according to EPA method 504 by analyzing seven replicate extracts of a low-level standard (0.02 µg/L). As shown in table 3, the MDLs were 0.002 and 0.003 µg/L for EDB and DBCP, respectively. These MDLs were three- to five-fold below those reported by EPA method 504

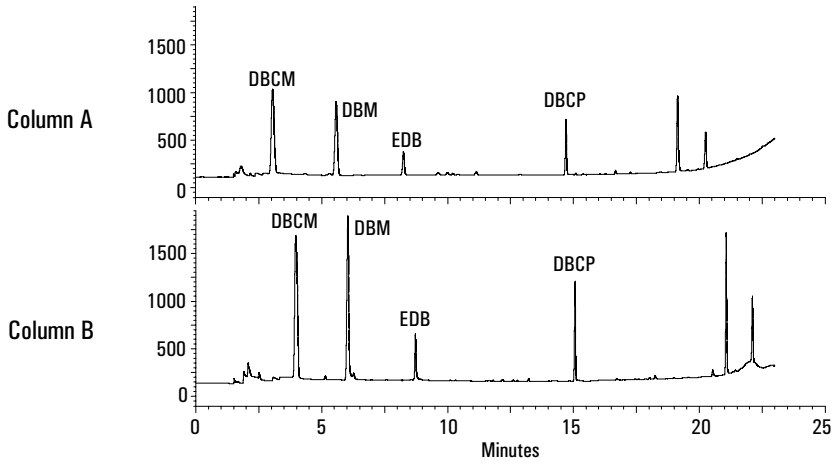

Figure 1. Hexane extract of a midpoint calibration standard (EDB/DBCP = 0.286 µg/L each).

Table 2. Typical Linearity on Column A*

Analyte	EDB	DBCP
Average response factor (RF)	4.66E-06	2.06E-06
Standard deviation, RF	2.19E-07	1.45E-07
%RSD, RF	4.69%	7.01%
Correlation coefficient	0.9992	0.9997

* Seven-level calibration at 0.0057, 0.020, 0.0571, 0.114, 0.286, 0.571, and 1.141 µg/L

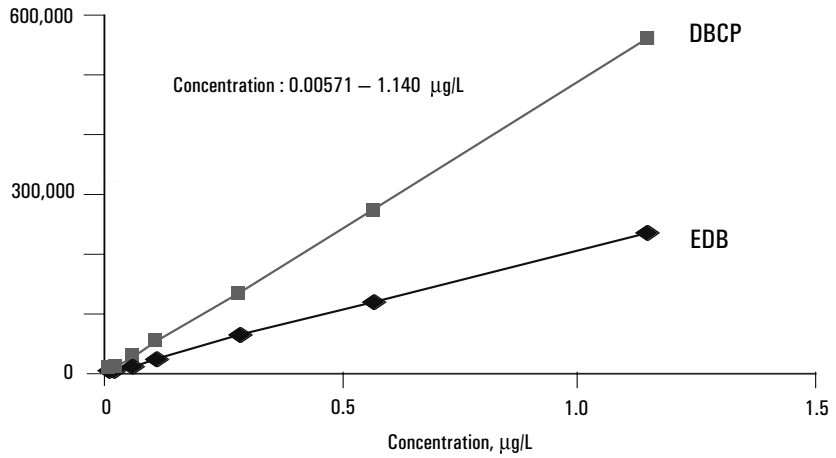

Figure 2. Typical calibration curves on column A

Table 3. MDLs, Precision, and Accuracy

Analyte	EDB	DBCP
Spiked concentration, µg/L	0.02	0.02
Number of replicates	7	7
MDL, µg/L	0.002	0.003
Spiked concentration, µg/L	0.20	0.20
Number of replicates	6	6
Average concentration, µg/L	0.202	0.205
Reproducibility, % RSD	5.3%	5.4%
% Recovery	101%	103%

and a Collaborative Study by K. W. Edgell and J. E. Longbottom.[2]

Six extracts of reagent water samples fortified with 0.20 µg/L of EDB and DBCP were analyzed. Both precision and accuracy were excellent, with reproducibility at 5% RSD and recovery of around 100% (see table 3).

Ruggedness of the 6890 Micro-ECD

For the detector to meet the low detection limit requirements, the chromatographic baseline must be clean and stable. In this study, the 6890 micro-ECD provided a clean baseline with no negative deflections during continuous operation over a period of 3 months. A variety of samples were also analyzed, including soil pesticide extracts that contained many late-eluting compounds (see figure 3). The 6890 micro-ECD showed rapid recovery even though this instrument had been switched from a drinking water method (EPA method 504) to solid waste methods (EPA method 8080/8081 and CLP method for pesticides and arochlors[3]), and back again.

EPA method 504 requires a continuous calibration (using a midlevel standard) for each 12-hour shift of operation or every 10- to 20-sample analyses. The retention times and the responses for these continuous calibration runs must match those from the initial calibration run with specific limits. The difference in responses (%D) between the later calibration run and the initial run must be less than 15%.

Table 4 presents the results of the sequence runs on the 1st, the 15th, and the 27th day of a month when samples were continuously analyzed according to EPA method 504. Responses of the 6890 micro-ECD proved to be quite stable over 3 to 4 weeks of continuous operation. The %D of EDB and DBCP did not vary by more than 10%, easily meeting the method requirement of 15%.

Conclusion

The Agilent 6890 Series GC with the micro-ECD can detect low levels of EDB and DBCP in drinking water and water supplies. All EPA method 504 criteria were easily met, yielding MDLs of 0.003 µg/L or less, reproducibility of 7% or less, and a linearity with correlation better than 0.999 over a concentration range greater than two orders of magnitude.

The system performance was stable for a long time (3 months), despite switching methods between EPA method 504 and CLP method for pesticides and arochlor. Stability, sensitivity, and linearity of the 6890 micro-ECD were significantly improved over the classical 6890 ECD.

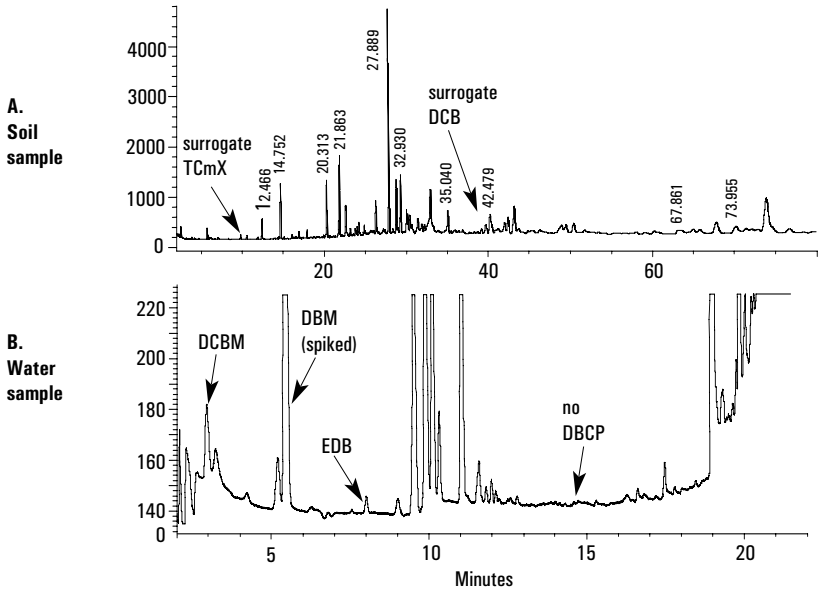

Figure 3. Typical chromatograms of sample extracts*

*The soil sample was analyzed according to EPA CLP method for pesticides along with 30 to 40 other samples in a sequence run.[4] No target pesticide was detected in this particular sample. The water sample was analyzed along with 20 other water samples based on EPA method 504 on the next day after the 6890 system was switched from the CLP method. No DBCP was found in any sample, and EDB was detected in only 3 to 4 samples. EDB in this sample was at the 0.01- to 0.02-ppb level. These chromatograms were plotted on different scales. Note the high signal for the soil sample. This demonstrates that it was possible to shift very quickly from analyzing dirty soil samples to analyzing low-level water samples using the 6890 system with micro-ECD .

Table 4. System Performance

	Run	Retention Time		Responses		%D	
	No.	EDB	DBCP	EDB	DBCP	EDB	DBCP
Day 1 Sequence							
Initial calibration	7	8.16	14.62	28486	70242		
Continuous calibration	19	8.16	14.62	29118	72434	2.2%	3.1%
Continuous calibration	30	8.16	14.61	28969	74268	1.7%	5.5%
Day 15 Sequence							
Initial calibration	7	8.11	14.58	30878	64439		
Continuous calibration	18	8.10	14.56	31684	66978	2.6%	0.8%
Continuous calibration	29	8.12	14.58	31241	71009	1.2%	6.9%
Continuous calibration	34	8.12	14.59	31219	70276	1.1%	5.8%
Continuous calibration	50	8.13	14.59	31689	72829	2.6%	9.6%
Continuous calibration	60	8.12	14.59	31627	72974	2.4%	9.8%
Day 27 Sequence							
Initial calibration	6	8.13	14.59	32203	76362		
Continuous calibration	19	8.13	14.59	31557	74711	–2.0% –	2.2%
Continuous calibration	28	8.13	14.59	31855	75417	–1.1% –	1.2%

* %D = (initial response — continuous calibration response) / initial response

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Printed in the USA 3/2000
5966-0495E

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Fast Screening of Pesticide and Endocrine Disrupters Using the Agilent 6890/5973N GC/MSD System, Part I

Application

Gas Chromatography
January 2000

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Abstract

Agilent Technologies' new, fast GC/MSD method can significantly speed up the screening of pesticides. Agilent's GC method translation software (available free from the Agilent Technologies Web site, http://www. chem. agilent.com/cag/servsup/usersoft/main.html#mxlator) was used in developing the new method based on the standard 42-min method. A 10 m x 0.1 mm x 0.1 µm HP-5 column was used to increase analysis speed up to fourfold. The time savings were implemented in increments (down to 10.5 minutes) to verify the predictability of scaling and the effect of scaling on the signal-to-noise ratio.

Key Words

RTL, pesticide, environmental, screening, fast GC, method translation, 5973, 6890, MTL

Introduction

Analysts want faster analyses to improve laboratory productivity. Often, when speeding up GC methods, an analyst will trade resolution for increased analysis speed. This loss of resolution can complicate peak identification, even with a mass selective detector (MSD).

Agilent Technologies has developed new techniques to solve the peak identification problem based on Agilent's retention time locking (RTL) software and a new mass spectral library that contains the locked retention times and characteristic ions for 567 of the most common pesticides and endocrine disrupters of concern worldwide. A GC/MSD method was developed based on the standard 42-min method[1] to screen for all 567 of the most common analytes. A specific combination of column stationary phase, carrier gas flow rate, and oven temperature programming is required to lock all the compounds to an expected retention timetable[2]. Compound identification based only on spectral searching alone is difficult when analyzing extracts containing significant sample matrix content because of overlapping peaks and noisy baselines.

The new screening tool, integrated within Agilent's ChemStation for MSD, searches for all 567 compounds by first checking and integrating four characteristic ions within the expected time window, and second by printing out a report showing "hits" and "possible hits" (ratios of characteristic ions that do not match the expected values in the library within specified limits).

In one application, the analysis time of the standard pesticide method was reduced by one half, two-thirds, and three-fourths. The faster methods were scaled exactly as predicted by using a combination of Agilent's method translation (MTL) and RTL software. Because scaling was exact, these faster methods can be used with precisely-scaled pesticide libraries, making the screening process even more powerful and adaptable to individual needs.

Experimental

The GC method translation software tool was used to find operating conditions for the faster methods. Figure 1 is a screen capture of MTL software data entry showing the original conditions and the new chromatographic conditions for a twofold speed gain. The column flow rate, which is helpful to avoid exceeding MSD pumping capacity[3], is also found in the table. A 16:1 split ratio was suggested in the table as a proportional scaling from the original column to the smaller i.d. column with corresponding lower capacity. The program also determined the required column head pressure and corresponding oven ramp. The Agilent 6890 GC fast oven option (220/240V in the U.S.) was required for the faster oven ramp used in this study.

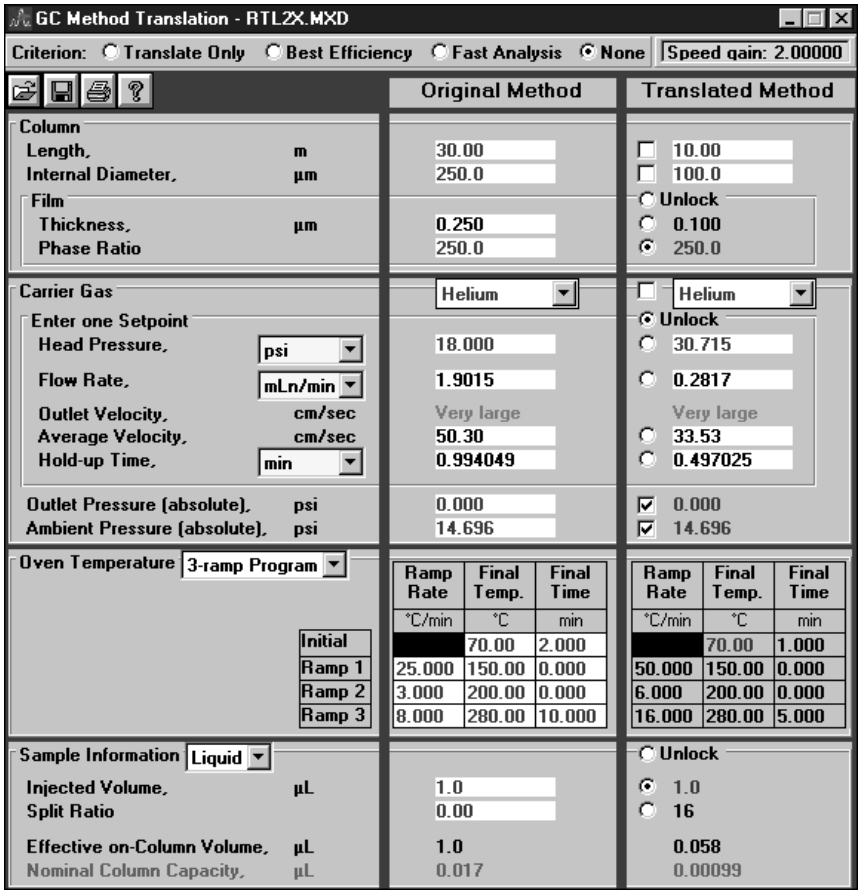

Figure 1. Screen capture showing the method translation (MTL) software data entry used in a twofold speed gain translation.

General chromatographic conditions are listed in table 1. The standard used was a mixture of 26 pesticides at 10 ppm. A 10 m x 0.1 mm x 0.1 µm HP-5 column (part number 19091J-141) was used. The head pressure determined by the method translation software (30.72 psi) was used as the starting point for retention time locking. The column head pressure required to lock retention times of the compounds to the library (the original retention time divided by 2) was determined using the automated RTL process integrated within the Agilent ChemStation for MSD. This process (first translate the method then lock the retention times) was repeated for the threefold and fourfold time reductions.

Table 1. Chromatographic Conditions

Speed	Onefold (1X)	Twofold (2X)	Threefold (3X)	Fourfold (4X)
GC	110 V	220/240 V		
Column	30 m x 0.25 mm x 0.25 µm HP5-MS (P/N 19091S-433)	10 m x 0.1 mm x 0.1 µm HP-5 (P/N 19091J-141)		
Injection mode	Splitless	16:1 split		
Column head pressure	18.0 psi	36.55 psi	63.17 psi	90.0 psi
Column flow (mL/min)	1.5	0.4	0.8	1.5
Inlet control mode	Constant pressure	Constant pressure		
Carrier gas	Helium	Helium		
Injector temperature	250 °C	250 °C		
Oven temperature	70 (2 min)	70 (1 min)	70 (0.67 min)	70 (0.5 min)
Ramp 1	25 °C/min	50	75	100
	150 (0 min)	150 (0 min)	150 (0 min)	150 (0 min)
Ramp 2	3 °C/min	6	9	12
	200 (0 min)	200 (0 min)	200 (0 min)	200 (0 min)
Ramp 3	8 °C/min	16	24	32
	280 (10 min)	280 (5 min)	280 (3.33 min)	280 (2.5 min)
Oven equilibration	2 min	2 min		
Injection volume	1 µL	1 µL		
Liner	5183-4647	5183-4647		
MS Conditions				
Solvent delay	3 min	1.8 min	1.2 min	0.9 min
Tune file	Atune.u	Atune.u		
Low mass	35 amu	35 amu		
High mass	500 amu	450 amu		
Threshold	150	250		
Sampling	2	2	1	1
Scans/sec	3.15	3.50	6.54	6.54
Quad temperature	150 °C	150 °C		
Source temperature	230 °C	230 °C		
Transfer line temperature	280 °C	280 °C		
Acquisition mode	Scan (EI)	Scan (EI)		

Figure 2 shows the results of the shortened analysis times. The three chromatograms look extremely similar, except that the time axis is scaled proportionally. Because MTL followed by RTL scales methods very precisely, scaled screening libraries for corresponding time reductions can be obtained by dividing the retention times in the library by the speed gain (which does not have to be an integer). The peak heights from all the methods are very similar. Although the sample was split 16:1 for the smaller column, the small column i.d. and faster oven ramp combination made the peaks narrower and higher, so there was minimal loss in the signal to noise ratio.

Conclusion

The highly accurate and reproducible pressure and temperature control of the Agilent 6890 GC allows precise scaling of the standard 42-min GC/MSD pesticide method. Run time was shortened to 10.5 minutes using a fast oven ramp rate and a 10-meter 100-micron column. The combination of MTL and RTL facilitated scaling and yielded exact scaling. RTL libraries can accurately be scaled to correspond to the faster analyses.

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2. H. Prest, P. L. Wylie, K. Weiner, and D. Agnew, "Efficient Screening for Pesticides and Endocrine Disrupters Using the HP 6890/ 5973 GC/MSD System," Agilent publication number 5968-4884E, April 1999.
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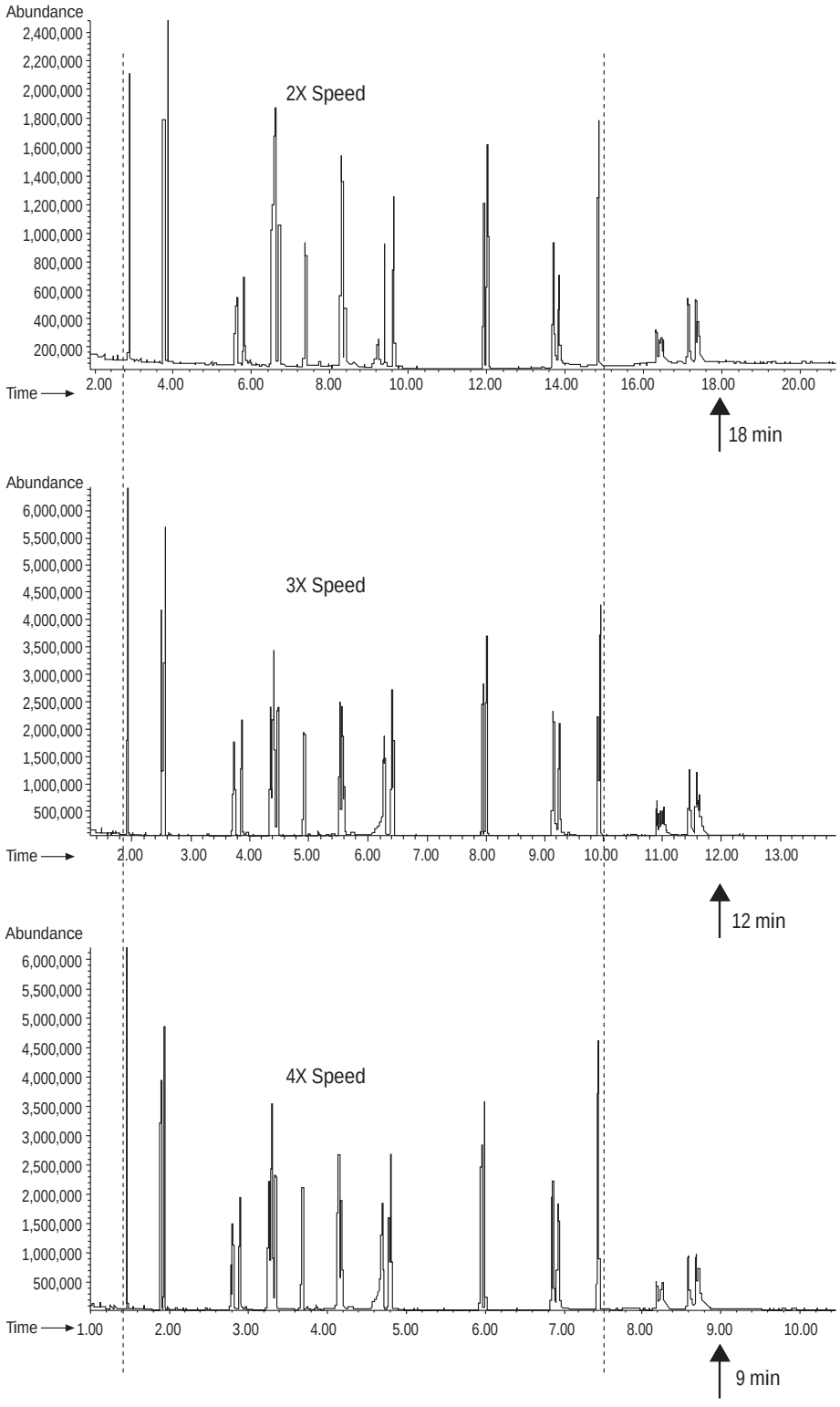

Figure 2. Three TICs of the 2X, 3X, and 4X speedups. The standard analysis (1X) was 42 minutes long. The two vertical lines on the figure are used as references to show the similarity of the TICs.

Printed in USA 2/00
5968-9220E

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Analysis of Bendiocarb and Metabolite by HPLC

Rainer Schuster

Environmental

Abstract

The bendiocarb insecticide can be extracted from soil either with Soxhlet equipment or by ultrasonic treatment in solution and from water by either a liquid–solid or a liquid–liquid technique.

Separation

Figure 1 shows the separation on a 2.1 mm internal diameter Hypersil ODS column. A constant oven temperature of 40 °C is important here.

- UV-visible detection
- Diode-array detection—for simultaneous multiple wave-lengths and peak identity confirmation by spectra.

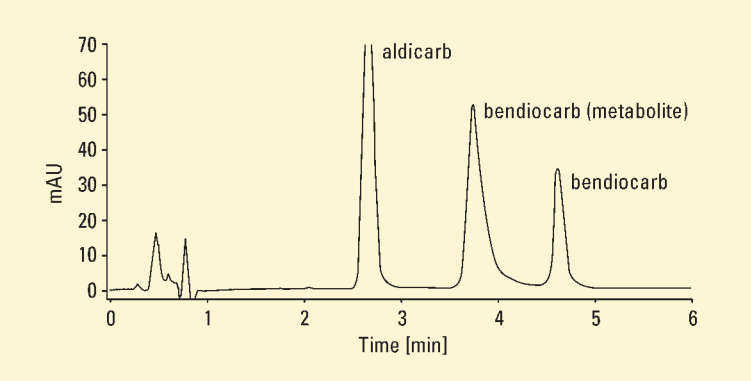

Figure 1
Separation of a 20 µl injection containing aldicarb, bendiocarb and metabolite monitored at 212 nm

Conditions

Column
100 x 2.1-mm Hypersil ODS C18, 5 µm
Mobile phase
Water–acetonitrile
(65:35 isocratic mixture)
Flow rate
0.36 ml/min
Temperature
40 °C
Detection
212 nm (16 nm bandwidth)
reference 450 nm (100 nm bandwidth)

Diode array detector performance
Detection limit 4 µg/l
(without sample enrichment

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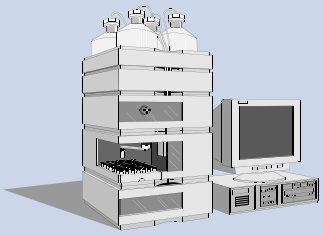
Sample preparation

Narrow-bore technology for
lowest solvent consumption and highest sensitivity.

Equipment

Agilent 1100 Series
- binary pump
- autosampler
- thermostatted column compartment
- diode array detector

Agilent ChemStation + software



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Released 10/97
Publication Number 5966-1876E

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Analysis of Paraquat and Diquat by HPLC

Rainer Schuster

Environmental

Abstract

The paraquat and diquat herbicides can be extracted from soil either with Soxhlet equipment or by ultrasonic treatment in solution and from water by either a liquid–solid or a liquid–liquid technique.

Separation

Figure 1 shows the separation on a 2.1-mm internal diameter Hypersil ODS column.

- UV-visible detection
- Diode-array detection—for simultaneous multiple wavelengths and peak identity confirmation by spectra.

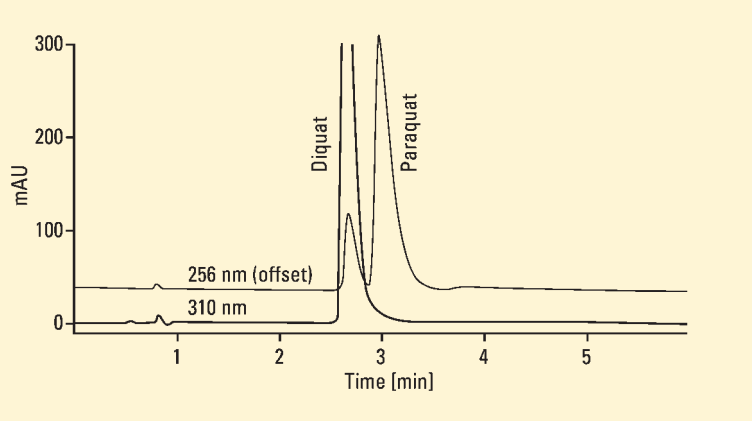

Figure 1
Separation of 10 µl injection of a paraquat and diquat standard

Conditions

Column
100 x 2.1 mm Hypersil ODS C18, 5 µm
Mobile phase
Hexane sulfonic acid
0.35 % triethylamine
pH 2.5 (H_3PO_4)
Flow rate
0.4 ml/min
Detection
256 nm (10 nm bandwidth),
310 nm (10 nm bandwidth)
reference 450 nm (100 nm bandwidth)

Diode array detector performance
Detection limit 4 µg/l
1 ng (absolute) with enrichment factor of 100

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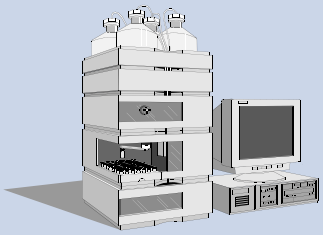
Sample preparation

Narrow-bore technology for lowest solvent consumption and highest sensitivity.

Equipment

Agilent 1100 Series
- binary pump
- autosampler
- thermostatted column compartment
- diode array detector

Agilent ChemStation + software



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Released 10/97
Publication Number 5966-1875E

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Gradient LC analysis of herbicides and polyaromatic hydrocarbons by isocratic Capillary Electrochromatography

Gordon Ross,
Thomas Adam
and Monika Dittmann

Environmental/chemical

Abstract
Capillary Electrochromatography (CEC) combines the separation principle of HPLC (partitioning between mobile and stationary phases) with the high efficiency of capillary electroseparation methods. In CEC the electroosmotic flow (EOF) inherent in capillary electrophoretic separations is used to transport solute and mobile phase through a packed capillary column. The properties of the EOF provides higher efficiencies than can be realized with LC. This can be sufficient to allow the transfer of methods conventionally performed by gradient LC to be performed by isocratic CEC.

Experimental
All CEC experiments were performed using the Agilent CE system, equipped for CEC operation and with a built in diode array detector. The system includes an Agilent ChemStation for system control, data collection and data analysis. CEC columns were supplied by Agilent Technologies. Buffer salts were of the highest purity available and organic solvents were HPLC grade. All buffers were filtered and degassed prior to use. Buffers/mobile phase were adjusted to pH prior to the addition of organic modifiers.

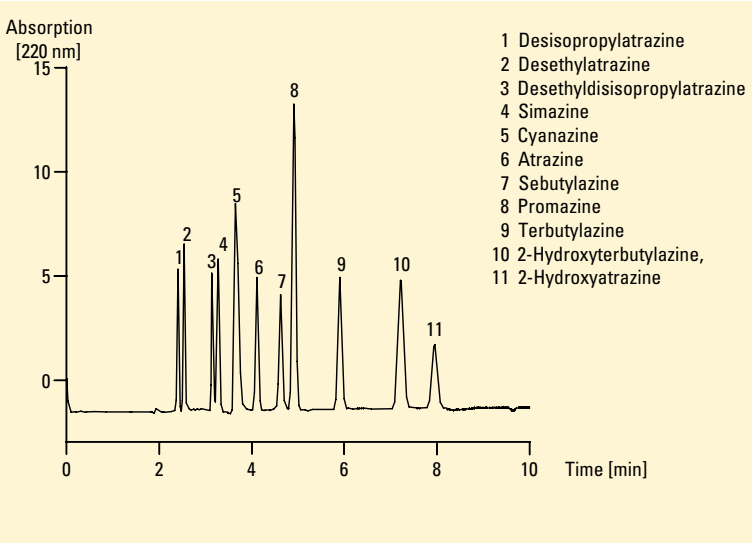

Figure 1
Isocratic CEC alternative to gradient HPLC separation of herbicides

Figure 1 shows the separation of a series of herbicides by CEC. The separation is normally achieved using gradient elution LC. The same is true for figure 2. Here the analysis is of polyaromatic hydrocarbons

Conditions

Column
250 mm × 100 µm; Sperisorb ODS1
Mobile Phase
60 % acetonitrile/40 % 25 mM TRIS pH 8
Voltage
30 kV
Temperature
15 °C

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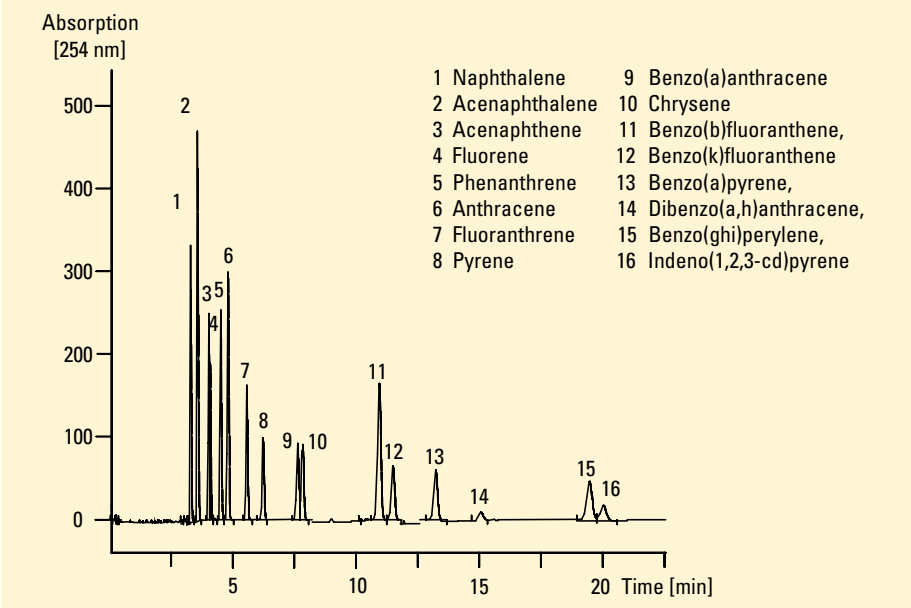


Figure 2
Fast CEC separation of EPA 16 PAH standard on CEC hypersil C18

which are of environmental significance and interest. Conventional analysis of these compounds can be achieved in a similar time however with isocratic CEC operation there is no inter-analysis time required for re-generation of the LC column.

Conclusions

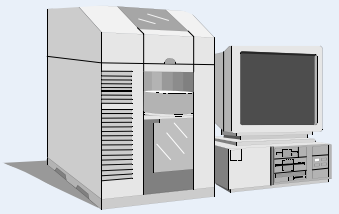
Some gradient LC separations can be succesfully performed using isocratic CEC. Very similar separations can be achieved in the same time frame. Time for re-equilibration of the LC column is not needed and therefore the overall analysis time is reduced.

Conditions

Column
CEC Hypersil C18, 250 mm (350 mm) × 0.1 mm i.d., 2.5 µm
Cell Standard
Eluent
90 % TRIS-HCl 50 mM, pH 8
Voltage 30 kV
Temperature 20 °C
Pressure 10 bar both sides

Equipment

- Agilent Capillary Electrophoresis System
- Agilent ChemStation + software



Monika Dittmann is an R&D chemist and Gordon Ross is an application chemists at Agilent Technologies, Waldbronn, Germany. Thomas Adam is at the University of Mainz, Germany.

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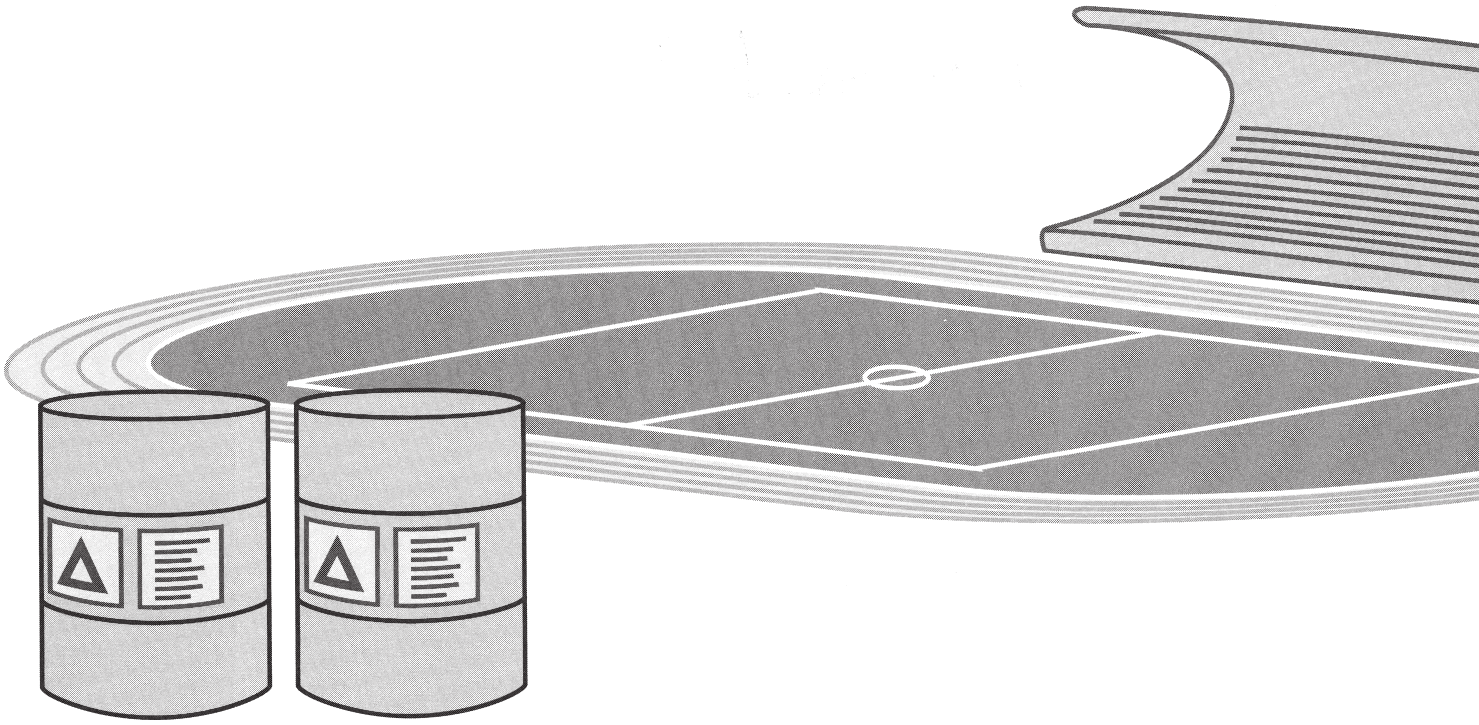
Released 06/97
Publication Number 5965-9032E

A Comparison of Pre- and PostColumn Sample Treatment for the Analysis of Glyphosate

Application Note

Environmental Analysis

R. Schuster and
A. Gratzfeld-Hüsgen



The active ingredient in several sports field weedkillers, glyphosate, and its main metabolite aminomethyl phosphonic acid (AMPA) can be analyzed to low (1–5) ppb levels by HPLC, although the absence of chromophores in both compounds makes labeling with fluorogenic reagents necessary. Precolumn derivatization with fluorenylmethyl chloroformate (FMOC) gives low detection limits and has the advantage of simplicity, since it uses the automated injector program facility of the HP 1050 Series autosampler. Post-column oxidation of the glyphosate with hypochlorite, followed by derivatization with *o*-phthalaldehyde (OPA) requires additional equipment, but has the advantage of superior selectivity. It is thus better suited to samples in complex matrices.

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Introduction

Glyphosate, the N-(phosphonomethyl) derivative of the amino acid glycine, is a widely-used broad-spectrum, non-selective and post-emergence herbicide. Its application to plants inhibits the production of specific enzymes so that the synthesis of aromatic amino acids is interrupted. Compared with triazines, glyphosate is strongly absorbed on soil particles and subsequently undergoes microbial degradation to the main metabolite, aminomethyl phosphonic acid (AMPA) (figure 1) and finally to ammonia and carbon dioxide.[1] It is the active ingredient of several commercial weedkillers, and is used against weeds, for example couch grass, in the farming of cereals, potatoes, vines and mushrooms; it is very often used to maintain sports fields and lawns.

The wide use of glyphosate in agriculture can result in its presence in ground water, vegetable matter and milk. Because of the suspected toxicity of glyphosate and its metabolite, tolerance levels have been set for food and drinking water. German regulations, for example, limit the maximum residue limits (MRL) to 80 mg/kg for mushrooms, 10 mg/kg for cereals, and 0.1 mg/kg for plant food, and the limit set by the European Community for drinking water is 0.1 µg/l.[2] The United States Environmental Protection Agency (EPA) has set a detection limit for glyphosate in drinking water of 6 µg/l.[3]

Because of the low MRLs and the wide variety of matrices, selectivity and sensitivity play an important role in the analysis of glyphosate. High selectivity is required for complex matrices such as food samples and the highest sensitivity is needed to monitor glyphosate and its metabolite in water samples.

Many different methods for the analysis of glyphosate and its metabolite AMPA have been described, including gas chromatography,[4] thin-layer chromatography[5] and high performance liquid chromatography (HPLC). All these methods, however, require special manual derivatization steps or tedious cleanup procedures making them time-consuming. For the HPLC analyses, the absence of chromophores makes it necessary for the analytes to be labeled with reagents either before the column[6] or after the column.[7] We have compared pre- and postcolumn derivatization methods in the light of the different requirements for the analyses of drinking water and agricultural produce.

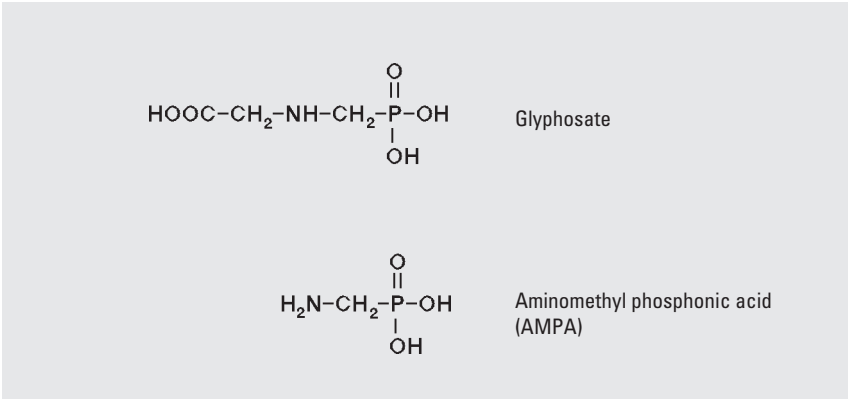

Figure 1
Glyphosate and its main metabolite aminomethyl phosphonic acid (AMPA)

Experimental

For the chromatographic analyses, we used the HP 1050 Series autosampler with variable-volume auto-injector, the HP 1050 Series quaternary pump with temperature-controlled column compartment and the HP 1046A programmable fluorescence detector. We used the programmable injection facility of the auto-injector for automated precolumn derivatization. For postcolumn derivatization, we used a Postcolumn Reaction System (PRS) from Pickering Laboratories. Figure 2 shows a schematic diagram of the complete analysis setup.

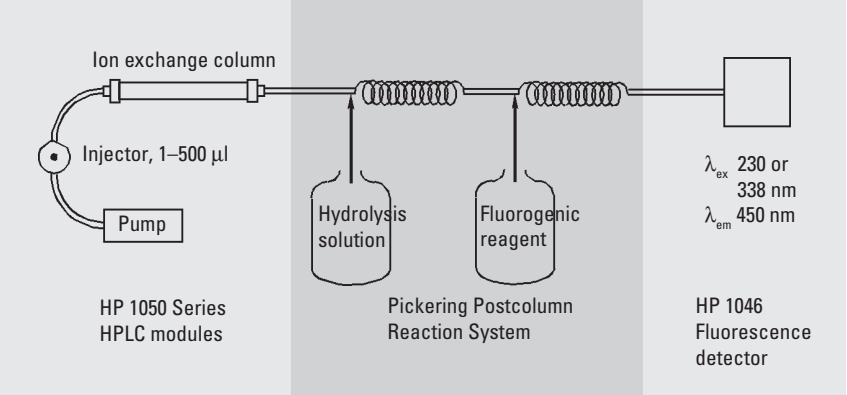

Figure 2
Analysis set-up for postcolumn derivatization

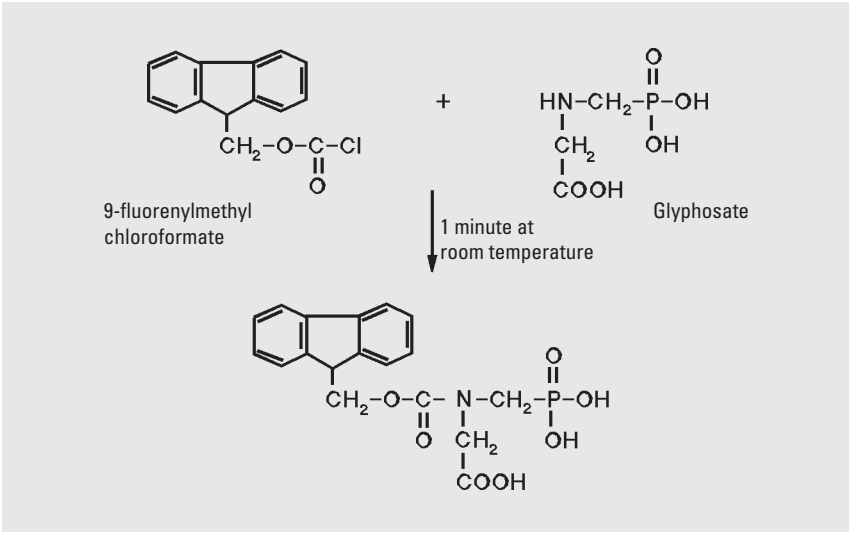

Figure 3
Precolumn reaction for glyphosate with FMOC

Automated precolumn derivatization

The reaction of glyphosate with fluorenylmethyl chloroformate FMOC,[8] used for the automated precolumn derivatization, is shown in figure 3. The reaction was performed automatically in the auto-injector, using an injector program. Table 1 lists the various program steps of the automated precolumn derivatization.

In step 1 of the program, a borate buffer (necessary to maintain the correct reaction pH of 10.4), was drawn into a capillary, then FMOC (step 3), sample (step 5) and again FMOC (step 7) were drawn into the capillary. Between each step, the surface of the needle was cleaned by washing with acetonitrile (steps 2, 4, 6 and 8). The sample and reagent volumes were mixed by moving them back and forth inside the capillary (step 9). The resulting FMOC derivatives of glyphosate and AMPA were injected after a 1 minute wait. Derivatization was performed at ambient temperature. The derivatives were separated on a reversed phase column and were detected with a fluorescence detector using an excitation wavelength of 266 nm and an emission wavelength of 305 nm, with a 280 nm cut-off filter.

1	**draw :**	**2 µl from vial 2**	draws borate buffer (0.4N, pH 10.4)
2	**draw :**	**0 µl from vial 3**	rinses tip by dipping in vial containing acetonitrile
3	**draw :**	**1 µl from vial 1**	draws FMOC (1mg/ml in acetonitrile)
4	**draw :**	**0 µl from vial 3**	rinses tip by dipping in vial containing acetonitrile
5	**draw :**	**1 µl from sample**	
6	**draw :**	**0 µl from vial 3**	rinses tip by dipping in vial containing acetonitrile
7	**draw :**	**1 µl from vial 1**	draws FMOC
8	**draw :**	**0 µl from vial 3**	rinses tip by dipping in vial containing acetonitrile
9	**mix :**	**7 µl cycles: 10**	mixes reagent and sample for derivatization reaction
		mixing speed: 300 µl/min	
10	**wait :**	**1 min**	
11	**inject**		

Table 1
Injector program for derivatizing glyphosate and its metabolite AMPA

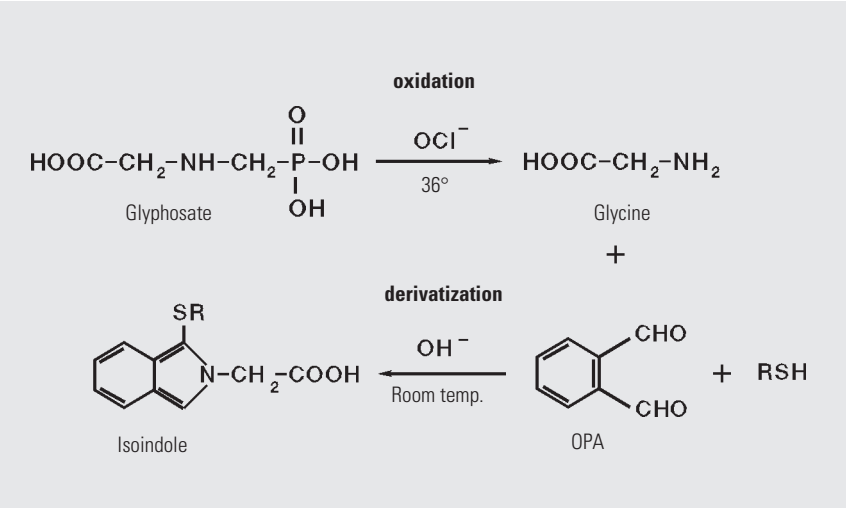

Figure 4
Two step postcolumn reaction for glyphosate

Postcolumn derivatization

For postcolumn derivatization experiments a Pickering glyphosate Postcolumn Reaction System EG5000XX (Pickering laboratories, Inc. 1951 Colony Street, Mountain View, CA 94043) was connected between the HPLC column and the fluorescence detector. After separation on the anion exchange column, the glyphosate underwent a two-stage derivatization which is shown in figure 4. The glyphosate was first hydrolyzed to glycine by an hypochlorite solution and the glycine then derivatized with *o*-phthalaldehyde (OPA) reagent to form a fluorescent isoindole. The amino group of AMPA (see figure 1) reacted directly with the OPA. The isoindole derivatives were detected by fluorescence with excitation wavelength of 230 nm or 338 nm and an emission wavelength of 450 nm with a

370 nm cut-off filter. Chemicals and solvents were obtained from Baker (FRG).

The hydrolysis solution was made up by dissolving 8 g dipotassium hydrogen phosphate, 5 g potassium chloride and 150 ml sodium hypochlorite solution (5% active chloride, supplied by Aldrich-Chemie D7924 Steinheim) in 1 liter of water. pH was measured as 9.1. If necessary, the sodium hypochlorite solution can be replaced by 15 mg calcium hypochlorite. The fluorogenic reagent can be either a premixed OPA solution (Fluoraldehyde®, Pierce), or a mix of 1 g *o*-phthaldaldehyde and 1 ml 2-mercaptoethanol dissolved in 10 ml methanol and added to 1 l of 0.05 N sodium borate solution (19.1 g sodium borate in water). pH was measured as 9.2.

The analysis was performed according to a method published by the EPA[3] for the analysis of glyphosate in drinking water. This method requires that the two compounds, glyphosate and AMPA, are monitored at maximum residue limits of 6 ppb (6 µg/l).

Because of the amphoteric character of glyphosate (–NH– and –COOH, $–PO_3H_2$), the separation of glyphosate and AMPA can be performed on either an anion exchange (SAX) with 0.0025 M potassium dihydrogen phosphate, adjusted to pH 2.1 with phosphoric acid, as mobile phase (elution order AMPA–glyphosate) or on a cation exchange (K^+ form) with 0.005 M potassium dihydrogen phosphate, adjusted to pH 2.0 with phosphoric acid, (elution order glyphosate–AMPA). We chose the anion-exchange column (SAX-300 100 × 4.6 mm id, part number 79919QA-754) for our experiments. At pH 2.1, any amino acids in the matrix (for example from food samples) behave like neutral molecules and are not retained. Additionally, if sample enrichment is necessary, glyphosate can be retained on an anion exchange column at neutral pH.[9]

Column		ODS Hypersil, 100 x 4.6 mm, 5 µm
Eluant	Channel A	0.002M KH_2PO_4 7% acetonitrile adjusted to pH 7.0
	Channel B	acetonitrile
Column temperature		40°C
Fluorescence detection		λ_{ex} 266 nm λ_{em} 305 nm with a cut-off filter at 280 nm.
Injection volumes		1 µl and 10 µl

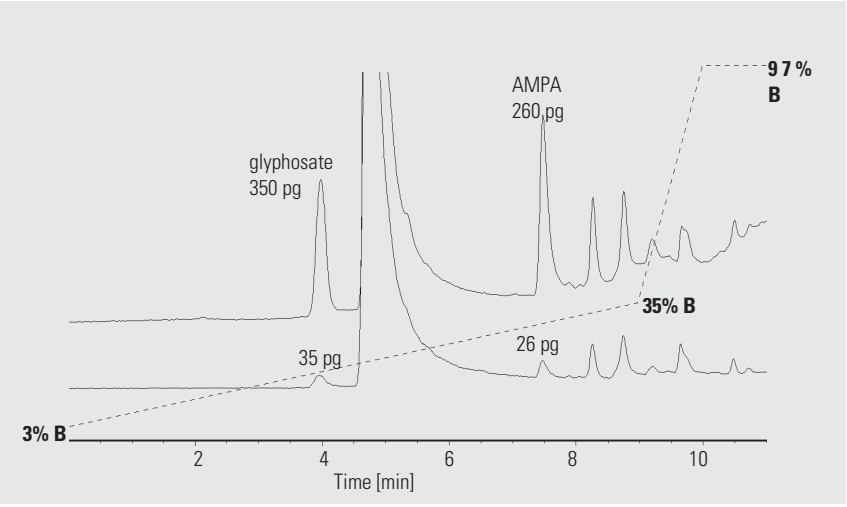

Figure 5
Analysis of two samples after precolumn derivatization (upper trace 1 µl injection of 350 ppb glyphosate and 260 ppb AMPA, lower trace 10 µl of a 100-fold dilution)

Results and discussion

Automated precolumn derivatization

Figure 5 (previous page) shows the separation of glyphosate and AMPA. The upper trace shows the results of a 1 µl injection of water containing 350 ppb glyphosate and 260 ppb AMPA. The lower trace shows the results of a 10 µl injection of water containing 3.5 ppb glyphosate and 2.6 ppb AMPA. The additional peaks in the chromatogram derive from either FMOC reagents or from by-products of the derivatization.

We found that the derivatization yield was constant for volumes of up to 10 µl of sample, resulting in a detection limit of approximately 1 ppb of each compound. Over eight runs, the method's repeatability was better than 0.5% RSD for retention times and better than 2.5% for peak areas when injecting 10 µl and better than 5% when injecting 1 µl sample volume.

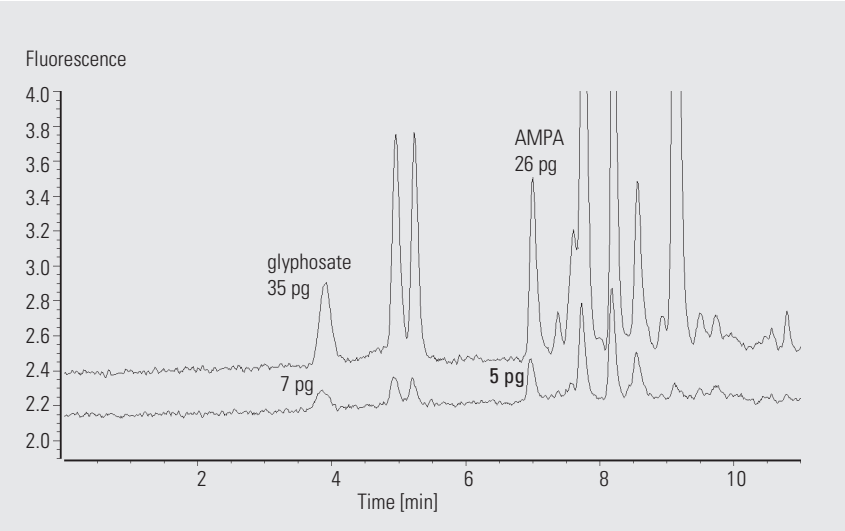

Figure 6
Analysis of 100 µl injection after manual derivatization of glyphosate and AMPA at different concentrations (upper trace 350 ng/l glyphosate, 260ng/l AMPA, lower trace 70 ng/l glyphosate, 52 ng/l AMPA)

For lower detection limits, larger sample volumes have to be used. There are three possible solutions :

a) 25 ml sample is derivatized manually with FMOC then extracted with two 30 ml aliquots of methylene chloride, as described by Gauch, Leuenberger and Mueller.[10] The methylene chloride extracts are concentrated and 20 µl are injected. Detection limits are 20 ng/l;

b) The sample is derivatized manually with FMOC, and a large volume (up to 500 µl) is injected without extraction or concentration. Figure 6 shows chromatograms of standards at different amounts, and figure 7 shows chromatograms of standards overlaid on a water sample taken from a well. The detection limits were below 50 ng/l;

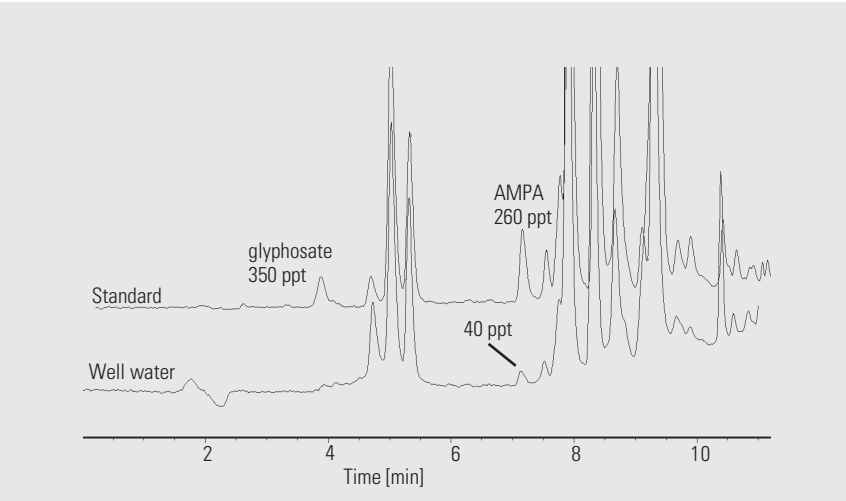

Figure 7
Analysis of 100 ml of well water overlaid with a standard

c) The sample is derivatized automatically with FMOC in the larger volume of a sample vial (rather than the capillary) using the HP 1050 Series autosampler with the extended 100 vial tray and rotary mixer accessory. Reagents and borate buffer (20 µl each) are added to a 500 µl sample vial and mixed in the vial using the rotary mixer; all steps are performed automatically using the injector program. The derivatization is completed within one minute, and volumes as large as 100 µl can be injected onto the column. An example of a large volume injection is shown in figure 8. Injection of volumes larger than 100 µl should be avoided because compounds from reagents may interfere with the analytes' derivatives. With the injection volume of 100 µl, the detection limit is about 50 ng/l for AMPA and 100 ng/l for glyphosate. Further investigations to improve the reproducibility of this method of derivatization are in progress.

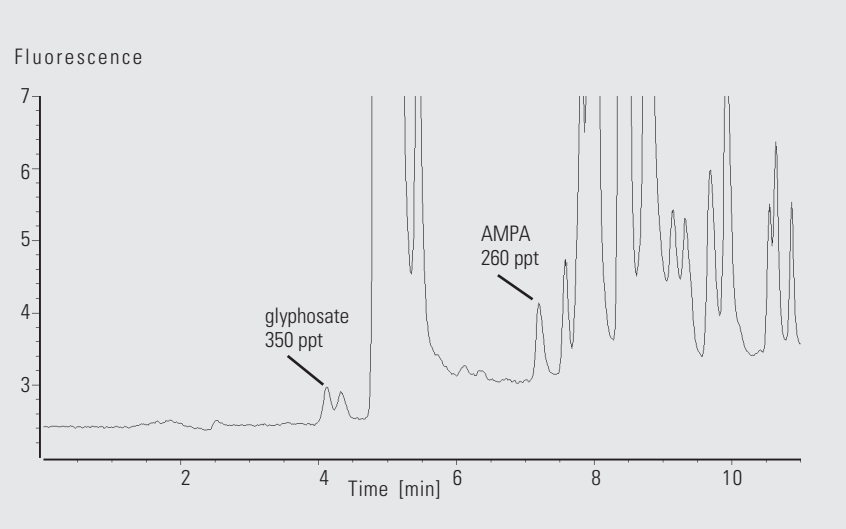

Figure 8
Analysis of 10 ml of sample after automated precolumn derivatization in the vial using a rotary mixer (35 pg glyphosate and 26 pg AMPA)

Postcolumn derivatization

Chromatograms of the separation of glyphosate and AMPA at different concentrations are shown in figure 9. In the lower chromatogram, 2.6 ng AMPA and 3.5 ng glyphosate in 500 µl are injected representing 5.2 µg/l AMPA and 7 µg/l glyphosate. Compared with the chromatograms obtained after precolumn derivatization, the selectivity is

Column	SAX 300, 100 x 4.6 mm
Eluant	0.34g/l KH_2PO_4, 0.1% H_3PO_4, adjusted to pH 2.1
Flow rate	0.8 ml/min
Column temperature	40°C
Fluorescence detection	λ_{ex} 230 nm λ_{em} 450 nm with a cut-off filter at 370 nm
Injection volume	500 µl

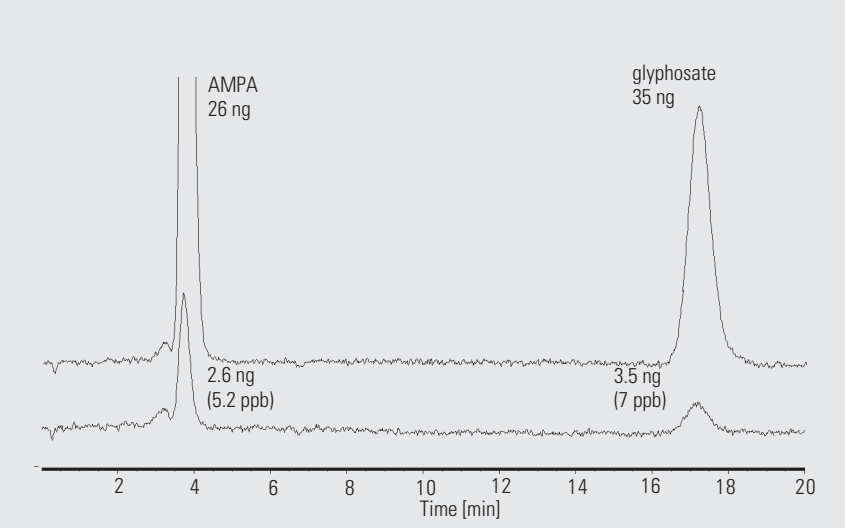

Figure 9
Chromatogram of glyphosate and AMPA with post-column derivatization

impressively high. No peaks from reagents or side reactions are visible. Detection limits are in the low ppb range. If lower detection limits are required, the sample can be enriched by passing it through an anion-exchange column at pH 7.2. A procedure for this has been described.[11]

Reproducibility of the method was measured over 10 runs. The standard deviation of the retention times was 0.8% RSD and the repeatability of the peak areas was 2.1% RSD for a 35 pg sample and 100 µl injection volume.

Conclusion

Glyphosate and its metabolite AMPA can be determined by either pre- or postcolumn automated derivatization techniques. Detection limits for precolumn derivatization are slightly better and the instrumentation is simpler, requiring no more than the HP 1050 Series autosampler with injector program. The advantage of the postcolumn reaction method is higher selectivity. This is important for the analysis of food samples, where the matrix contains amino acids that are derivatized during the precolumn derivatization and interfere with glyphosate and AMPA in the chromatography. Thus the determination of glyphosate and its metabolite in water samples is dealt with adequately by precolumn derivatization, while more complex matrices are better handled by the postcolumn derivatization technique.

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Quantitative Analysis of Perchlorate by Ion Chromatography MS/MS

Application

Environmental

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Abstract

Perchlorate in water is analyzed using a Metrohm Ion Chromatography system interfaced to an Agilent 6410 Triple Quadrupole (QQQ) Mass Spectrometer. Quantitation is based on the sum of two multiple reaction monitoring (MRM) transitions corresponding to both chlorine isotopes (^{35}Cl and ^{37}Cl) of perchlorate. Excellent linearity among calibration standards ranging from 0.5 to 25 ppb is established using the Metrohm conductivity detector with a resulting coefficient of linearity of $R^2 > 0.999$. Linearity for the QQQ mass spectrometer is $R^2 > 0.998$ over the range of 0.01 to 10 ppb. Reproducibility among seven replicate injections of standards is also excellent for the QQQ, with seven replicates at the 0.1 ppb level resulting in a peak area relative standard deviation (RSD) of only 5.33%.

Confirming the presence of perchlorate by QQQ mass spectrometry involves measuring the peak area ion ratio of the two MRM transitions, corresponding to the ^{35}Cl and ^{37}Cl isotopes. The transitions are 101 > 85 and 99 > 83. The sum of the two is used for quantitation and the latter is used as a qualifier so that as long as all analyzed samples have qualifier to quantifier peak area ion ratios within ± 20% of the value determined using one of the calibration standards, the presence of perchlorate is confirmed.

Perchlorate in the presence of the total dissolved solids (TDS) of chloride, carbonate, and sulfate in reagent water is also analyzed. For example, at the 3,000 ppm TDS concentration, reproducibility among seven replicate injections of perchlorate at 1 ppb is only 0.2%, when using the IC conductivity detector. Tandem IC conductivity and MSMS yields very similar results. The advantage of MSMS is to have confirmation and Metrohm Suppressor de-salts the matrix to yield better sensitivity for MSMS.

When using the QQQ mass spectrometer to analyze perchlorate in the presence of salt water, the reproducibility is very good. For example, at 1 ppb perchlorate in 1,000 ppm salt water, the peak area reproducibility among three injections is only 0.63% RSD.

Introduction

Perchlorate is commonly used as an oxidant in solid fuel propellants for rockets and missiles. Recently, perchlorate contamination was found in many aquifers associated with the Colorado River (CA). Other sites were also identified, but by far the widest contamination problem is in California, Nevada, and Arizona. Perchlorate was also found at elevated levels in crops that use contaminated water for irrigation. Ion chromatograph (IC) with conductivity detection can be used to measure perchlorate levels in drinking and waste waters (as per EPA Method 314). The method is reliable to approximately 1 to 5 ppb in drinking water, but sensitivity decreases dramatically as the complexity of the matrix is increased (such as in surface and waste waters). Both false positive and false negative results may occur due to matrix effects and co-eluting substances detected by nonspecific conductivity detection. Lower detection limits (DLs) for perchlorate are needed as the EPA and state environmental agencies are looking to target levels in the 1 to 2 ppb range. Reliability of the measurement in heavy matrix samples is also important.

The use of a mass spectrometer as a detector for perchlorate at much lower DLs (50 to 100 ppt) has shown promise; however, reliability issues and problems related to suppression of the electrospray ionization (ESI) signals in typical matrices are well documented phenomena. The key to reducing suppression is to ensure that analyte and high concentrations of matrix are well separated and do not enter the ion source and interface at the same time.

In addition to ion suppression in the source, the *m/z* attributed to the perchlorate anion (99 and 101) have isobaric interferences that can be attributed to minor sulfate isotopes and from organic material that can be present and bleed from the column used for IC and the associated cation suppressor. The selection of separation column and suppressor are critical for reduction of sample bleed and for efficient separation of high levels of interfering ions, particularly sulfate.

Two USEPA methods (EPA Method 332.0 and SW-846 Method 6860) have been developed and published for this analyte using ion chromatography mass spectrometry (ICMS) and/or ICMSMS. The advantage of using tandem mass spectrometers is that interference from the hydrogen sulfate ion (HSO_4^{-1}, *m/z* 99) can be completely eliminated because in secondary fragmentation, the perchlorate transition is 99 > 83 (loss of ^{16}O) but sulfate (HSO_4^{-1}) ion is completely destroyed and does not even interfere. This helps to quantify the perchlorate ion with an excellent isotope ratio for chlorine ($^{35}Cl/^{37}Cl$) without any distortion. Data in this application is demonstrated. This application is also applicable to complex matrices like green leafy vegetables, fruits, plants (bio-accumulation of perchlorate), wines, and liquid medicines.

The chemical structure of perchlorate is shown in Figure 1. The compound is an excellent candidate for negative polarity electrospray ionization as it exists as an ion in solution.

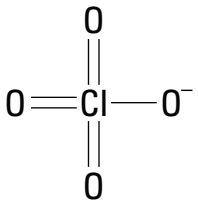

Figure 1. Chemical structure of perchlorate.

Experimental

Sample Preparation

The following samples are prepared for use in the analysis:

Perchlorate stock standard (1,000 ppm) is prepared from sodium perchlorate (Sigma p/n S-1513, MW 122.4, CAS 7601-89-0). The stock standard is spiked into reagent-grade water to make up both calibration levels and lowest concentration minimum reporting limits (LCMRL). The LCMRL samples are used as quality controls and a reproducibility evaluation is made from replicate injections at differing concentrations.

Total dissolved solid (TDS) samples, which contain 3,000 ppm each of chloride, carbonate, and sulfate, dissolved in reagent water, are made up using ACS-grade sodium chloride, sodium carbonate, and sodium sulfate purchased from Aldrich (Milwaukee, WI).

Isotopic (^{18}O) enriched sodium perchlorate is used as internal standard and spiked into all TDS samples, calibrators, and LCMRLs at 10 ppm. The internal standard solution is purchased commercially from SPEX (Metuchen, NJ).

IC/MS/MS Details

Metrohm IC components

Metrohm Model 818 Inert Dual Piston Pump

Metrohm Model 819 Conductivity Detector

Metrohm Model 820 Separation Center (with two injection valves)

Metrohm Model 830 Interface

Metrohm Model 833 MSM-II

Metrohm Model 853 Sequential Suppressor

Metrohm Model 838 Auto Sample Processor

Metrosep ASUPP7-250 column (4.0 mm id × 250 mm length)

Metrosep RP Guard disc

Ion Chromatography Conditions

Eluent	10 mM sodium carbonate (Na_2CO_3) + 35% acetonitrile
Column temperature	45 °C
Column flow rate	0.7 mL/min
Injection volume	100 µL
Conductivity detector	Range and full scale = 100 µSiemens/sec

The mass spectrometry component is the **Agilent 6410 Triple Quadrupole Mass Spectrometer**.

Mass Spectrometry Conditions

Mode	Negative ESI using the Agilent G1948B ionization source
Nebulizer	45 psig
Drying gas flow	12 L/min
Drying gas temp	350 °C
V_{cap}	1750 V
Resolution (FWHM)	Q1 (unit) = 0.7 amu; Q2 (unit) = 0.7 amu
Fragmentor	120 V
Collision energy	30 V
Dwell time	200 msec

MRM transitions:
Perchlorate (sum of both Cl^{35} and Cl^{37} isotopes) = *m/z* 99 > 83 + 101 > 85
Perchlorate internal standard (O^{18}) = *m/z* 107 > 89

Results and Discussion

Part 1: Calibration Levels and LCMRLs

The conductivity calibration curve using the signal generated from the Model 819 conductivity detector of the Metrohm IC system is shown in Figure 2. Conductivity is measured in units of micro-Siemens per sec (µS/sec). The calibration curve fit has a coefficient of linear regression $R^2 > 0.999$ over the calibration level range of 0.5 to 25 ppb.

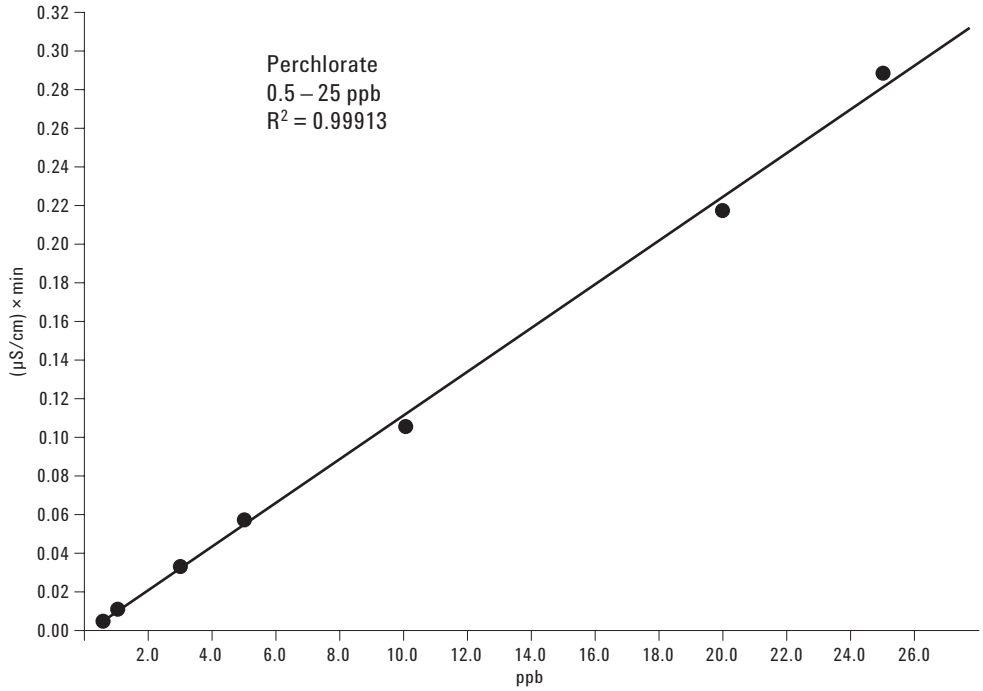

Figure 2. Conductivity calibration curve.

The calibration curve for the QQQ mass spectrometry analysis of this work is shown in Figure 3. The measured signal is the sum of two MRM transitions coming from both the ^{35}Cl (99 > 83) and ^{37}Cl (101 > 85) isotopic ion contributions. The lowest three levels are expanded to demonstrate excellent accuracy with respect to the curve as well as excellent reproducibility of the lowest level LCMRL samples at 0.1 ppb. Reproducibility is further detailed in Table 1 as the percent relative standard deviation (% RSD) of the calculated concentrations for seven replicate injections. The reproducibility is also demonstrated in Figures 4a through 4c as overlaid chromatographic traces.

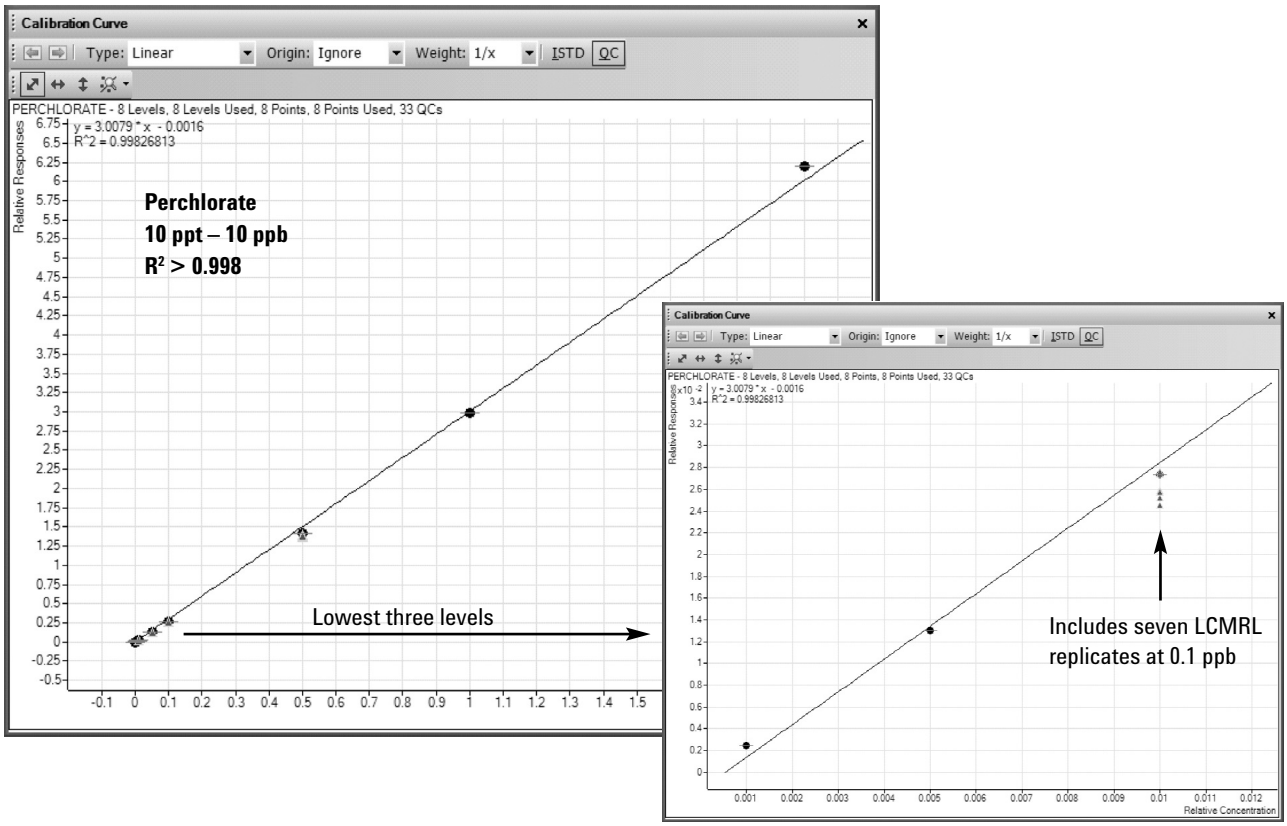

Figure 3. Good MS linearity for perchlorate over three orders of magnitude with excellent accuracy shown at lower end.

Table 1 Reproducibility of Calculated Concentrations for LCMRLs

LCMRL replicate	0.1 ppb Calc. conc.	0.5 ppb Calc. conc.	1 ppb Calc. conc.
1	0.1191	0.5546	1.0713
2	0.1089	0.5267	1.0863
3	0.1110	0.5149	1.0897
4	0.1140	0.5206	1.0809
5	0.1073	0.5417	1.1000
6	0.1087	0.5406	1.0888
7	0.1002	0.5534	1.0975
Standard dev	0.006	0.016	0.010
Average	0.110	0.536	1.088
% RSD	5.33	2.92	0.90

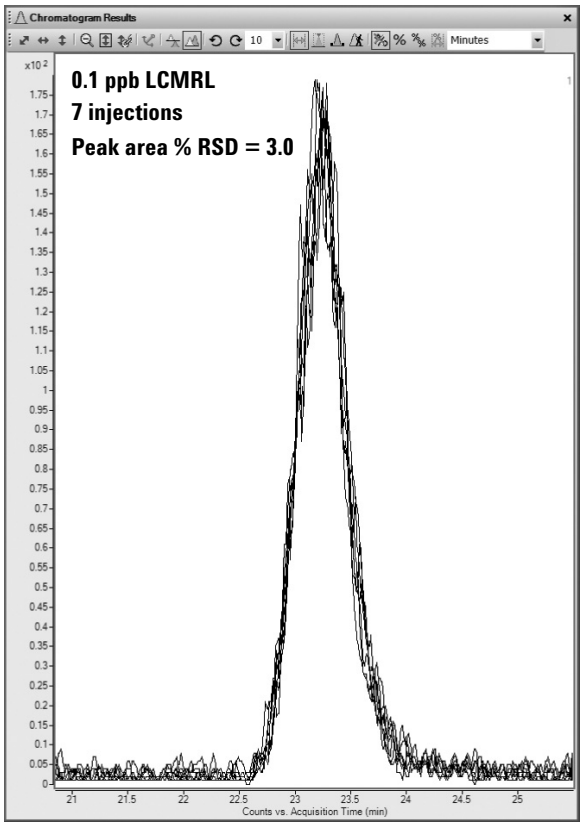

Figure 4a. MS chromatographic overlay of 0.1 ppb LCMRL.

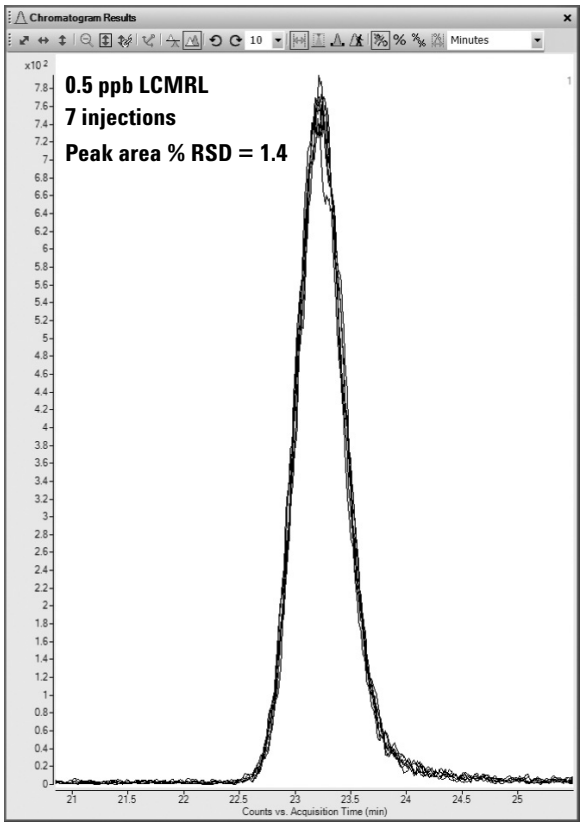

Figure 4b. MS chromatographic overlay of 0.5 ppb LCMRL.

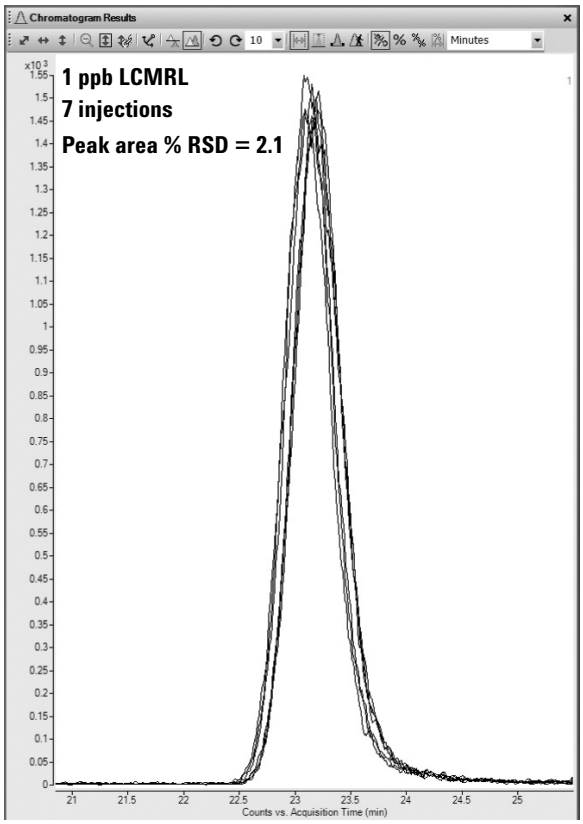

Figure 4c. MS chromatographic overlay of 1 ppb LCMRL.

For confirming the presence of the perchlorate ion the less intense ^{37}Cl MRM transition can be used as a qualifier ion. As long as all samples have ion ratios within ± 20% of the expected value, the presence of perchlorate in that sample is confirmed. An example involving one of the 0.5 ppb LCMRL injections is shown in Figure 5.

Part 2: TDS Samples

These samples are made up of reagent water, spiked perchlorate analyte, and internal standards, as well as 3,000 ppm each of chloride, carbonate, and sulfate.

Figure 6 shows the Metrohm conductivity trace of perchlorate in the presence of the 3,000 ppm TDS and the need to have a relatively long elution time of approximately 21 minutes in order to chromatographically separate the perchlorate from the TDS matrix. The reproducibility of seven replicate injections is demonstrated in Figure 7, with corresponding peak area reproducibility shown in Table 2.

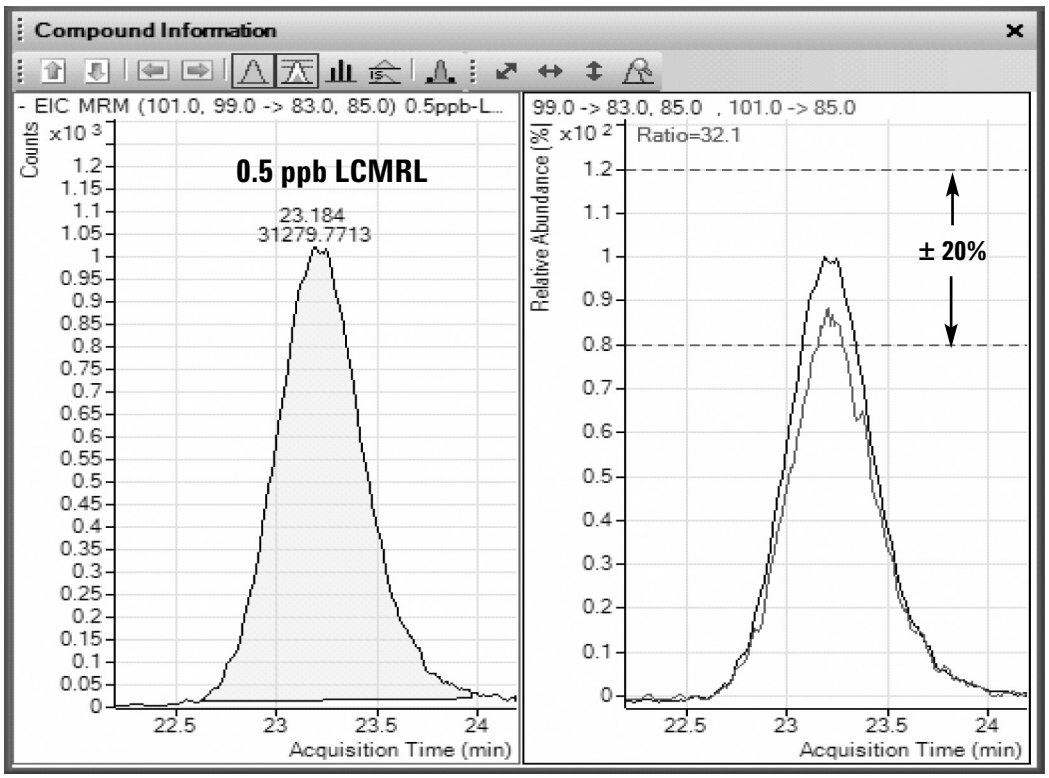

Figure 5. Confirmation of perchlorate using qualifier/quantifier peak area ion ratios.

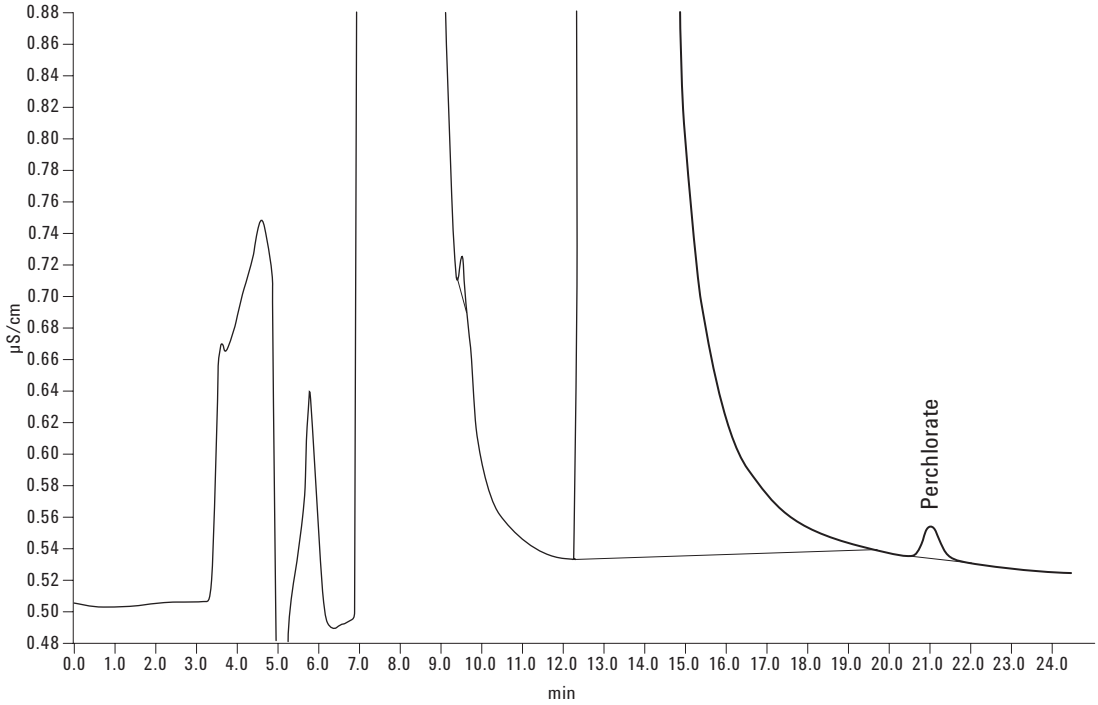

Figure 6. Chromatographic trace of 1 ppb perchlorate (RT = 21 min) in the presence of 3,000 ppm TDS. Plot of conductance (µS/sec) vs. time (min).

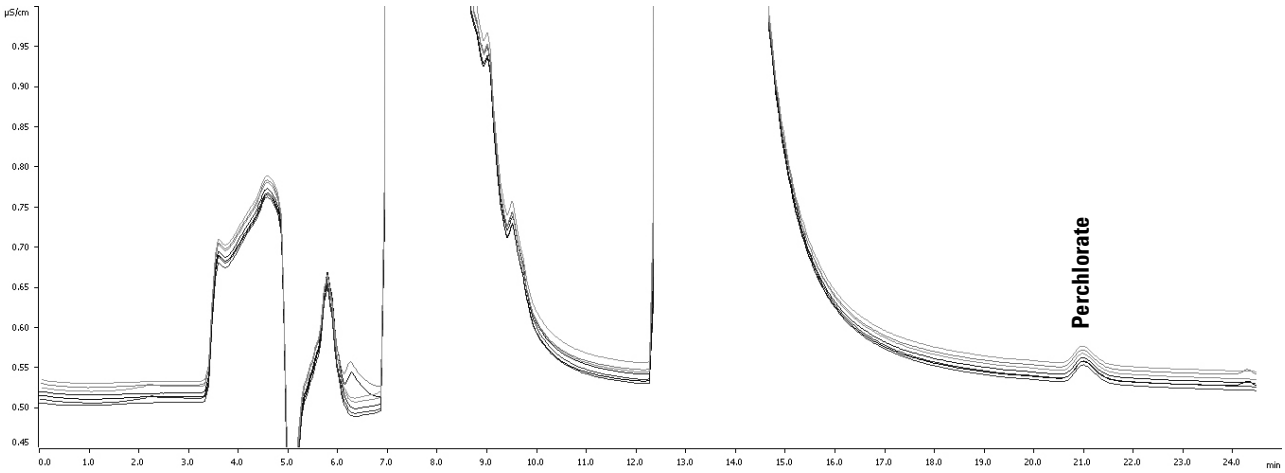

Figure 7. Overlay of seven 1-ppb perchlorate replicate injections in 3,000 ppm TDS.

Table 2. Reproducibility of Seven Replicate Injections at 1 ppb Perchlorate in 3,000 ppm TDS (Calculated concentrations based on conductivity calibration of Figure 2.)

Sample id	Perchlorate ug/L
MDL-1	1.220
MDL-2	1.221
MDL-3	1.223
MDL-4	1.221
MDL-5	1.216
MDL-6	1.220
MDL-7	1.219
Average	1.220
Standard dev	0.002
% RSD	0.177
True value	1.200
% Recovery	101.67

Iin Figure 8, three of the injections for perchlorate in the 3,000 ppm TDS are shown. The peak area reproducibility is not very good and is obviously affected by the presence of the chloride, carbonate, and sulfate, dissolved in reagent water. The peak area RSD is about 41%, even though the minimum peak-to-peak signal-to-noise ratio (p-p S/N) is 25:1.

And yet, when using the QQQ mass spectrometer to analyze perchlorate in the presence of salt water, the reproducibility is very good. For example, at 1 ppb perchlorate in 1,000 ppm salt water the peak area reproducibility among three injections is only 0.63% RSD, as shown in Figure 9.

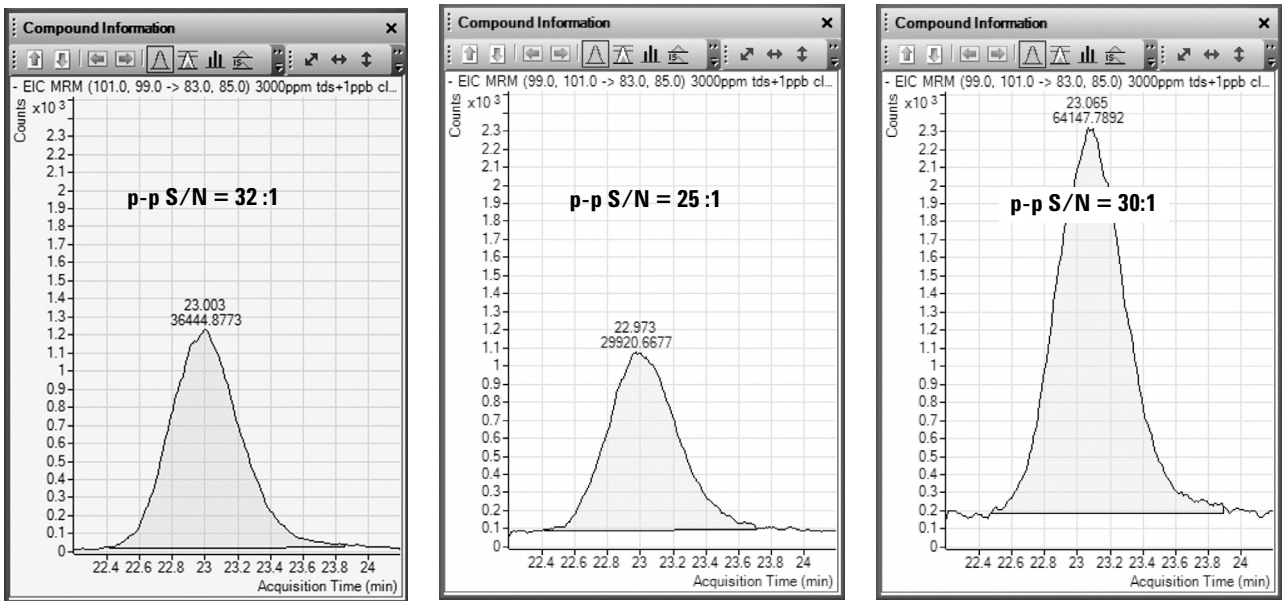

Figure 8. Peak area reproducibility for three injections of 1 ppb perchlorate in 3,000 ppm TDS is 41% RSD.

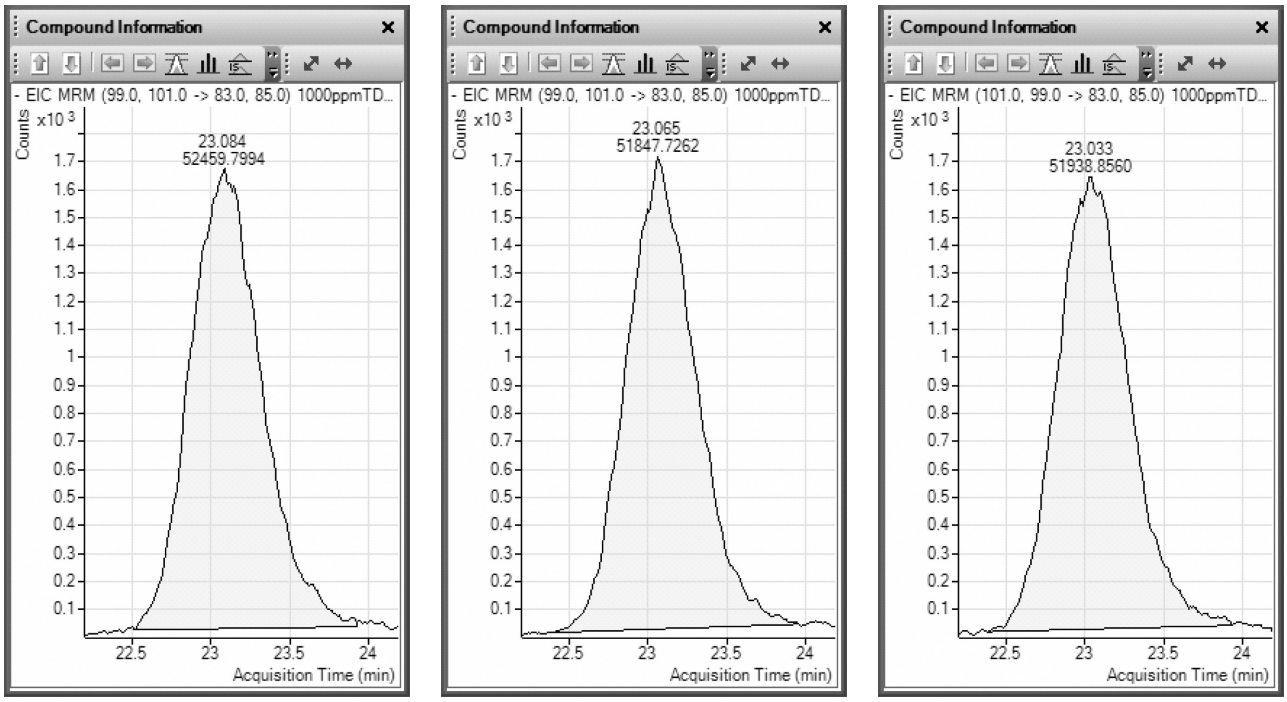

Figure 9. Peak area reproducibility for three injections of 1 ppb perchlorate in 1,000 ppm salt water is 0.63% RSD.

Conclusions

The analysis of perchlorate in water using a Metrohm Ion Chromatography system interfaced to an Agilent 6410 QQQ Mass Spectrometer is carried out on several calibration standards in reagent water and at various concentrations in the presence of TDS consisting of chloride, carbonate, and sulfate and also in the presence of reagent water. A linearity coefficient of $R^2 > 0.999$ is established for perchlorate standards ranging in concentration from 0.5 to 25 ppb. Linearity for the QQQ mass spectrometer is $R^2 > 0.998$ over the range of 0.01 to 10 ppb. Reproducibility among seven replicate injections of standards is also excellent for the QQQ, with seven replicates at the 0.1 ppb level resulting in a peak area RSD of only 5.33%.

The confirmation of perchlorate by QQQ mass spectrometry is also done by using qualifier ion ratios.

Perchlorate at the 3,000 ppm TDS concentration shows excellent reproducibility among seven replicate injections of 1 ppb perchlorate with an RSD of only 0.2% when using the IC conductivity detector. However, in negative ion electrospray ionization mode, the QQQ mass spectrometer shows a 41% RSD at the same level, even though all peaks have a peak-to-peak signal-to-noise ratio of at least 25:1.

When using the QQQ mass spectrometer to analyze perchlorate in the presence of salt water, the reproducibility is very good. For example, at 1 ppb perchlorate in 1,000 ppm salt water, the peak area reproducibility among three injections is only 0.63% RSD.

Acknowledgements

M. Johnson gratefully acknowledges the support of David Neleigh, Rick McMillin, and Dr. Melvin Ritter of EPA Region 6.

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For more details concerning the ion chromatography portion of this application, please contact Jay Gandhi at Metrohm-Peak, LLC.

For more details concerning the mass spectrometry portion of this application, please contact Sheher Mohsin at Agilent Technologies, Inc.

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Printed in the USA
February 4, 2008
5989-7907EN

Agilent Technologies

High-Speed Environmental Analysis Using the Agilent 7500cx with Integrated Sample Introduction System – Discrete Sampling (ISIS–DS)

Application Note

Environmental

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Abstract

Agilent has further improved the sample throughput capabilities of its 7500cx ICP-MS with Octopole Reaction System (ORS) using a newly configured Integrated Sample Introduction System–Discrete Sampling (ISIS-DS) accessory, and helium collision mode. Employing this new methodology, a complete suite of 30 or more elements can be analyzed in compliance with USEPA criteria (spectrum mode, three replicates, and sub-ppb MDLs) in approximately 75 seconds, sample to sample, with excellent removal of polyatomic interferences. Performance data showing stability, interference control, accuracy, precision, and washout are presented. The new system is applicable to labs requiring extremely high sample throughput and with its low sample consumption of ~2.2 mL/sample, for applications where sample volume is limited.

Agilent Technologies

Introduction

The Agilent Integrated Sample Introduction System (ISIS) has always permitted the highest sample throughput, using either high-speed uptake with constant flow nebulization or discrete sampling using a six-port valve and sample loop with time-resolved acquisition. In keeping with Agilent's continual focus on product enhancement, ISIS Discrete Sampling (ISIS-DS) has been reconfigured to further improve productivity. The resulting enhanced ISIS-DS sampling mode takes advantage of the ability of the 7500cx ICP-MS to analyze environmental samples using a single collision cell mode (He mode). This new mode of operation permits USEPA-compliant analysis (spectrum mode, three replicates, and sub-ppb MDLs) of a complete suite of 30 or more elements in approximately 75 seconds, sample to sample.

ISIS Configuration

Figure 1 shows the ISIS configuration used. It is a typical discrete sampling configuration with a couple of important modifications. Pump 1 (P1) is the large ISIS sample uptake peristaltic pump. Pump 2 (P2) is the standard 7500 nebulizer pump.

The ISIS uptake pump (P1), which is located downstream of the valve, draws the sample from the autosampler into the sample loop. As a result, the sample loaded in the sample loop is never exposed to peristaltic pump tubing, thereby eliminating a common source of contamination and carryover. This high-speed, high-capacity peristaltic pump is capable of rinsing and filling the sample loop in approximately 10 seconds when using the Cetac ASX-520 autosampler with the wide-bore 0.8 mm id probe. The other modification is the addition of the tee joint between the valve and nebulizer to allow the use of online internal standard addition. By minimizing both the length and diameter of the tubing between the valve and nebulizer, the time from rotation of the valve (sample injection) to the realization of a constant analyte signal is less than 15 seconds. A 300-µL loop is sufficient to allow more than 30 seconds of continuous spectrum mode acquisition. Larger loop sizes can be used to achieve any duration of acquisition required. After acquisition has completed, the valve returns to the load position, flushing any remaining sample to waste and rinsing the nebulizer and spray chamber with clean rinse solution. At this point, approximately 15 seconds is required for the signal to return to baseline in preparation for the next analysis (Figure 2).

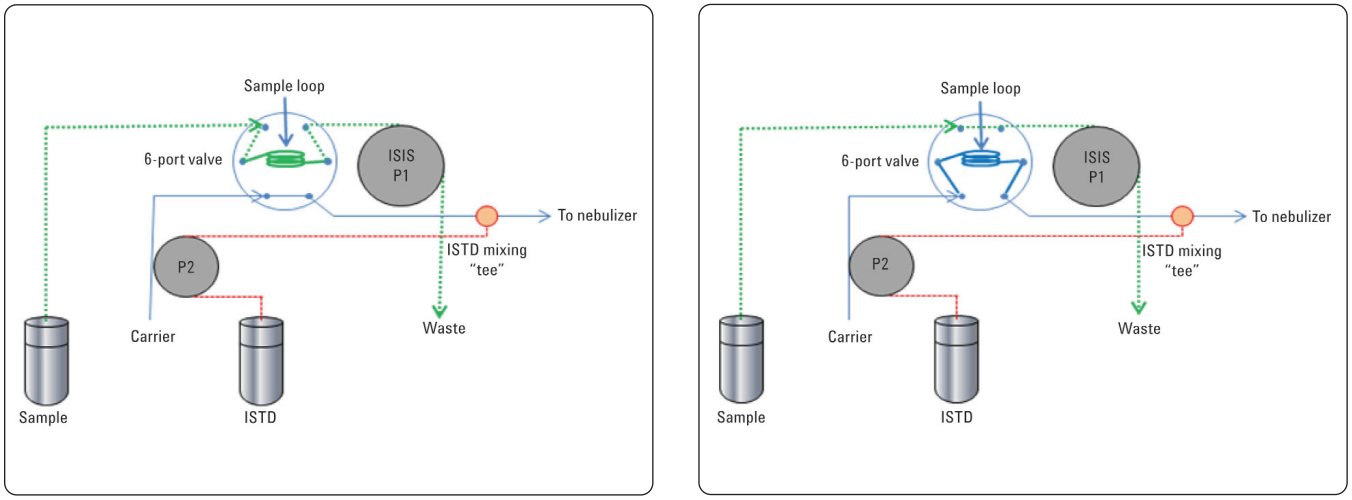

Figure 1. ISIS-DS sampling with online internal standard configuration. Valve in "load" position on left and in "inject" position on right.

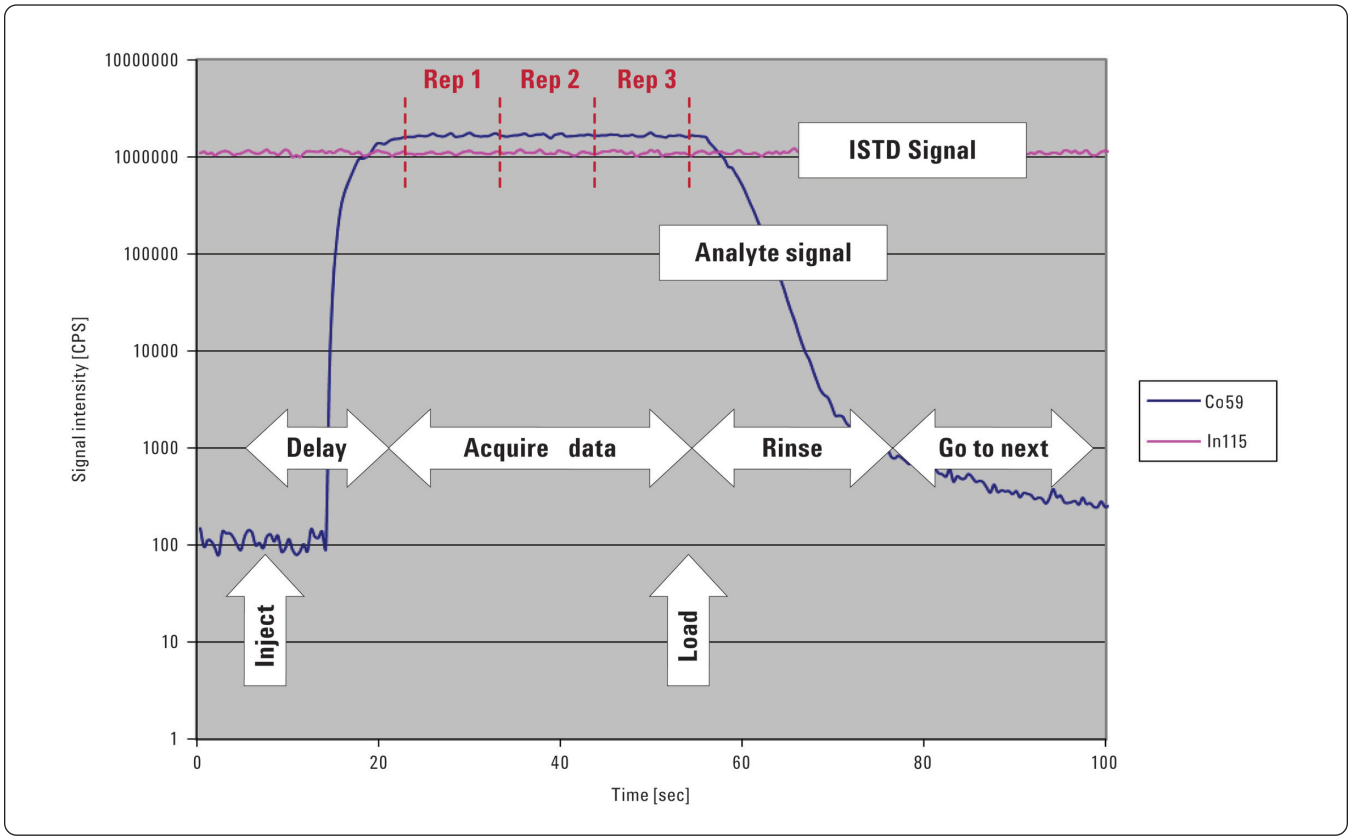

Figure 2. Analyte and internal standard profile during discrete sampling analysis (log scale for signal intensity). Time 0 – autosampler probe enters the sample and loop is loaded. Inject at 10 seconds, 15 seconds acquisition delay, 30 seconds acquisition, 10 seconds rinse, 10 seconds overhead. Total time is approximately 75 seconds.

Experimental

A sequence of 216 samples was analyzed in 4 hours 29 minutes using the new ISIS-DS sampling configuration depicted in Figure 1 and the acquisition conditions listed in Table 1. The 7500cx was operated in a single cell mode (He collision mode) resulting in both excellent removal of polyatomic interferences and very fast acquisitions since no cell gas switching or stabilization was required[1]. The sequence consisted of a single initial calibration at 0.1, 1, 10, and 100 ppb for all elements, followed by repeated (n = 26) analyses of a block of samples consisting of:

- 50 ppb calibration check (CCV)
- NIST 1643e water
- CCB (blank)
- USEPA Interference Check Solution – A (ICS-A)
- Blank
- USEPA ICS-AB (spiked with all analytes at 100 ppb to monitor carryover)
- Blank
- Blank

[1] This is a key benefit over reaction cell ICP-MS instruments that have to operate in multiple cell modes to cover all analytes. While it is possible to use multiple cell modes with discrete sampling, the resulting acquisition time is significantly lengthened, minimizing the benefits in terms of both run time and matrix exposure. If multiple cell modes are employed using the Agilent Octopole Reaction System, the small cell volume and very rapid gas switching reduce the cost in time and matrix exposure.

Table 1. ISIS/7500cx ICP-MS Acquisition Conditions for Spectrum Mode Discrete Sampling Analysis

Plasma	Robust mode – 1550 watts
Nebulizer	Glass concentric (standard)
Number of elements (including internal standards)	31
ORS mode	Helium - 4 mL/min (single mode)
Integration time per point	0.1 seconds (all elements)
Points per peak	1
Replicates	3
Total acquisition time (3 replicates)	29 seconds
Loop volume	300 µL
Loop rinse and fill time	10 seconds
Acquisition delay (after valve rotation to inject)	15 seconds
Steady state signal time (before valve rotation to fill again)	30 seconds

Results

Total Run Time and Sample Consumption

The resulting run-to-run time was measured at approximately 75 seconds per sample. Total sample consumption was determined by weighing each sample before and after analysis and was calculated to be 2.2 mL per sample per analysis. The method thus lends itself to samples in which the volume available for analysis is limited, and because small amounts are used, waste disposal costs are reduced. The small sample consumption also permits samples to be automatically reanalyzed by intelligent sequencing if needed from a 10-mL autosampler vial while allowing the ASX-520 to be configured for the maximum possible number of samples.

Stability

Long-term stability was monitored using internal standards. The abstracted internal standard data are illustrated in Figure 3, and show no downward drift, even after repeated (52 total) injections of ICS-A and ICS-AB. Only ^{6}Li demonstrated matrix suppression greater than 10% in the highest matrix samples, otherwise internal standard recoveries were within ~ ± 10% for the entire sequence. Calibration stability was monitored by measuring a 50-ppb CCV once in each 8-sample block (Figure 4). USEPA limits for CCV recovery are ± 10%. No CCV failures occurred; in fact, nearly all CCV recoveries were within ± 5% for the entire sequence.

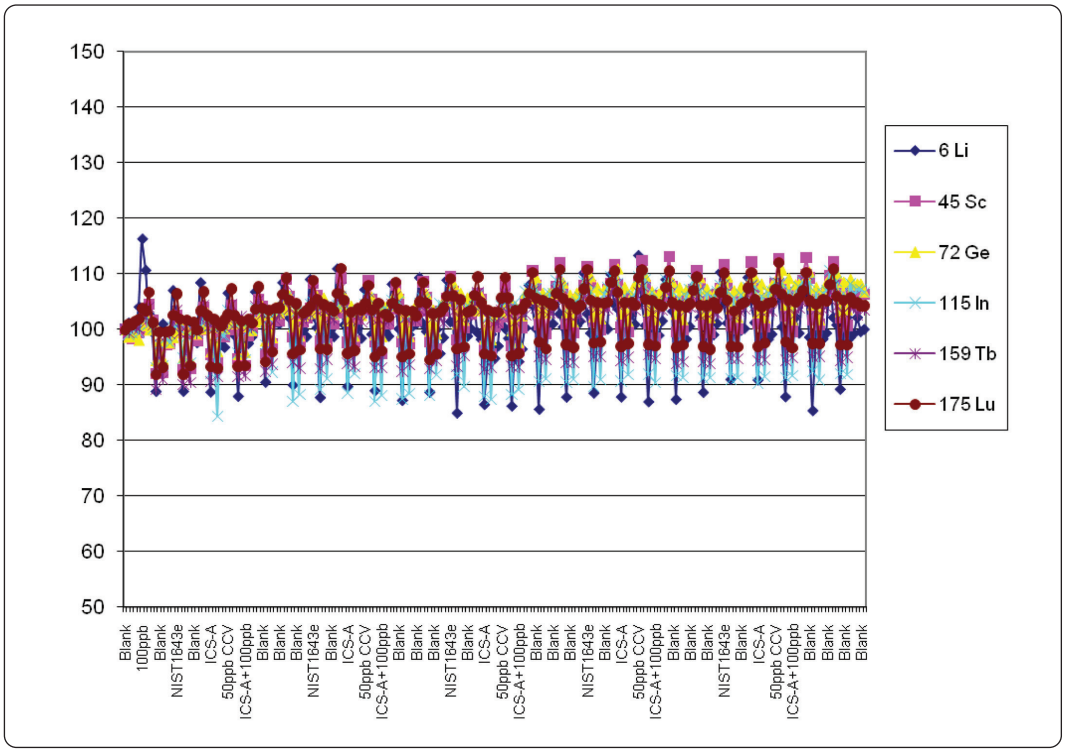

Figure 3. Internal standard recoveries compared to calibration blank for all 216 samples.

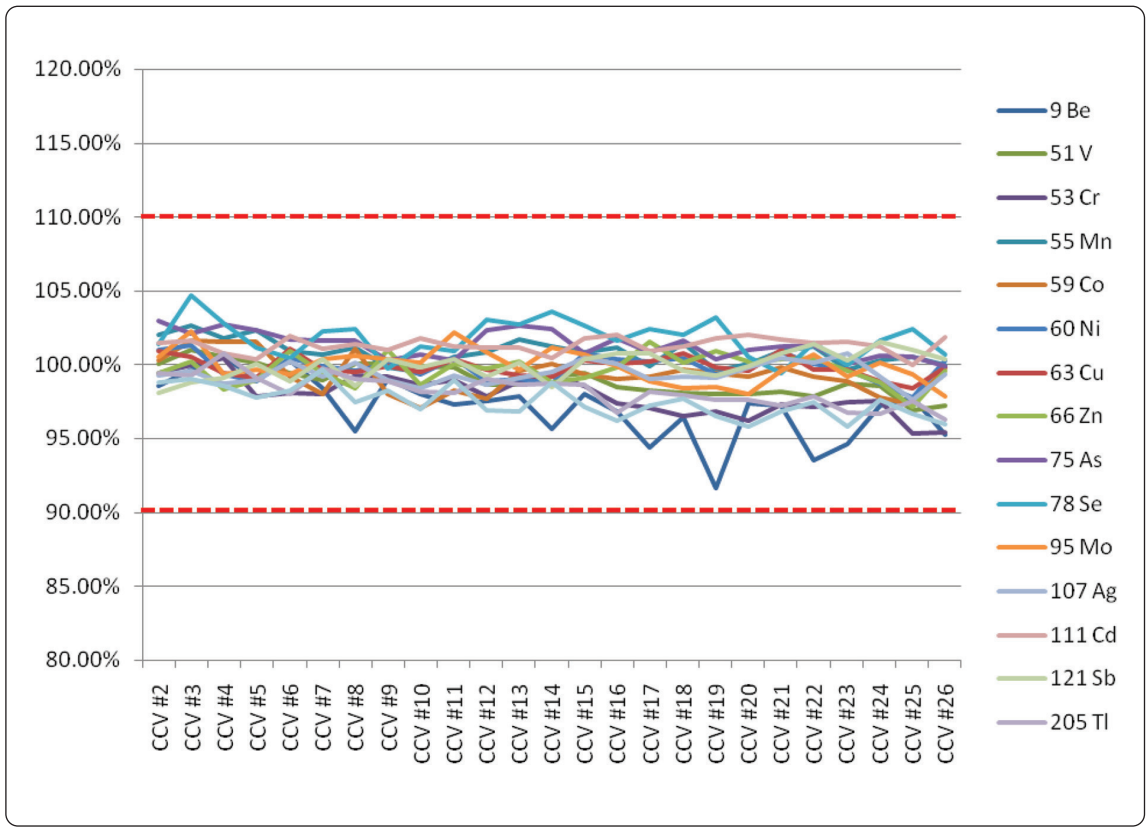

Figure 4. CCV recoveries (50 ppb) for entire sequence. USEPA limits for CCV recoveries in both Method 200.8 and 6020 are ± 10% (shown in red).

Accuracy

Long-term accuracy and precision were also determined through repeated analysis of NIST 1643e Certified Reference Water (n = 26). Results are tabulated in Table 2, showing recoveries within ± 10% or better of certified values and relative standard deviations near 1% for most elements. Be and Se had higher %RSDs due to the short integration times used and slightly lower count rates for these elements in He mode. Longer integration times can be used if higher counts or better precision are required for these elements. When ultimate DLs for Se are required (low ppt), H_2 mode is recommended. Due to the fast switching time of the ORS, Se can be measured in H_2 mode with only ~30 seconds added to the sample to sample run time.

Table 2. Precision (%RSD) and Mean Recovery of NIST 1643e Water for 26 Separate Analyses

Mass/ element	Mean measured value (µg/L)	RSD (%)	Certified value (µg/L)	Mean recovery (%)
9 Be	13.8	2.5	14.0	101.0
23 Na	22689.2	2.0	20740.0	109.4
24 Mg	7300.3	2.1	8037.0	90.8
27 Al	142.3	3.3	141.8	100.4
39 K	1837.8	1.1	2034.0	90.4
43 Ca	32170.1	0.7	32300.0	99.6
51 V	37.8	1.1	37.9	99.8
53 Cr	19.2	1.7	20.4	93.9
55 Mn	38.0	0.9	39.0	97.6
56 Fe	98.1	3.9	98.1	100.0
59 Co	28.8	0.7	27.1	106.4
60 Ni	59.2	0.8	62.4	94.9
63 Cu	23.2	0.8	22.8	101.9
66 Zn	70.0	0.5	78.5	89.2
75 As	54.3	0.9	60.5	89.8
78 Se	10.0	3.4	12.0	83.2
95 Mo	121.7	1.1	121.4	100.3
107 Ag	1.1	1.4	1.1	101.1
111 Cd	6.2	0.8	6.6	94.3
121 Sb	59.5	0.9	58.3	102.0
205 Tl	7.4	0.8	7.4	100.0
208 Pb	19.6	0.9	19.6	99.7

Table 3. Washout Performance (Mean value of 26 ICS-AB spikes [100 ppb], each immediately followed by two consecutive blanks. Percent reduction calculated as 1-([mean Blank]/[mean ICS-AB]) in percentage.)

Mass/ element	ICS-AB spike Mean	Blank 1 Mean	Percent reduction Mean	Blank 2 Mean	Percent reduction Mean
9 Be	94.9315	0.0199	99.979	0.0097	99.990
23 Na	96707.6923	19.6032	99.980	13.5090	99.986
24 Mg	79238.8462	14.2332	99.982	9.8046	99.988
27 Al	75758.0769	11.7913	99.984	7.8004	99.990
39 K	82694.2308	17.6441	99.979	13.2657	99.984
43 Ca	9092.8462	1.4105	99.984	0.9697	99.989
53 Cr	95.7327	0.0419	99.956	0.0441	99.954
55 Mn	94.8977	0.0132	99.986	0.0069	99.993
56 Fe	77021.9231	12.5122	99.984	8.0837	99.990
57 Fe	75266.5385	12.0863	99.984	7.7304	99.990
59 Co	106.8577	0.0140	99.987	0.0092	99.991
60 Ni	101.3692	–0.0161	100.016	–0.0129	100.013
63 Cu	98.5700	0.0163	99.984	0.0043	99.996
66 Zn	99.9350	0.0055	99.994	0.0011	99.999
75 As	95.8615	0.0290	99.970	0.0171	99.982
78 Se	94.0162	0.0841	99.911	0.0428	99.955
95 Mo	1862.3077	1.4281	99.923	0.6278	99.966
107 Ag	96.8769	0.0181	99.981	0.0098	99.990
111 Cd	104.0538	0.0134	99.987	0.0084	99.992
121 Sb	109.1346	0.2629	99.759	0.1077	99.901
205 Tl	93.4731	0.0339	99.964	0.0131	99.986
208 Pb	92.4704	–0.0175	100.019	–0.0241	100.026

Washout

Washout is always a concern in high sample throughput applications, particularly when analyzing high-matrix, variable samples. In order to evaluate the washout for each element, two sequential blank samples were measured immediately after each spiked ICS-AB sample. The spiked ICS-AB contained 100 ppb of all calibrated elements, plus very high concentrations of Na, Mg, Al, K, and Fe. Memory effects were determined by measuring the blank immediately following the ICS-AB. Any subsequent carryover was measured in the second blank (Table 2). In all cases, greater than 3 orders of magnitude reduction (> 99.9%) was achieved before the first blank, even for the high-concentration matrix elements. The second blank showed nearly no additional reduction, indicating that essentially complete washout was achieved during the configured sample uptake and rinse-out steps of the analysis. Even "sticky" elements, such as Mo, Sb, and Tl, demonstrated the same high degree of washout. This level of washout is comparable to or better than standard peristaltic pumped systems using much longer rinse times.

Conclusions

The results of this simple experiment illustrate that discrete sampling in spectrum mode (as opposed to time-resolved mode) using the Agilent Integrated Sample Introduction System can achieve extremely high sample throughput for typical environmental analyses using USEPA criteria. These data highlight that this novel method, using ISIS, easily exceeds the demanding USEPA requirements for stability, interference control, accuracy, precision, and washout.

The ISIS-DS system offers several advantages over other discrete sampling systems: Full integration into the ICP-MS mainframe, fully integrated software, compatibility with the industry standard ASX 520 autosampler, no vacuum pump and associated pump valve to wear and replace, very low sample consumption (~2.2 mL/sample), and the flexibility to use the ISIS for other supported sample-introduction tasks, such as constant-flow nebulization, autodilution, or hydride generation.

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Printed in the USA
March 11, 2009
5990-3678EN

Agilent Technologies

Rapid Analysis of High-Matrix Environmental Samples Using the Agilent 7500cx ICP-MS

Application

Environmental

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Abstract

The new Agilent 7500cx with Octopole Reaction System (ORS) is capable of analyzing most typical environmental samples using only one mode of analysis: helium mode. For the first time, it is possible to analyze an entire environmental suite of elements, including Hg and the major elements such as Na, K, Ca, Mg, Al, and Fe, in less than 2.5 minutes per sample, under conditions that remove or reduce practically all matrix-based interferences.

Introduction

Contract analytical laboratories, particularly those focused on the analysis of environmental samples, face challenges that are significantly different from those of research institutes in government and academia. The samples are typically more numerous, unknown and highly variable in composition, and generally require rapid turnaround. Despite these challenges, the environmental laboratory must ensure that the data produced is of high quality and is supported by extensive analytical quality control (AQC) in order to remain productive and profitable. The recent great improvement in productivity (and, consequently, profitability) of the metals section in contract environmental labs is largely due to the increasing use of ICP-MS, with its rapid multi-element capability, wide elemental coverage and dynamic range, low detection limits, and ease of use. With the advent of collision/reaction cell (CRC) ICP-MS, the ability of the technique to eliminate or significantly reduce the effects of polyatomic interferences in complex matrices has further improved its usability for many applications. However, until recently, the improved accuracy delivered by CRC ICP-MS came at a significant cost to productivity. Typical CRC ICP-MS systems must use reactive cell gases to specifically target known interferences, which requires time-consuming, matrix-specific method development. Furthermore, multiple cell conditions are necessary depending on the matrix and analyte list, which can add minutes to each sample analysis.

Agilent pioneered the use of helium (collision) mode coupled with kinetic energy discrimination (KED) on the 7500c instrument, allowing most polyatomic interferences to be removed using a single set of cell conditions. Subsequent advances in instrument design and in the understanding of the collision mechanisms involved have resulted in the 7500cx, an ICP-MS capable of analyzing typical environmental samples using only helium mode. By eliminating the need for both hydrogen (reaction) mode[1] and no-gas mode, sample throughput is significantly improved and routine operation is greatly simplified. Coupled with improvements in uptake and rinse-out speed through various hardware and software innovations, it is now possible to analyze an entire environmental suite of elements, including Hg and the major elements such as Na, K, Ca, Mg, Al, and Fe, in less than 2.5 min-

[1] Trace level selenium analysis (i.e., below 0.2 ng/mL) requires the use of hydrogen mode to eliminate the Ar_2^+ interferences on the preferred isotopes at mass 78 and 80.

utes per sample, under conditions that remove or reduce all matrix-based interferences. This application documents the performance of the 7500cx for the high-throughput analysis of long sequences of typical high-matrix environmental samples.

Instrumentation

A standard Agilent 7500cx ICP-MS with a glass concentric nebulizer was used for all analyses. The instrument was tuned for standard robust plasma conditions (Table 1) and the ORS was operated in helium mode only. This means that all elements were measured under identical helium mode collision conditions and no mode switching was necessary. Furthermore, the helium mode conditions used are generic and do not have to be set up or modified for specific sample matrices. Method parameters are shown in Table 1.

Table 1. Instrument Tune and Acquisition Conditions Used

Instrument	**7500cx**
Sampler	Ni (standard)
Skimmer	Ni (standard)
Nebulizer	MicroMist (standard)
Plasma torch	Quartz, 2.5 mm (standard)
Integration Time	
Li, Be, As, ^{78}Se, ^{111}Cd	0.3 sec x 1 point
All other	0.1 sec x 1 point
Tune Parameters	
RF power	1550 W
Sample depth	8.5 mm
Carrier gas	0.80 L/min
Makeup gas	0.23 L/min
Extract 1	0V
Extract 2	–120 V
Energy discrimination	2 V
Reaction gas	He 5.0 mL/min
CeO/Ce	0.52%
Ce^{++}/Ce	2.06%

Sensitivity in Helium Mode

Real sensitivity, as determined by practical limits of detection (LOD), is a function of signal to background (high signal, low background) and the precision of the background measurement. The greatest analytical benefit in using helium mode will be realized for analytes that suffer from polyatomic ion overlaps (essentially every isotope of every element from mass 45 to 82). However, it is important to assess the possible degradation in performance for elements that do not suffer from polyatomic interference where helium mode is used for all analytes. Poorer signal to noise for noninterfered elements is a possibility, as any ICP-MS operating with the cell pressurized (in collision or reaction mode) will suffer some loss of signal for low-mass elements when compared with no-gas mode. This signal loss occurs as a result of collisions between analyte ions and gas molecules in the cell. However, in most cases, the reduction in background more than compensates for the loss of signal, so real detection limits for noninterfered elements are not significantly impacted. In order to measure actual sensitivity under helium conditions, signal-to-background ratios and 3 sigma instrument detection limits (IDLs) were determined in helium mode for all commonly measured elements[2]. For nearly all elements, IDLs are in the low- to sub-ppt range. More important for environmental applications are the background equivalent concentrations (BECs) and IDLs for those elements that typically suffer from interferences in high-matrix samples. Table 2 compares the BECs, IDLs, and equivalent concentration of interferences for several critical elements in no-gas and helium mode in USEPA Interference Check Solution (ICS-A[3] - see Table 3 for composition). Note the significant reduction in all three measurements for all isotopes, showing that helium mode is capable of simultaneously removing interferences on multiple elements (and even multiple isotopes) in complex matrices.

Interference Removal in Helium Mode

USEPA Method 6020 specifies an interference check sample (ICS-A) designed specifically to monitor the effect of polyatomic interferences resulting from high concentrations of common matrix components. Traditionally, these interferences have been compensated for through the use of mathematical correction equations. However, experienced ICP-MS users know that in the case of multiple interferences on a single analyte or interferences from uncommon matrix components, mathematical correction is unreliable. Additionally, many polyatomic interferences cannot be corrected mathematically because of the lack of a free mass at which to monitor the interferent. A common example is the interference from $^{40}Ar^{23}Na$ on ^{63}Cu. This is a significant interference in saline matrices, but because Na is monoisotopic (at mass 23), it is not possible to derive a mathematical cor-

[2] Performance characteristics of the Agilent 7500cx ICP-MS. Agilent application note 5989-6663EN.

[3] ICS-A is the USEPA-specified "Interference Check Solution" designed to alert the user to the possibility of isobaric, doubly charged, polyatomic and memory interferences in high-matrix samples. ICS-AB is the same high-matrix solution spiked with 100 to 200 ppb of each analyte element in order to measure the effects of high matrix on analyte recovery. In this work, the target analytes were spiked much lower (20 ppb, ICS-AB Modified) in order to test the effectiveness of interference removal at trace analyte levels.

Table 2. Comparison of No-Gas Mode and Helium Mode on BEC, IDL, and Measured Concentration in ICS-A Solution (Note the much higher measured concentration values obtained in no-gas mode due to polyatomic interferences. Se 77 and 78 values do not agree in no-gas mode, and V gives a negative concentration reading.)

	No Gas Mode			Helium Mode		
	BEC (ppt)	IDL (ppt)	Measured conc (ppb)	BEC (ppt)	IDL (ppt)	Measured conc (ppb)
^{51}V	1461	143	–1.35	107	45	0.13
^{75}As	1945	186	3.23	120	149	0.70
^{77}Se	9973	540	12.31	401	204	0.50
^{78}Se	9738	313	3.84	342	162	0.43

rection based on the abundance of a second ArNa polyatomic ion. This has typically led ICP-MS users to select the alternative (and much lower abundance) Cu isotope at mass 65. However, ^{65}Cu suffers from a much higher level of S-based interferences (S_2 and SO_2) than ^{63}Cu as well as a significant $^{25}Mg^{40}Ar$ interference, so switching to ^{65}Cu to avoid the ArNa overlap can result in compromised data quality in many sample types.

Cr is another example of an element that commonly suffers from polyatomic interferences ($^{40}Ar^{12}C$, $^{35}Cl^{16}OH$, $^{36}Ar^{16}O$, and $^{38}Ar^{14}N$ on ^{52}Cr, and $^{37}Cl^{16}O$, $^{40}Ar^{13}C$, and $^{36}Ar^{16}OH$ on ^{53}Cr), which cannot be reliably corrected mathematically due to the lack of a free reference mass. For these reasons, helium mode, with its ability to remove all polyatomic interferences regardless of sample matrix composition, is vastly more reliable and more widely applicable than the use of mathematical corrections[4].

Figure 1 shows overlaid spectra for USEPA ICS-A, measured from mass 73 to 82 in no-gas, helium, and hydrogen modes. The spectra have been normalized on the bromine peak at *m/z* 79 to compensate for differences in sensitivity between modes. The differences in spectral complexity are clear, with almost every mass showing some level of interference in no-gas mode, while helium mode has reduced all of these interferences to background levels.

Table 3. Composition of ICS-A and ICS-AB (modified)[3] (ICS-AB was prepared by spiking ICS-A with a 20-ppb standard containing all analyte elements of interest.)

Component	ICS-A concentration (mg/L)	ICS-AB concentration (mg/L)
Al	100.0	100.0
Ca	300.0	300.0
Fe	250.0	250.0
Mg	100.0	100.0
Na	250.0	250.0
P	100.0	100.0
K	100.0	100.0
S	100.0	100.0
C	200.0	200.0
Cl	2000.0	2000.0
Mo	2.0	2.0
Ti	2.0	2.0
As	0.0	0.02
Cd	0.0	0.02
Cr	0.0	0.02
Co	0.0	0.02
Cu	0.0	0.02
Mn	0.0	0.02
Hg	0.0	0.02
Ni	0.0	0.02
Se	0.0	0.02
Ag	0.0	0.02
V	0.0	0.02
Zn	0.0	0.02

[4] Note that because helium mode works only on polyatomic interferences, it is not capable of removing elemental isobaric interferences (e.g., ^{40}Ar on ^{40}Ca) or doubly charged interferences. Fortunately, these types of interferences are rare, and simple methods are available to avoid them, such as choosing an alternative analyte isotope.

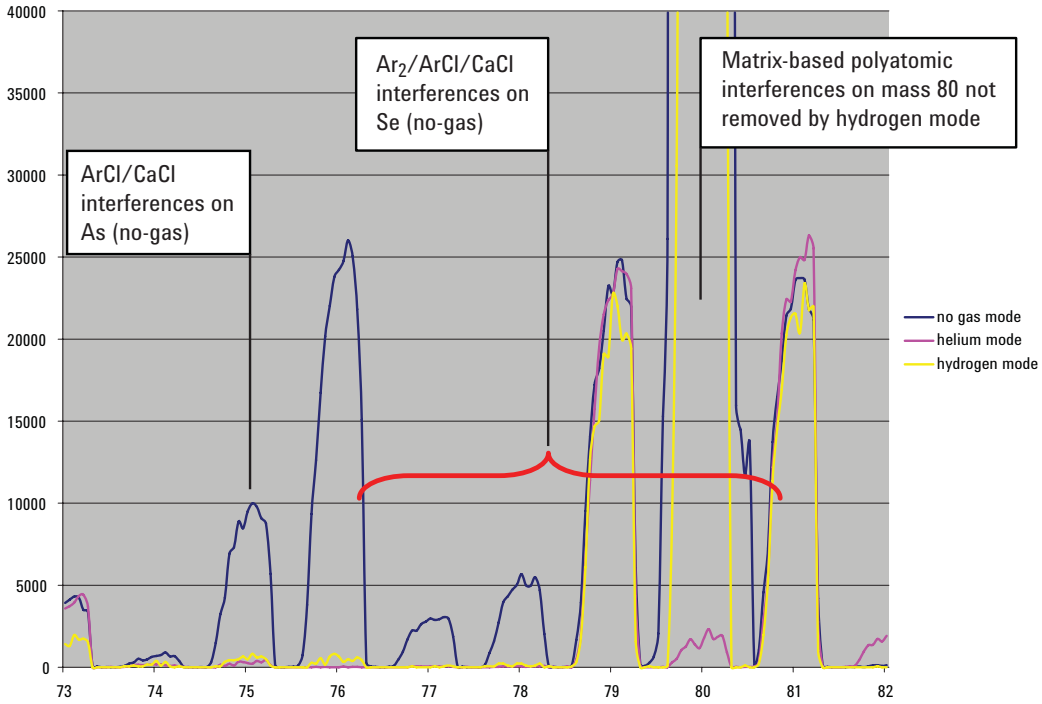

Figure 1. Overlaid spectra of ICS-A obtained in no-gas, hydrogen, and helium modes over the range from mass 73 to 82 to show interferences on As and Se. Spectra normalized on Br signal at *m/z* 79. Note that while H_2 mode is effective for the removal of the Ar_2^+ overlap at mass 80 (main isotope of Se) in simple matrices, it is not effective for several other interferences at this mass in ICS-A (ArCa, Ca_2, S_2O, SO_3, etc.). Ar_2^+ is completely removed by H_2 mode at *m/z* 78, which is therefore the preferred isotope.

Experimental

A 12-hour, 300-sample sequence, representing a typical environmental batch, was analyzed after a single initial calibration consisting of a blank and standards at 1, 10, 50, and 100 ppb (Figure 2). The sequence consisted of repeated blocks of 10 samples, including NIST 1640 standard reference water, ICS-A, ICS-AB, and two commercially available high total dissolved solids (TDS) mineral water samples. After each block, blank check and calibration check samples (USEPA sample types continuing calibration blank [CCB] and continuing calibration verification [CCV]) were automatically inserted to check for memory effects and calibration accuracy. No recalibrations were performed during the 12-hour run.

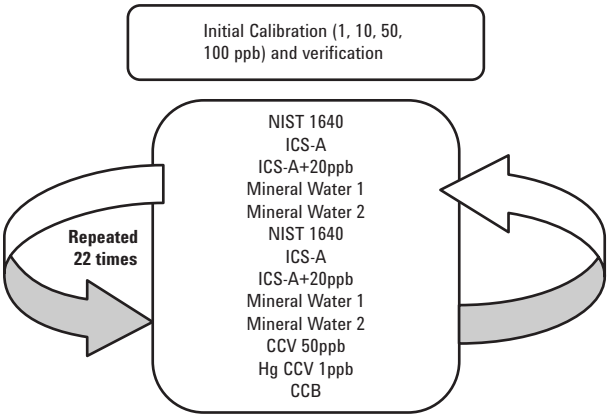

Figure 2. Schematic of analytical sequence. 300 sample analyses were performed, including an initial calibration and 22 repeated analyses of a block of samples containing 10 samples followed by 2 CCV samples and a CCB.

Long-Term Stability

Analysis of CCV Samples

As a check on calibration stability for all analyte elements, a CCV standard (50 ppb for all analytes except Hg – 1 ppb) was analyzed repeatedly throughout the sequence. USEPA Methods 200.8 and 6020 require that the measured CCV values fall within ± 10% of the true value in order to report samples. Figure 3 shows the results of 25 measurements of the CCV sample over the 12-hour sequence, indicating no failures throughout the run, despite the fact that no recalibrations were performed after the initial calibration.

Analysis of High-Matrix Samples

In order to simulate difficult, high-matrix sample types, ICS-A and ICS-AB were each analyzed twice in each 10-sample block (giving a total of 48 replicate analyses of each), in addition to the two high-TDS mineral water samples. ICS-A and ICS-AB were selected because they are well characterized and were specifically designed by the USEPA to challenge the ICP-MS's ability to handle high-matrix samples in terms of controlling interferences, managing ionization suppression, eliminating memory effects, and maintaining long-term stability. Long-term precision and accuracy for trace-level measurement in high-matrix samples can be determined by examining the results of repeated analysis of ICS-AB. Recoveries ranged from 97 to 104% with %RSDs ranging from less than 1% to approximately 5% over the 12-hour sequence (Figure 4).

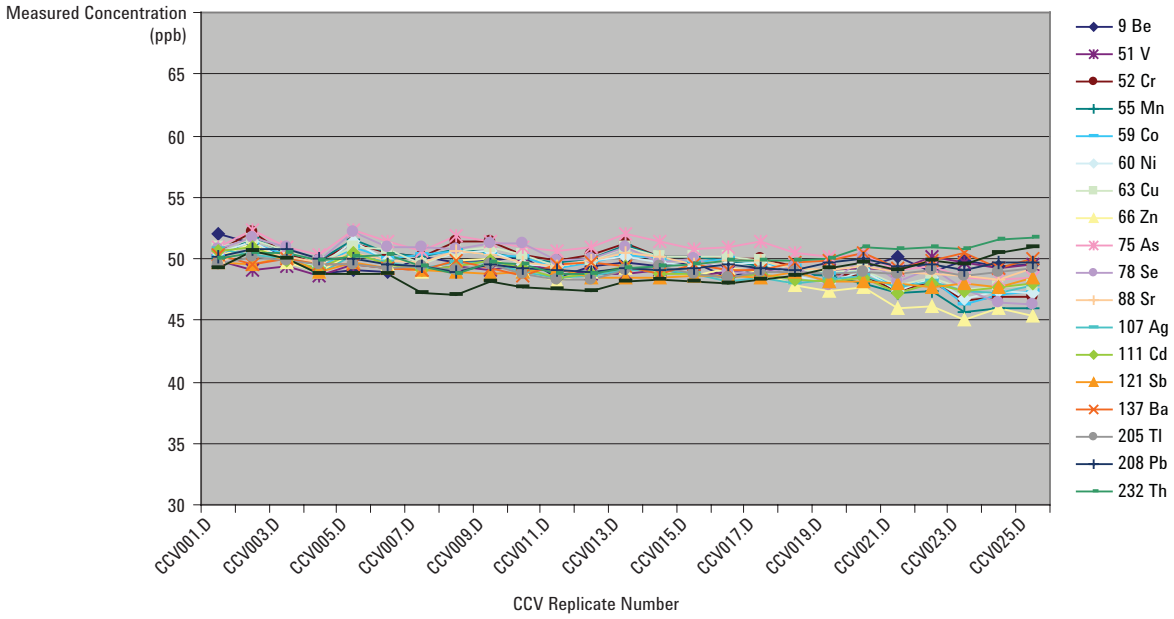

Figure 3. Measured values of 50 ppb CCV samples (n = 25) over the course of the sequence. USEPA criteria are ± 10% (i.e., 45 to 55 ppb).

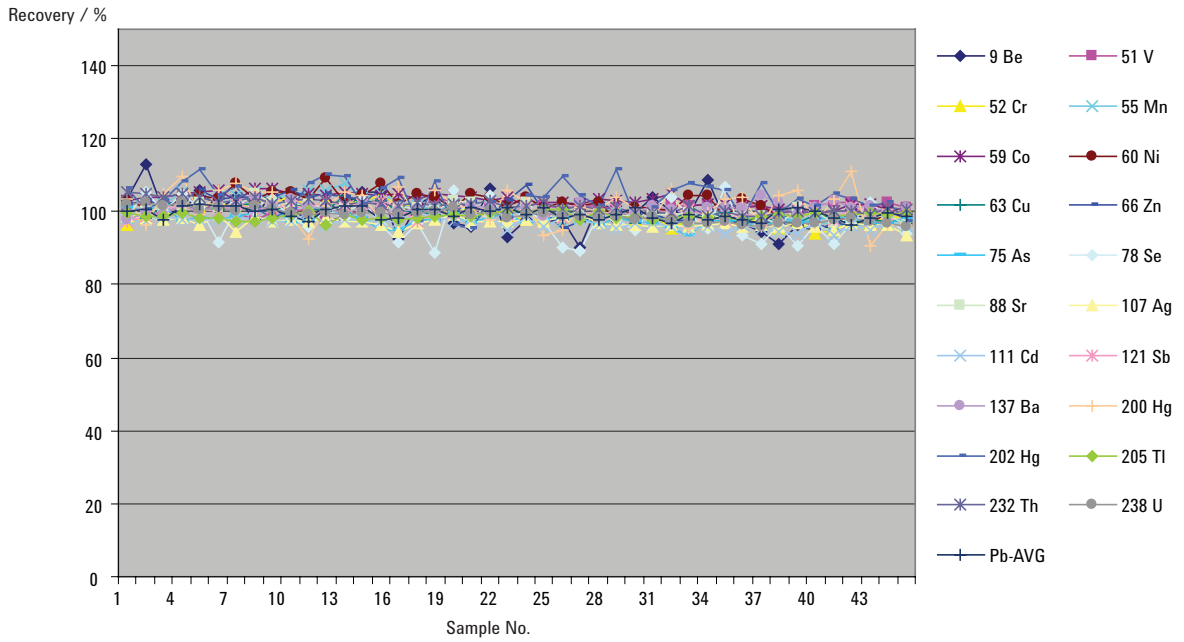

Figure 4. Spike recovery (20 ppb, 1 ppb Hg) for ICS-AB over 12 hours (n = 44).

Analysis of Certified Reference Material

NIST 1640 certified reference water was analyzed repeatedly (n = 44) as part of the sequence. Results are shown in Table 4.

Table 4. Results of Repeated Analysis of NIST 1640 (n = 44) Over a 12-Hour Sequence

Element	Mean (n = 44)	RSD (%)	Certified value (µg/L)	Recovery (%)
^{9}Be	32.36	4.72	34.94	92.6
^{27}Al	48.62	3.90	52.00	93.5
^{42}Ca	6652.25	2.59	7045	94.4
^{51}V	12.66	1.40	12.99	97.4
^{52}Cr	36.14	3.19	38.60	93.6
^{55}Mn	114.96	3.87	121.50	94.6
^{59}Co	19.64	2.27	20.28	96.8
^{60}Ni	26.76	2.86	27.40	97.7
^{63}Cu	84.95	2.16	85.20	99.7
^{66}Zn	52.64	2.66	53.20	99.0
^{75}As	25.28	1.52	26.67	94.8
^{78}Se	20.69	4.61	21.96	94.2
^{88}Sr	118.03	1.31	124.20	95.0
^{107}Ag	7.15	1.67	7.62	93.8
^{111}Cd	21.31	1.26	22.79	93.5
^{121}Sb	13.48	1.68	13.79	97.7
^{137}Ba	140.78	1.03	148.00	95.1
^{200}Hg	0.10	12.23	—	—
^{202}Hg	0.10	9.83	—	—
^{204}Pb	26.98	3.62	27.86	96.9
^{205}Tl	0.01	54.91	—	—
^{206}Pb	25.04	1.06	27.86	89.9
^{207}Pb	26.94	1.11	27.86	96.7
^{208}Pb	26.17	0.86	27.86	94.0
^{232}Th	0.05	45.36	—	—
^{238}U	0.73	2.90	—	—

Average Analysis Time

One of the major goals of using a single ORS mode is to improve productivity. To ensure that this end was met:

- Integration times were kept short, typically 0.1 second per point.
- A single point per mass was used.
- Intelligent and pre-emptive rinse functions were employed (minimizes wasted time in uptake and rinseout and ensures that carryover could not occur).

Figure 5 graphically shows the time savings possible. In a conventional CRC system, after sample uptake and initial stabilization, acquisition occurs in the first of several CRC modes, followed by cell evacuation, repressurization, and restabilization (top). The process continues until all necessary modes have been completed (typically 3). In the 7500cx helium mode (bottom), initial uptake and stabilization are the same. After that, helium mode acquisition can begin immediately, since no cell evacuation or repressurization is necessary, followed by rinse. Pre-emptive rinsing begins up to 60 seconds before acquisition has finished, and intelligent rinse monitors rinseout, ensuring complete washout without any wasted time. The total acquisition time for all analytes and internal standards was 9.7 seconds per replicate. Three replicates were acquired according to USEPA methods,

resulting in a total acquisition time of 29.2 seconds. Overall, the average run-to-run time based on 300 runs beginning at 4:44 p.m. and ending at 5:04 a.m. the following morning was 2.46 minutes per run. As the data in Table 4 illustrate, despite the short acquisition time, precision was not compromised and all data returned excellent %RSDs over the 12-hour period.

Conclusions

Since helium mode is universal, all interferences are removed without prior sample knowledge. Tuning is simplified and problems associated with reactive cell processes such as the creation of new interferences or loss of analyte or internal standard are avoided. Stability is not compromised since cell conditions are static and run times are markedly improved through the elimination of multiple cell conditions along with the associated stabilization times.

For many applications, particularly commercial analysis of high-matrix environmental samples, the use of helium mode offers significant benefits in productivity, data reliability, and ease of use.

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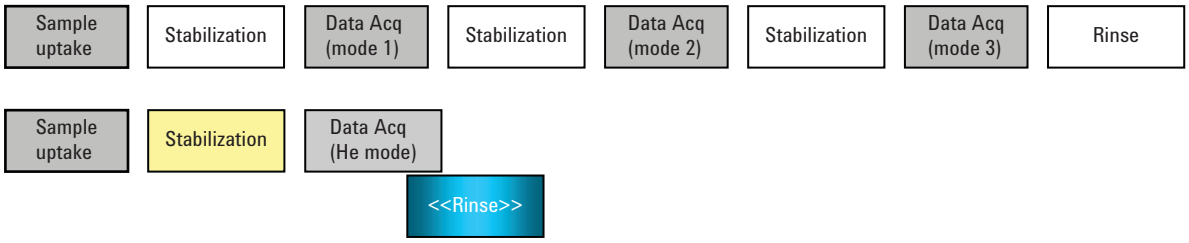

Figure 5. Typical multimode CRC operation (top), and 7500cx using helium mode and pre-emptive rinse software (bottom).

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Printed in the USA
September 20, 2007
5989-7297EN

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Determination of Organic and Inorganic Selenium Species Using HPLC-ICP-MS

Application

Environmental

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Abstract

A methodology based on coupling isocratic high-performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS) with optimized collision/reaction cell conditions has been developed for the simultaneous analysis of organic and inorganic selenium species in natural water samples. Selenium concentrations found in total and speciation analysis of a number of water samples showed good agreement. Because HPLC-ICP-MS coupling is easily automated, the method can be considered robust and applicable to the routine monitoring of selenium species in environmental and nutritional samples.

Introduction

In the last 20 years, there has been increasing interest in the determination of the different chemical forms in which an element can exist, that is, in the determination of its speciation. Indeed, knowledge of total concentrations of elements is not sufficient to assess their effects on human health or the environment. Among the elements of concern, there is a growing interest in selenium. Selenium is a very important element from an ecotoxicological point of view due to the narrow concentration range between its essential and toxic effects. Selenium compounds are distributed throughout the environment as a result of human activities (industrial and agricultural uses) and natural processes (weathering of minerals, erosion of soils, and volcanic activity). In waters, concentrations can vary from 2 ng/L to 1,900 µg/L depending on the system [1]. The natural cycle of selenium shows its existence in four oxidation states (-II, selenide; 0, elemental selenium; +IV, selenite; and +VI, selenate) and in a variety of inorganic and organic compounds. The organically bound Se(-II) compounds include seleno-amino acids and volatile forms (dimethylselenide and dimethyldiselenide), which are less toxic relative to other species and result from various detoxification pathways. The toxic dose of selenium as a function of its chemical form is shown in Table 1.

Table 1. Selected Selenium Compounds and Their Toxicity

Compound	Formula	Lethal dose–LD-50*	Ref.
Dimethylselenide (–II)	$(CH_3)_2Se$	1600 mg/kg (Int.)	[2]
Hydrogen selenide (–II)	H_2Se	0.02 mg/L (Resp.)	[3]
Trimethylselenonium (–II)	$(CH_3)_3Se^+$	49 mg/kg (Int.)	[3]
Selenocystine (–I)	$[HO_2CCH(NH_2)CH_2Se]_2$	35.8 mg/kg (Or.)	[4]
Selenomethionine (–II)	$CH_3Se(CH_2)_2CH(NH_2)CO_2H$	4.3 mg/kg (Int.)	[3]
Selenite (+IV)	SeO_3^{2-}	3.5 mg/kg (Int.)	[5]
Selenate (+VI)	SeO_4^{2-}	5.8 mg/kg (Int.)	[5]

*Lethal doses obtained on mice or rats by intraperitoneal (Int.), oral (Or.), or respiratory (Resp.) absorption.

A number of analytical procedures exist for the determination of selenium and its various species in samples from different environmental sources. Existing methods can be divided in three groups, depending on selenium concentration:

- Total selenium
- Selenite species
- Species including inorganic and organic forms of selenium

Various redox reactions are often used to determine selenite species. However, the series of required reagents and pretreatment steps increases the possibility of element loss and contamination. Speciation results can also be distorted as back-oxidation of selenite to selenate may occur during sample pretreatment. Moreover, selenite and selenate are distinguished by two separate analyses, which is not the case for individual organic selenium species that remain unidentified. Hence, methods able to separate and quantify different selenium species simultaneously, in a single analysis, are preferred and are becoming more widespread.

In this application, the coupling of high-performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS) is presented for selenium speciation analysis with emphasis on its application to natural water samples.

Instrumentation

A 7500ce ICP-MS from Agilent Technologies (Tokyo, Japan), equipped with an Octopole Reaction System (ORS) cell, was used for this study; see Table 2 for operating parameters. The sample introduction system consisted of a concentric nebulizer (Meinhard Associates, California, USA) and a Scott double-pass spray chamber cooled to 2 °C. Nickel sampler and skimmer cones were used.

Table 2. Instrumental Parameters for Agilent 7500ce ORS ICP-MS

Parameter	Value
RF power	1590 W
Ar plasma gas flow	15.0 L/min
Ar auxiliary gas flow	0.86 L/min
Ar nebulizer gas flow	1–1.1 L/min
Spray chamber temperature	2 °C
Integration time per isotope for speciation analysis	400 ms
m/z ratio monitored	77 to 82
Integration time per isotope for elemental analysis	100 ms

Chromatographic separation was carried out using the Agilent 1100 Series HPLC pump, equipped with an autosampler and variable volume sample loop. The analytical column was a Hamilton PRPX-100, 10 µm particle size, 25 cm length × 4.1 mm internal diameter (id). The chromatographic separation of selenocystine (SeCyst), selenomethionine (SeMet), selenite (SeIV), and selenate (SeVI) was adapted from Ge et. al. [6] and performed using a 5 mmol/L ammonium citrate buffer with pH adjusted to 5.2. Injection volume was fixed at 100 µL. Methanol (2% v/v) was added to the mobile phase to improve sensitivity [7]. The mobile phase was delivered at 1 mL/min isocratically. The HPLC-ICP-MS interface consisted simply of polyetheretherketone (PEEK) tubing.

Polyatomic Interference Removal

ICP-MS is the detector of choice for trace element analysis due to its high sensitivity and selectivity. It is also one of the most often used detection systems for total and speciation analyses of selenium. Nevertheless, selenium detection limits obtained with a conventional ICP-MS (quadrupole filter without collision/reaction cell system) are not sufficient when dealing with selenium determinations in natural waters. Difficulties in Se determination by ICP-MS are mainly due to its high first ioniza-

tion potential (9.75 eV) compared to argon (15.75 eV) and, as a consequence, its low ionization in an Ar plasma (around 33% [8]). Secondly, argon polyatomic interferences, especially $^{40}Ar^{40}Ar^{+}$ and $^{40}Ar^{38}Ar^{+}$ dimers, prevent selenium determination from its most abundant isotopes ^{80}Se (49.6% abundance) and ^{78}Se (23.8% abundance). Hence, the less interfered and less abundant ^{82}Se isotope (9.2% abundance) is generally monitored. The problem of argon-based polyatomic interferences can be solved with the use of ICP-MS systems equipped with a collision/reaction cell (CRC). A 10- to 20-fold improvement in total Se and speciation analysis detection limits was observed using the ORS cell of the Agilent 7500ce. Speciation analysis detection limits are below 15 ng/L based on monitoring ^{80}Se (see Table 3). Better detection limits were achieved for ^{80}Se compared to ^{78}Se because the 7500ce was optimized on ^{80}Se.

Table 3. Optimization of ORS Operating Conditions

Instrument	**Agilent 7500ce**	
Cell gases	5.5 mL/min H_2 0.5 mL/min He*	
Elemental Analysis		
	^{78}Se	^{80}Se
Detection limit (ng/L)	6	4
Repeatability (%)	2	2
HPLC Coupling		
	^{78}Se	^{80}Se
Detection limit (ng/L)	14–30	7–15
Repeatability (%)	2	2

*Addition of He is optional. Similar detection limits should be achievable without He.

The use of CRC technology allows efficient removal of argon-based interferences, resulting in improved ICP-MS detection power for selenium by permitting monitoring of its most abundant isotope, ^{80}Se. However, such improvements are mitigated, in some cases, by reaction cell induced interferences. Indeed, hydrogen, or impurities contained in gases, can cause hydride formation from elements such as bromine, selenium, or arsenic [9-11]. Therefore, in samples containing bromine, as in the case of natural waters, there would be an interference on ^{80}Se and ^{82}Se from bromine hydride. As a result, the ^{78}Se signal should be monitored to avoid misinterpretation of the results and alleviate the need for correction equations.

Selenium concentrations determined in different mineral and spring waters, under the ICP-MS operating conditions described in Table 3, are summarized in Table 4. Results for certified simulated rain water (TM-Rain 95 from National Water Research Institute, [Ontario, Canada]) are also given. Total Se was established by measuring the ^{78}Se isotope without correction equations.

Experimental

Figure 1 shows a chromatogram of 1 µg(Se)/L per species standard obtained using HPLC-ICP-MS. The method was then applied to the mineral and spring water samples previously analyzed for their total selenium content. The results of selenium species concentrations are summarized in Table 4, together with the total selenium data.

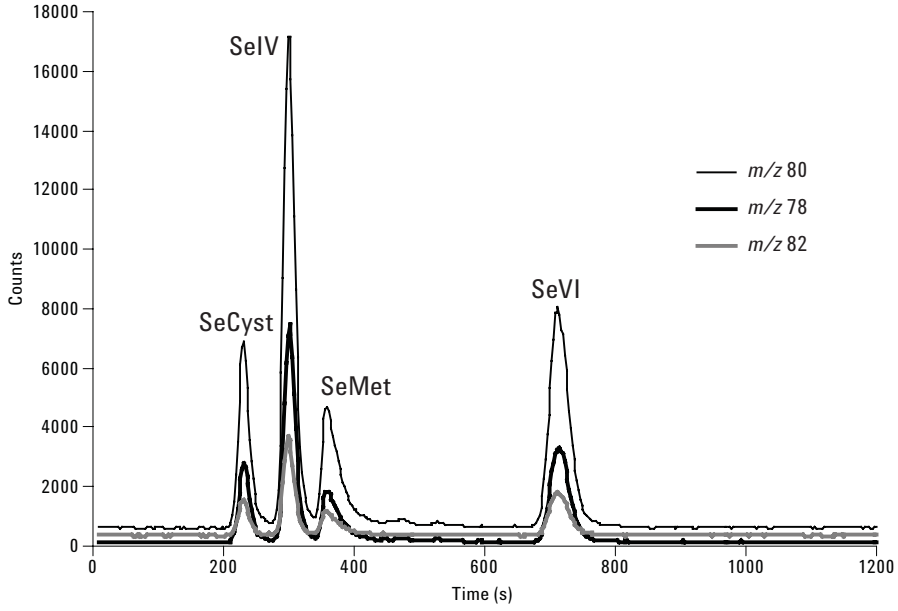

Figure 1. Chromatogram of standard, 1 µg(Se)/L per species; 100 µL injected, Hamilton PRP X-100 column, citrate buffer pH 5.2 and 2% methanol as mobile phase.

Table 4. Selenium Concentrations Determined in Different Natural Waters [units: ng(Se)/L]

	Elemental Analysis	HPLC Coupling			
		^{78}Se		^{80}Se	
Natural Water	^{78}Se	SeIV	SeVI	SeIV	SeVI
TM-Rain 95	622 ± 19*	629 ± 7	< DL	615 ± 8	< DL
A	67 ± 1	< DL	69 ± 2	< DL	72 ± 6
B	142 ± 24	< DL	140 ± 9	< DL	143 ± 4
C	240 ± 20	< DL	232 ± 13	< DL	267 ± 13
D	467 ± 17	< DL	475 ± 4	< DL	492 ± 5
E	1890 ± 160	55 ± 2	1840 ± 30	57 ± 6	1920 ± 20

*Certified value 740 ± 290 ng(Se)/L

Concentrations found in total and speciation analyses are in complete agreement, showing the suitability of the method when applied to natural water samples. Although the bromine hydride interference on *m/z* 80 is present, it is separated chromatographically without overlapping with the selenium species. The chromatogram of water sample "C" (Figure 2) shows bromine elutes after the selenate peak.

Selenate, commonly found in oxygenated waters, was determined in commercial waters A-D. Selenite was identified in TM-Rain 95 water, which is only certified for its total selenium content. Only water "E," a noncommercial ground water, contained both inorganic selenite and selenate species (see Figure 3).

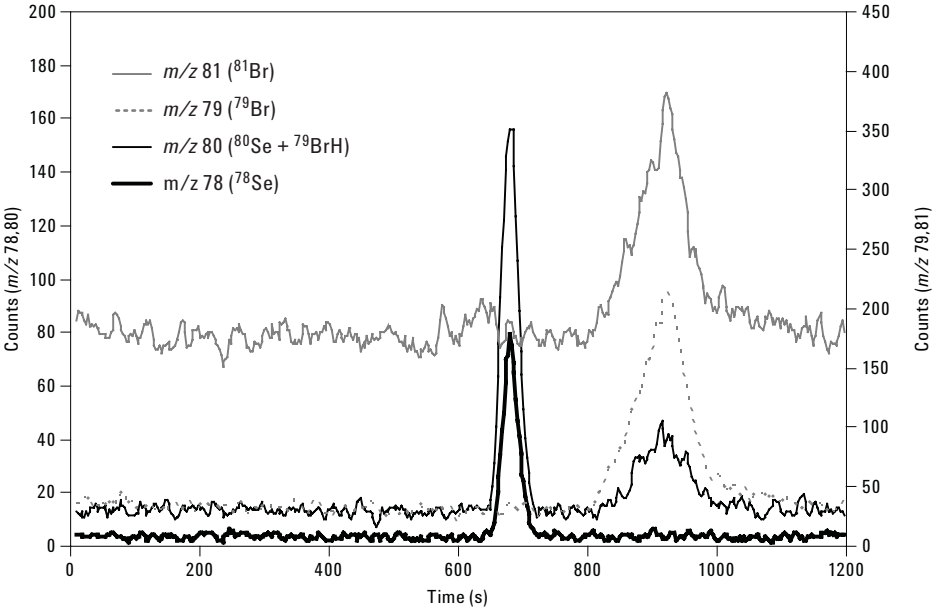

Figure 2. Chromatogram of natural water "C" showing reaction cell induced interference from bromine hydride elutes after the selenate peak.

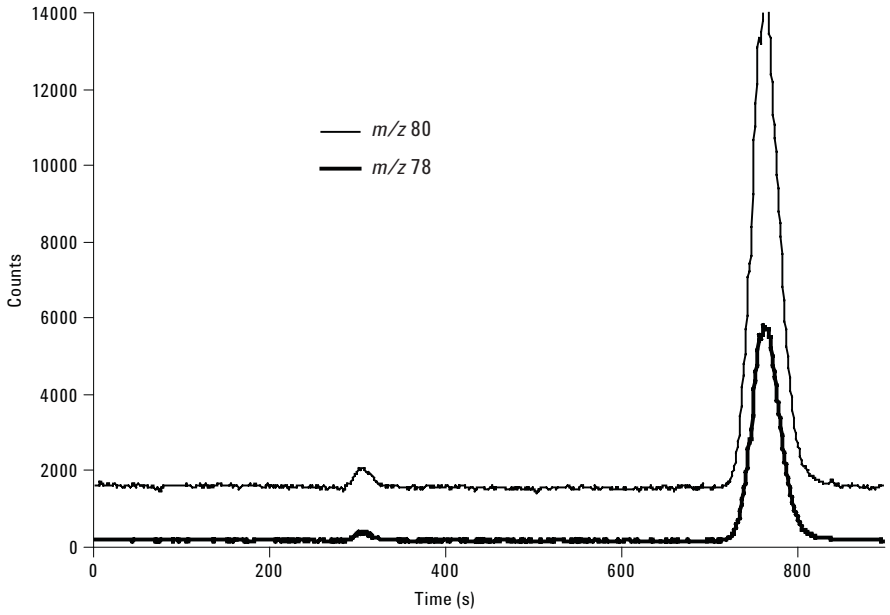

Figure 3. Chromatogram of natural water "E," the only sample to contain both inorganic species. First peak is SeIV, second peak is SeVI.

Conclusions

Interest in selenium speciation has grown in recent years due to its characteristics as both an essential and toxic element. However, the complete speciation of selenium, including organic and inorganic forms, is still a major challenge. This is particularly true when exploring selenium speciation in natural waters due to the low levels of Se present. A hyphenated technique consisting of isocratic HPLC coupled to ICP-MS with optimized collision/reaction cell conditions allows for a quick and precise simultaneous analysis of organic and inorganic selenium species. Moreover, as HPLC-ICP-MS coupling is easily automated, it can be considered a robust routine method to monitor selenium species levels in environmental and nutritional samples.

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Printed in the USA
July 25, 2007
5989-7073EN

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Determination of Butyltin Compounds in Environmental Samples by Isotope Dilution GC-MS

Application

Environmental

Authors

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Abstract

A GC-MS with electron impact ionization was used for the development of a speciation method for the simultaneous determination of monobutyl-, dibutyl-, and tributyltin in environmental samples (waters, sediments, and biota). The method is based on the use of a mixed spike containing ^{119}Sn-enriched monobutyltin (MBT), dibutyltin (DBT), and tributyltin (TBT) for isotope dilution analysis. The mixed ^{119}Sn-enriched spike was applied to the simultaneous determination of MBT, DBT, and TBT in waters, sediments, and mussel tissue samples with satisfactory results. A single injection allows the concentration of all three butyltin compounds in the sample to be computed quickly using standard spreadsheet software.

Introduction

Recently, the EU has included TBT in the list of compounds to be measured regularly in fresh waters. Thus, analytical methods for the determination of organotin compounds should provide enough sensitivity, selectivity, and accuracy to be applied routinely by testing laboratories. Most reported methods so far combine a separation technique such as gas chromatography (GC) hyphenated to element-specific detection systems, including atomic absorption spectrometry (AAS), flame photometric detection (FPD), mass spectrometry (MS), or inductively coupled plasma mass spectrometry (ICP-MS).

Isotope dilution (ID) methodologies, on the other hand, can produce superior accuracy and precision compared to more common calibration strategies provided that the first solid-liquid extraction step is quantitative or true isotope equilibration between the added spike and the analyte is achieved. Since quantitation is done by ratio measurements, subsequent nonquantitative analyte recoveries do not affect the final results. Identification for trace element speciation has been widely applied using ICP-MS for detection after HPLC or GC separation. However, ICP-MS is an expensive and not generally available instrument and similar results can be obtained with standard GC-MS instrumentation using electron impact ion sources. This application describes this analytical method, which could be applied routinely by testing laboratories.

Experimental

Reagents

Tributyltin (TBT) chloride (96%), dibutyltin (DBT) dichloride (96%), and monobutyltin (MBT) trichloride (95%) were obtained from Aldrich (Steinheim, Germany). Stock solutions were prepared by dissolving the corresponding salt in a 3:1 mixture of acetic acid (Merck, Darmstadt, Germany) and methanol (Merck). All organometallic standards solutions were kept in the dark at –18 °C and diluted working solutions were prepared by weight daily before the analysis. Acetic acid (Merck) and methanol (Merck) were used for the extraction of the organotin compounds from the solid matrices. Ethylation of the butyltin species was performed using sodium tetraethylborate (Galab, Geesthacht, Germany).

The spike solution (^{119}Sn-enriched butyltin mixture) was obtained from ISC-Science (Oviedo, Spain), diluted by weight with a mixture of methanol and acetic acid (3:1), and stored in the dark at –18 °C. Table 1 shows the isotopic composition as well as the concentration of the butyltin species in the spike solution.

Table 1. Isotope Composition and Concentration of MBT, DBT, and TBT in the ^{119}Sn-Enriched Butyltin Mix (Uncertainty Corresponds to 95% Confidence Interval)

Isotopic composition					
Isotopes	116	117	118	119	120
Abundance (%)	0.029	0.114	14.33	82.40	3.127
Uncertainty	0.008	0.005	0.12	0.15	0.032
Concentration (µg Sn/g)					
MBT	**DBT**		**TBT**		
0.121 ± 0.005	0.748 ± 0.009		1.019 ± 0.017		

Reference materials tested were PACS-2 purchased from National Research Council of Canada (NRCC) (Ottawa) and CRM 477 obtained from Bureau Communautaire de Référence (BCR). Sea and fresh water samples were spiked with natural abundance butyltin compounds to check for recovery.

Instrumentation

Chromatographic analysis was performed with an Agilent Technologies gas chromatograph model 6890N, fitted with a split/splitless inlet and an HP-5MS capillary column (cross-linked 5% phenyl methyl siloxane, 30 m × 0.25 mm id × 0.25 µm coating). The gas chromatograph was equipped with an Agilent mass spectrometric detector model 5973 Network MSD (quadrupole based).

Helium was employed as the carrier gas with a constant flow of 1.2 mL/min. The column temperature was initially held at 60 °C for 1 min, increased at 30 °C/min to a final temperature of 300 °C. Injection was performed using a split/splitless inlet in splitless mode. The transfer line and ion source temperatures were at 280 °C and 230 °C, respectively. Electron impact ionization was performed at an electron energy of 70 eV. A mass range from *m/z* 40 to 400 was recorded in the full-scan mode to check for spectral interferences. The measurement of isotope ratios for each butyltin compound was performed on the M-29 molecular ion (loss of an ethyl group) using 10-ms dwell-time per mass. Five *m/z* values were used for the selective ion monitoring (SIM) mode for each butyltin compound. Daily optimization of the GC-MS conditions was performed using the "autotune" option of the software supplied with the GC-MS instrument. For this purpose, perfluorotributylamine (PFTBA) was used as the tuning compound for all GC-MS autotunes, because it provides ions at 31, 50, 69, 100, 131, 219, 264, 414, 464, 502, 576, and 614 amu. Using this option, mass calibration and sensitivity optimization over the entire mass range was performed using the *m/z* 69, 219 and 502.

Procedures

Extraction and Derivatization of Organotin Compounds from Sediments

Approximately 0.2 g of sample was spiked with a diluted solution of the ^{119}Sn-enriched mixture of MBT, DBT, and TBT. A 4-mL mixture of acetic acid and methanol (3:1) was immediately added. The resulting slurry was exposed to ultrasound (30 W) for 8 minutes. A volume of 200 µL of the extract was derivatized as described below.

Extraction and Derivatization of Organotin Compounds from Mussel Tissue

Approximately 0.2 g of sample was spiked with a diluted solution of the ^{119}Sn-enriched mixture of MBT, DBT, and TBT. A 4-mL mixture of acetic acid and methanol (3:1) was immediately added. The resulting slurry was heated in a water bath at 37 °C for 1 hour. A volume of 250 µL of the extract was derivatized as described below.

Derivatization of Sn Compounds

Ethylation of the tin species was carried out in 7 mL clear glass vials with screw caps (Supelco, Bellefonte, PA). The pH was adjusted to 5.4 with

4 mL of 1 M acetic acid/sodium acetate buffer. Ethylation was performed using 0.5 mL of 2% w/v sodium tetraethylborate in 0.1 M NaOH. After 10 min of manual shaking, the organic layer was transferred to a glass vial and stored at –18 °C until measurement. The organic layer was then evaporated under a gentle stream of nitrogen to approximately 10 µL. Finally, 1 µL was injected in the GC instrument.

Extraction and Derivatization of Organotin Compounds from Water Samples

A sample of 100 mL of seawater is measured in a precleaned all-glass volumetric flask and mixed with a diluted solution of the mixed ^{119}Sn-enriched spike. In order to correct for volumetric errors, the amounts of sample and spike added were controlled gravimetrically. The spiked sample was shaken manually and left to equilibrate for 15 min prior to derivatization. Then, 1 mL of acetate buffer, to adjust the pH to 5.4, and 100 µL of a 2% w/v sodium tetraethylborate in 0.1M NaOH were added for the ethylation of the organotin compounds. Finally, 1 mL of hexane was introduced into the flask in such a way that it remained in the narrow neck of the flask. All these procedures can be performed under clean-room conditions to reduce the blank levels. The volumetric flasks were shaken manually for 10 min, the phases allowed to separate, and most of the organic layer was transferred to a 2-mL chromatographic vial with the help of a Pasteur pipette. Then, the hexane phase was evaporated under a gentle stream of nitrogen to approximately 10 µL. Finally, 1 µL of this volume was injected in the GC instrument.

Results and Discussion

Isotope Ratio Measurements by GC-MS

While elemental isotope ratios can be easily obtained with ICP-MS, in GC/MS the isotopic pattern in molecular ions is different from that of the naturally occurring elements due to the contributions from the organic groups attached to the metal because of the presence of ^{13}C. The contribution of ^{13}C to the observed m+1 and m+2 ions can be calculated in a fairly straightforward way, by applying equations 1 and 2:

$$I_{m+1} = I_m \cdot nX_{13_C} \quad (1)$$

$$I_{m+2} = I_m \cdot 1/2 \cdot n(n-1)^2 \cdot X^2{}_{13_C} \quad (2)$$

where x_{13_C} is the relative abundance of ^{13}C with respect to ^{12}C (0.0111/0.9899), n is the number of C atoms in the molecular ion and I is the intensities of the ions m, m+1 and m+2, respectively. The contributions to m+1 and m+2 of the butyltin compounds were corrected by monitoring five molecular ions for each analyte, corresponding to the ^{116}Sn, ^{117}Sn, ^{118}Sn, ^{119}Sn, and ^{120}Sn isotopes. The measured signal intensities at the different masses were corrected taking into account the ^{13}C contributions to m+1 and m+2. The intensity (I) correction equations used were:

$$^{116}Sn = {}^{116}I \quad (3)$$

$$^{117}Sn = {}^{117}I - x(^{116}Sn) \quad (4)$$

$$^{118}Sn = {}^{118}I - x(^{117}Sn) - y(^{116}Sn) \quad (5)$$

$$^{119}Sn = {}^{119}I - x(^{118}Sn) - y(^{117}Sn) \quad (6)$$

$$^{120}Sn = {}^{120}I - x(^{119}Sn) - y(^{118}Sn) \quad (7)$$

Where x is the contribution factor m+1 and y the contribution factor m+2. The contributions of ^{114}Sn and ^{115}Sn can be neglected owing to their very low natural abundances, and therefore the signal intensity measured for ^{116}Sn can be considered free of m+1 and m+2 contributions. The selected molecular clusters for the measurement of MBT, DBT, and TBT by GC-MS and the contribution factors x and y are given in Table 2. The selected molecular cluster for TBT (*m/z* = 287 to 291) in the sample, spike, and mixture can be observed in Figure 1.

Table 2. Monitored Masses and Contribution Factors for MBT, DBT, and TBT

Corresponding tin isotope	MBT ($BuEt_2Sn^+$)	DBT (Bu_2EtSn^+)	TBT (Bu_3Sn^+)
116	231	259	287
117	232	260	288
118	233	261	289
119	234	262	290
120	235	263	291
x (M + 1)	0.088	0.110	0.132
y (M + 2)	0.0038	0.0060	0.0086

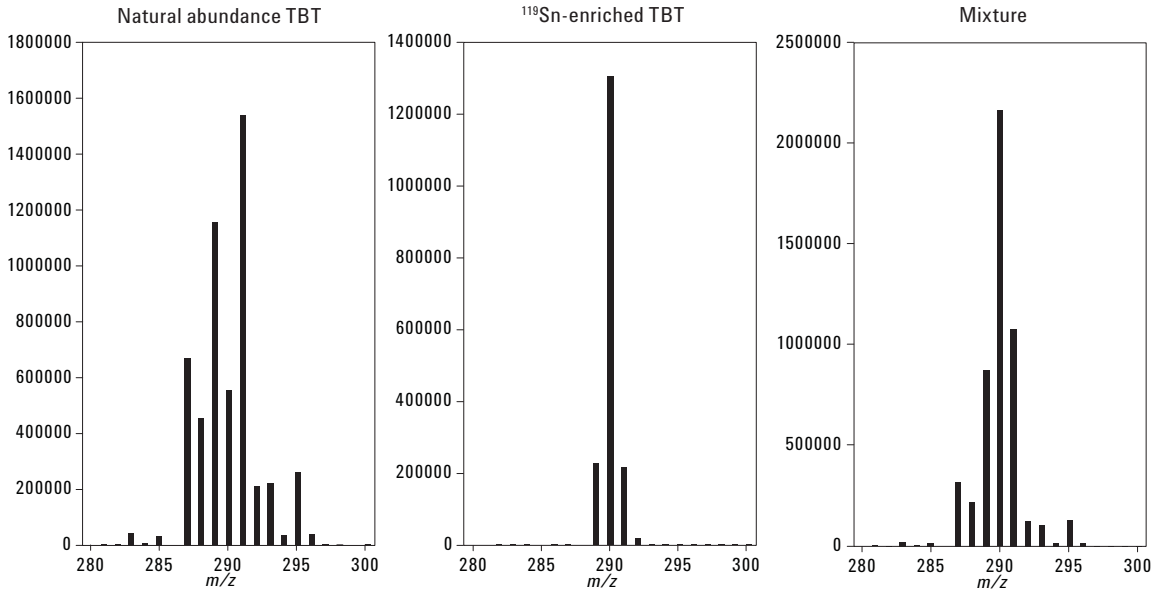

Figure 1. TBT mass spectra for the selected molecular clusters (*m/z* = 287 to 291) in the sample, spike, and mixture.

Analytical Characteristics of the Method

The analytical characteristics of the method are listed in Table 3. Method detection limits were calculated as three times the standard deviation of the blanks after measuring the concentration of MBT, DBT, and TBT in nine blank replicates by isotope dilution analysis following the methodology given in the procedures. The reproducibility of the method was studied by analyzing a natural seawater sample containing approximately 10 ng/kg of TBT. Recovery studies for seawater analysis were performed at three different levels to cover the range of concentrations that could be found in the real samples. The two high levels were obtained by addition of a natural MBT, DBT, and TBT standard to a real sample of coastal seawater (low content of butyltins) and the low level by addition of the natural standard to an artificial seawater sample. As can be observed, quantitative recoveries were obtained for all compounds at all concentration levels studied.

Table 3. Analytical Characteristics of the Method for the Analysis of Seawater Samples

			MBT	DBT	TBT
Limit of detection (3 σ) (ng/Kg)			0.2	0.1	0.2
Limit of quantification (10 σ) (ng/Kg)			0.8	0.5	0.7
Reproducibility (% RSD) (n = 9)			1.4	1.6	2.8
Recoveries (%)					
Sample Concentration (ng/Kg)					
Artifical seawater	2	(n = 3)	98 ± 7	101 ± 2	102 ± 9
Seawater	20	(n = 3)	103 ± 3	97 ± 2	104 ± 1
Seawater	100	(n = 3)	101 ± 2	98 ± 2	101 ± 3

Analysis of reference materials

Mono-, di- and tributyltin were determined in two reference materials: a sediment (PACS-2) and a mussel tissue (CRM 477), by the proposed ID procedure. Three independent spiking experiments were made on each certified reference material and each sample was injected three times in GC-MS systems. The overall results obtained for the two reference materials by GC-MS are summarised in Table 4 (PACS-2) and Table 5 (CRM 477).

The concentration values for TBT and DBT obtained for PACS-2 (Table 4) were within the certified range (890 ± 105 ng/g for TBT and 1047 ± 64 ng/g for DBT). Values found for MBT were substantially higher than the original certified value (450 ng g^{-1}) at the time the analyses were made. Recently, PACS-2 was recertified for MBT and the new "recommended value" for MBT is 600 ng/g – close to the values found by our method. The corresponding results obtained for CRM-477 certified mussel tissue (Table 5) show an excellent agreement between the certified and found values for each individual butyltin species.

Table 4. Determination of MBT, DBT, and TBT in PACS-2 Using the 120/119 Isotope Ratio for Quantitation; data in ng/g as Sn

Replicate	MBT	DBT	TBT
1	623 ± 2	1022 ± 7	872 ± 9
2	605 ± 4	971 ± 10	863 ± 2
3	617 ± 17	996 ± 1	849 ± 10
Average	**615 ± 9**	**996 ± 25**	**849 ± 10**
RSD (%)	1.5	1.5	1.5
Certified values	*600**	*1047 ± 64*	*890 ± 105*

**information value only due to lack of independent methods*

Table 5. Determination of MBT, DBT, and TBT in CRM- 477; data in ng/g as Sn

Replicate	MBT	DBT	TBT
1	1154 ± 9	761 ± 7	816 ± 6
2	1173 ± 18	766 ± 3	817 ± 2
3	1207 ± 19	787 ± 4	841 ± 2
Average	**1178 ± 27**	**772 ± 14**	**825 ± 14**
RSD (%)	2.3	1.9	1.7
Certified values	*1014 ± 182*	*786 ± 61*	*902 ± 78*

Conclusions

A fast, precise, and accurate method for the simultaneous determination of mono-, di-, and tributyltin in water, sediments, and mussel tissue has been developed. The detection at masses corresponding to ^{116}Sn and ^{117}Sn permits one to correct for the m+1 and m+2 contributions of ^{13}C on the ^{118}Sn, ^{119}Sn, and ^{120}Sn masses with simple mathematical equations. A single injection allows the concentration of all three butyltin compounds in the sample to be computed quickly without the need for time-consuming calibration, standard addition or recovery correction procedures. The method corrects for all possible errors in the speciation of butyltin compounds, provides extremely low detection limits, and is fast and simple to apply by non-trained personnel. The price of the enriched spike is no longer a limitation as a single determination in water samples requires less than 2 ng of the enriched compounds. In brief, the proposed ID-GC-MS technique appears to be a practical alternative to other measurement procedures, such as GC-AED or GC-FPD. The results obtained by this method are similar to those obtained by GC-ICP-MS.

The advantages provided by the proposed methodology are not only the less expensive instrumentation and high-quality analytical results, but also a drastic minimization of the time required both in the sample preparation steps and in the analytical measurement. Using such isotope dilution analysis methods and a GC-MS equipped with an autosampler, more than 15 samples can be analyzed by one operator per day from sample reception to analysis report. These advantages have been demonstrated in practice with the implementation of the proposed methodology in several routine testing laboratories and its subsequent accreditation according to the requirements of UNE-EN ISO/IEC 17025 by the Spanish National Accreditation Body.

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Printed in the USA
July 23, 2007
5989-7001EN

Agilent Technologies

Performance Characteristics of the Agilent 7500cx

Evaluating Helium Collision Mode for Simpler, Faster, More Accurate ICP-MS

Application

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Abstract

The new Agilent 7500cx collision/reaction cell (CRC) ICP-MS was designed to improve the speed and accuracy of multielement analyses in even the most complex, unknown sample matrices. While most CRC instruments require the use of reactive cell gases or gas mixes to remove interfering species, the 7500cx employs an Octopole Reaction System (ORS) with inert helium gas (helium mode). Being nonreactive, helium forms no new interferences in the cell and no analytes are lost by reaction. This application outlines the performance benefits of the Agilent 7500cx ICP-MS using helium collision mode for trace level multielement analysis in different matrices.

Introduction

Collision/reaction cell (CRC) technology revolutionized ICP-MS by virtually eliminating the problems associated with polyatomic interferences for most elements in most matrices. However, it became apparent when using reaction-based systems, that in the majority of cases, the conditions required to eliminate a specific interference in a specific matrix were, in fact, specific. Different interferences, different matrices—or both—typically require different CRC conditions.

This requirement for multiple conditions compromises the multielement capability and productivity of CRC ICP-MS. Most CRC ICP-MS systems require at least two or more distinct acquisition steps for a typical multielement suite. Techniques devised to overcome the disadvantages associated with multiple CRC conditions include the use of mixed gases, compromised cell conditions, and automated mode switching. However, these compromised CRC conditions cannot achieve optimum interference removal or the throughput of a non-cell instrument. The interference removal of a CRC instrument combined with the productivity of a non-cell instrument can only be achieved through the use of a single cell mode. Because reactive CRC processes work only for specific analytes in specific matrices, only nonreactive mechanisms can be used reliably with unknown samples.

A CRC process using a nonreactive collision gas, helium (helium mode), with kinetic energy discrimination (KED) is capable of universally removing all polyatomic interferences, regardless of the matrix. In addition, helium mode does not produce new interferences due to reaction with matrix components or cause specific loss of analyte or internal standard due to reaction processes. The purpose of this application is to demonstrate the

performance benefits in both speed and accuracy of the Agilent 7500cx ICP-MS using *only* helium mode with KED for trace level multielement analysis.

Experimental

All work was performed using a standard Agilent 7500cx ICP-MS fitted with a glass concentric nebulizer and standard autosampler. The Agilent 7500cx ICP-MS is the successor to the highly successful 7500ce. The 7500cx was designed for the high-throughput commercial laboratory that demands absolute confidence in results in the most demanding of matrices with the simplest possible operation and highest possible throughput.

With these goals in mind, the 7500cx has been optimized to operate efficiently using only helium mode. It can also be operated in no-gas mode, which will give slightly improved DLs for low-mass, uninterfered elements, such as Li, Be, and B. In special cases where the measurement of selenium at less than 100 ppt is required, the optional hydrogen cell gas kit can be installed, which enables reaction mode using hydrogen. Hydrogen mode also offers improved LODs for some other elements, such as Si and Ca, by allowing access to their most abundant isotopes, but this is not typically required for most sample types. A comparison of the performance of the instrument in hydrogen, helium, and no-gas modes has shown that for routine labs, the productivity gains through the use of a single mode (helium mode), significantly outweigh the small DL improvements for a few elements that can be achieved by the use of multiple gas modes. Like the 7500ce, the 7500cx can also take advantage of additional hardware and software features such as a second peripump option as well as intelligent and pre-emptive rinse to maximize throughput [1].

Table 1. Instrument Conditions Used to Measure IDL Values for All Masses Between 6 and 238 (Only helium mode was used for all elements)

Acquisition Parameters	
Instrument	**Agilent 7500cx**
Cones	Ni
Nebulizer	Glass concentric
Integration Time (total - 3 points)	
Cl(35,37), Ca(43,44), As(75), Se(78,82), Hg(200,201,202)	3.0 sec
All other	1.0 sec
Tune Parameters	
RF power	1,550 W
Sample depth	8.5 mm
Carrier gas	0.90 L/min
Makeup gas	0.23 L/min
Energy discrimination	2 V
Cell gas	He 5.0 mL/min

Results and Discussion

Measured Instrument Detection Limits in Helium Mode

Full scan acquisitions using a single set of helium mode conditions were performed for all elemental masses between 6 and 238. The conditions listed in Table 1 were selected for both optimum performance and throughput.

Three sigma instrument detection limits (IDLs) in parts per trillion in ultrapure water are shown in Table 2.

Table 2. Three Sigma IDLs in Ultrapure Water Using Helium Mode

Mass	Element	3σ IDL (ppt)	Mass	Element	3σ IDL (ppt)
7	Li	18.0	114	Cd	0.27
9	Be	8.8	115	In	0.35
11	B	88.0	118	Sn	0.87
23	Na	490	121	Sb	1.0
24	Mg	1.6	126	Te	5.2
27	Al	26.0	127	I	20.0
28	Si	360	133	Cs	0.50
31	P	560	137	Ba	0.85
34	S	19,600	139	La	0.13
35	Cl	4,040	140	Ce	0.10
39	K	400	141	Pr	0.07
44	Ca	21.0	146	Nd	0.35
45	Sc	1.3	147	Sm	0.43
47	Ti	3.7	153	Eu	0.19
51	V	0.28	157	Gd	0.23
52	Cr	0.53	159	Tb	0.07
55	Mn	0.79	163	Dy	0.20
56	Fe	9.4	165	Ho	0.06
59	Co	0.50	166	Er	0.13
60	Ni	1.7	169	Tm	0.04
63	Cu	2.0	172	Yb	0.33
68	Zn	3.1	175	Lu	0.11
69	Ga	0.47	178	Hf	0.83
72	Ge	1.3	181	Ta	0.09
75	As	1.4	182	W	1.1
78	Se	35.0	185	Re	0.24
79	Br	130	189	Os	2.7
82	Se	26.0	193	Ir	0.53
85	Rb	0.87	195	Pt	1.1
88	Sr	0.35	197	Au	0.97
89	Y	0.09	202	Hg	0.56
90	Zr	0.17	205	Tl	0.71
93	Nb	0.25	208	Pb	0.29
95	Mo	1.1	209	Bi	0.33
103	Rh	0.10	232	Th	0.77
105	Pd	3.3	238	U	0.16
107	Ag	0.72			

Because helium is a light, inert gas, and KED has little effect on monatomic ions, IDLs are excellent across the entire mass range. Even low-mass, high-ionization-potential elements like beryllium yield single-digit ppt IDLs. Overall, of the 73 elements measured, 57% show IDLs less than 1 ppt and 80% less than 10 ppt. Only sulfur and chlorine had IDLs higher than 1 ppb. If needed, sulfur can be analyzed at ppt levels using the optional xenon cell gas option.

Accuracy of Helium Collision Mode

To test the accuracy of helium mode, a certified reference water standard (NIST 1640) was analyzed using standard, high-throughput conditions and helium collision mode for all elements. The results are displayed in Table 3. No interference correction equations were used, since all polyatomic interferences are removed, and no analytes are lost to reactions within the cell. Even elements that are normally run in no-gas mode, such as Be, and Se, which is normally run in hydrogen mode, showed excellent recoveries.

Table 3. Results of Analysis of NIST 1640 in Helium Collision Mode (with ISTD)

Element	Certified (ppb)	Measured (ppb)	Recovery (%)
9 Be	34.94	34.48	98.7%
11 B	301.1	300.3	99.7%
23 Na	29.35	30.42	103.6%
24 Mg	5.819	5.60	96.2%
27 Al	52.0	50.97	98.0%
39 K	994.0	1,016.0	102.2%
42 Ca	7,045.0	7,018.0	99.6%
51 V	12.99	12.95	99.7%
52 Cr	38.6	37.17	96.3%
55 Mn	121.5	125.0	102.9%
56 Fe	34.3	33.88	98.8%
59 Co	20.28	20.38	100.5%
60 Ni	27.4	27.39	100.0%
63 Cu	85.2	85.88	100.8%
66 Zn	53.2	53.96	101.4%
75 As	26.67	27.20	102.0%
78 Se	21.96	22.98	104.6%
88 Sr	124.2	125.9	101.4%
95 Mo	46.75	47.56	101.7%
107 Ag	7.62	7.13	93.6%
111 Cd	22.79	22.59	99.1%
121 Sb	13.79	13.67	99.1%
137 Ba	148.0	147.3	99.5%
208 Pb	27.89	25.98	93.2%

Comparing the Effectiveness of Helium Mode for Selenium, Arsenic, and Vanadium in Variable Matrices

Of all the elements typically measured in environmental or other high-matrix samples, only selenium benefits from the use of hydrogen mode compared to either no-gas mode or helium mode. Because selenium is subject to common spectroscopic interferences on all of its six isotopes, it is difficult to measure in no-gas mode. While hydrogen reaction mode is very effective at removing the Ar_2^+ polyatomic at masses 78 and 80, resulting in low-ppt IDLs in most matrices, helium collision mode is also very efficient, resulting in an IDL between 35 and 150 ppt at mass 78, depending on the matrix. Helium collision mode is also effective at removing the $ArCl^+$ and $CaCl^+$ interferences at mass 77 even in high-chloride matrices, freeing up a second isotope with sub-ppb IDL. Helium mode also provides superior detection limits for both arsenic and vanadium, which also suffer from chloride-based interferences in high-chloride matrices (Table 4).

Table 4. Results of Analysis of 1/50 Diluted Aquaregia (0.5 vol% HNO_3 + 1.5 vol% HCl) and EPA 6020 Interference Check Solution A (ICS-A) to Determine the Background Equivalent Concentration (BEC) and Instrument Detection Limit (IDL) in Each Matrix

BEC (ppt)

	1/50 aquaregia		ICS-A	
Element	No-gas mode	He mode	No-gas mode	He mode
77 Se	26,700	630	10,000	400
78 Se	5,700	130	9,700	340
51 V	11,300	330	1,500	110
75 As	7,500	130	1,900	120

3σ IDL (ppt)

	1/50 aquaregia		ICS-A	
Element	No-gas mode	He mode	No-gas mode	He mode
77 Se	1,300	270	540	200
78 Se	270	150	310	160
51 V	830	91	140	45
75 As	600	84	190	150

Performance Advantages in Real-World Samples

In order to test the expected advantages in simplicity, speed, and accuracy, a sequence composed of typical environmental samples was analyzed for 12 hours after a single initial calibration. Acquisition parameters are shown in Table 5. In all, 300 analyses were performed, including replicate ICS-A samples, commercial mineral waters, and replicates of NIST 1640. NIST 1640 was analyzed 48 times over the course of the sequence.

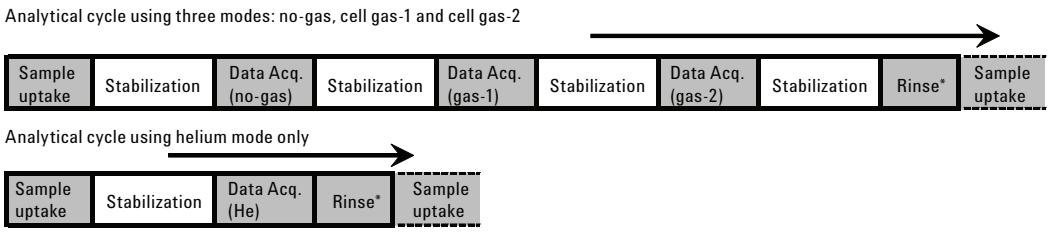
The primary advantages of using only helium collision mode over multiple modes are speed and simplicity. Tuning is reduced to a single set of standardized conditions that work well for any analyte in any matrix. No special optimizations are required, and the need to generate and store tune conditions for multiple modes is eliminated. During acquisition, a single set of instrument conditions is used, eliminating the gas changeover and stabilization time required when switching between modes (Figure 1). The result is reduced setup time and significantly reduced acquisition times, making the 7500cx the most productive ICP-MS available.

An additional benefit from the use of a single mode is improved long-term stability. There are several reasons for this. First, since the sample analysis time is shorter than multimode analysis, the interface is exposed to less sample matrix, which reduces drift due to sample cone deposition when high-matrix samples are analyzed. Additionally, maintaining static gas and pressure conditions within the cell eliminates a common source of instability associated with gas changes. Figure 2 is a normalized plot showing the long-term stability of NIST 1640 recoveries over the 12-hour, 300-sample sequence, which also included high-TDS mineral water samples (n = 96) and EPA interference check solutions A and AB (n = 48 each).

Table 5. Method Parameters Used for the 12-Hour Sequence (Average run time 2.46 minutes.)

Average sample-to-sample time	**2.46 minutes**
Number of isotopes	29
Integration time	
Li, Be, As, Se(78), Cd(111)	0.3 sec
All others	0.1 sec
Points per peak	1
Replicates per sample	3
Total acquisition time	9.72 seconds
Uptake time and flow rate	20 sec at 0.3 rps
Total rinse time and rinse flow rate	30 seconds at 0.3 rps
Preemptive* rinse	On, time = 28 seconds

*Preemptive rinse begins rinsing before acquisition has finished, using the sample remaining in the sample and peripump tubing to complete the acquisition, thereby reducing the total time by as much as 30 to 60 seconds per run.



*In both cases, rinse time can be shortened by using preemptive rinse.

Figure 1. A comparison of acquisition time and complexity between a system using three cell modes and the Agilent 7500cx using helium collision mode.

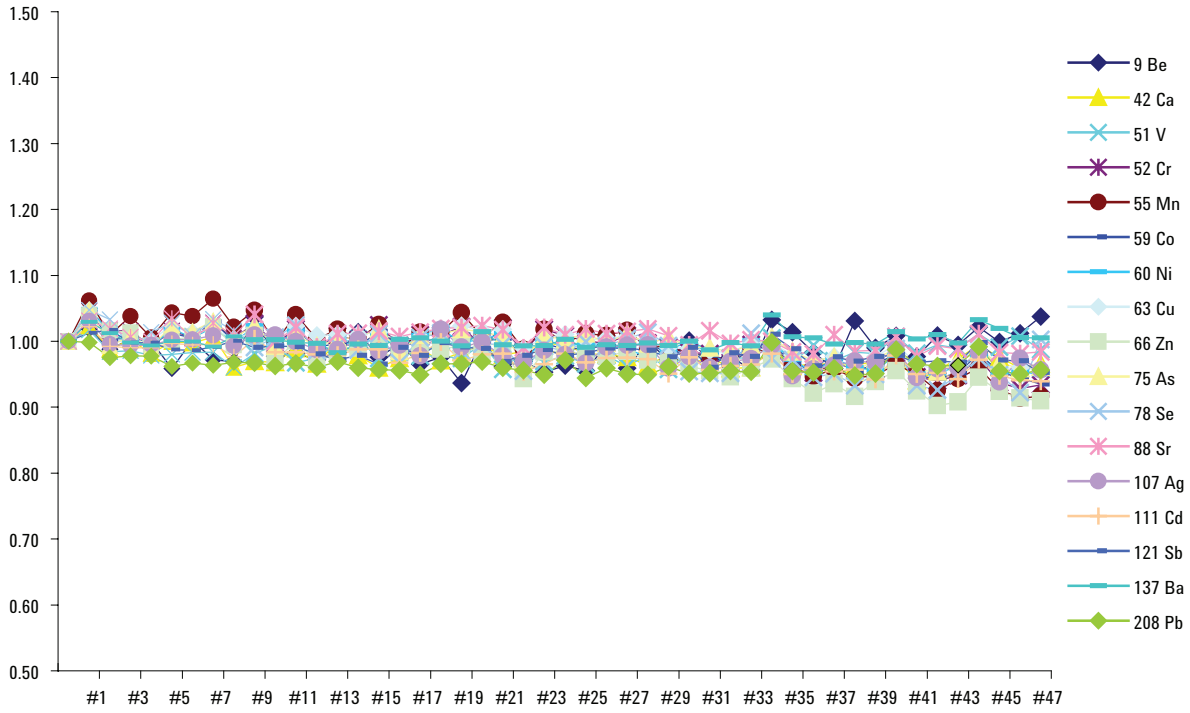


Figure 2. Normalized recovery of NIST 1640 components (n = 48) over a 12-hour 300-sample sequence in helium mode.

Conclusions

Helium mode with KED as implemented on the Agilent 7500cx ICP-MS has been shown to be a simple, fast, and effective solution to the problems associated with polyatomic interferences in ICP-MS. Instrument detection limits, measured in helium mode, across the mass range are sub-ppb for all elements except sulfur and chlorine. The addition of the optional xenon flow controller kit can be used if sub-ppb IDLs are required for sulfur. Most other elements, including selenium, exhibit IDLs in the low- to sub-ppt range. The optional hydrogen kit can provide single-digit-ppt DLs for selenium if needed. By eliminating the requirement for multiple collision cell modes, the 7500cx operating only in helium collision mode significantly reduces the run time and complexity of CRC ICP-MS. A single, universal tune is utilized for all analytes in all matrices. No time is spent acquiring data, such as internal standards, in more than one mode, and stabilization time after mode changes is completely eliminated. The result is a significant reduction in run time. Coupled with software enhancements such as preemptive and intelligent rinse, a full suite of environmental metals can be analyzed in less than 2.5 minutes per sample. Furthermore, data integrity in unknown or complex matrices is also significantly improved compared with systems that depend on either the use of mathematical corrections or reactive cell gases.

Reference

1. Achieving Optimum Throughput in ICP-MS Analysis of Environmental Samples with the Agilent 7500ce ICP-MS. Agilent Application Note 5989-5001EN, 2006.

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Printed in the USA
May 11, 2007
5989-6663EN

Agilent Technologies

Faster, Simpler, More Accurate Semiquantitative Analysis Using the Agilent 7500cx ICP-MS

Application

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Abstract

The new Agilent 7500cx allows the user to achieve the full potential of ICP-MS for semiquantitative elemental screening of a wide range of sample types. Complex, unknown samples can be analyzed with better speed, accuracy, and data integrity than ever before, since all matrix interferences are removed in the Octopole Reaction System (ORS) using helium collision mode. Results are presented for three different certified reference materials.

Introduction

Semiquantitative elemental analysis (semiquant) by ICP-MS is a powerful tool for quick screening of unknown samples for a wide range of trace elements. The ability to perform accurate semiquant is a strength of ICP-MS that is not shared by other elemental analysis techniques. It is based on the fact that the relative response of any element can be estimated from the response of any other element under a given set of conditions. These relative responses are determined by the unique properties of each element as well as the instrument and operating conditions, and can be stored in a semiquant response factor database. The use of internal standards or other calibration elements allows the database to be updated as needed to reflect the specific acquisition and matrix conditions. In practice, however, spectral interferences have limited the usefulness of semiquant for a number of elements in many common matrices.

Collision/Reaction Cell ICP-MS and Semiquant

In most collision/reaction cell (CRC) instruments, specific information about the matrix and target analytes is required in order to set up the correct collision/reaction chemistry to eliminate the interferences. Additionally, the conditions required to eliminate one interference in one matrix are generally not effective for all analytes in all matrices. For this reason, multiple sets of collision/reaction conditions are typically used. However, accurate semiquant response factors cannot be determined for elements acquired under different CRC conditions. As a result, it has not previously been possible to use CRC technology to reduce interferences in semiquant in the same way as in full quantification. However, the unique ability of Agilent's Octopole Reaction System (ORS) to eliminate polyatomic interferences using carefully controlled kinetic energy discrimination (KED) in helium collision mode permits all elements to be acquired under a single, universal set of CRC conditions.

KED eliminates the transmission of the larger polyatomic ions from the collision cell to the quadrupole by placing an energy barrier between the collision cell and quadrupole. Since polyatomic ions are always larger than atomic (analyte) ions of the same mass (Figure 1), they undergo more energy-reducing collisions with the helium cell gas than do the smaller atomic ions. As a result, the polyatomic ions have insufficient residual energy to cross the energy barrier at the cell exit, and so are excluded from the ion beam. Figure 2 depicts the effects of KED on ion energy. Only the high-energy atomic ions exceed the stopping potential

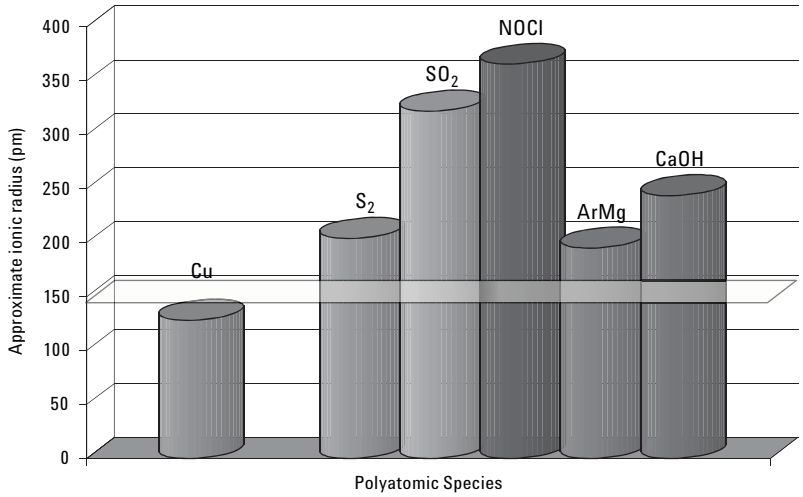

Figure 1. Graphic representation of the relative diameter of an atomic ion (Cu) compared with the polyatomic ions that can interfere. Most elemental ions are smaller than 150-picometer radius, while most polyatomic ions are larger.

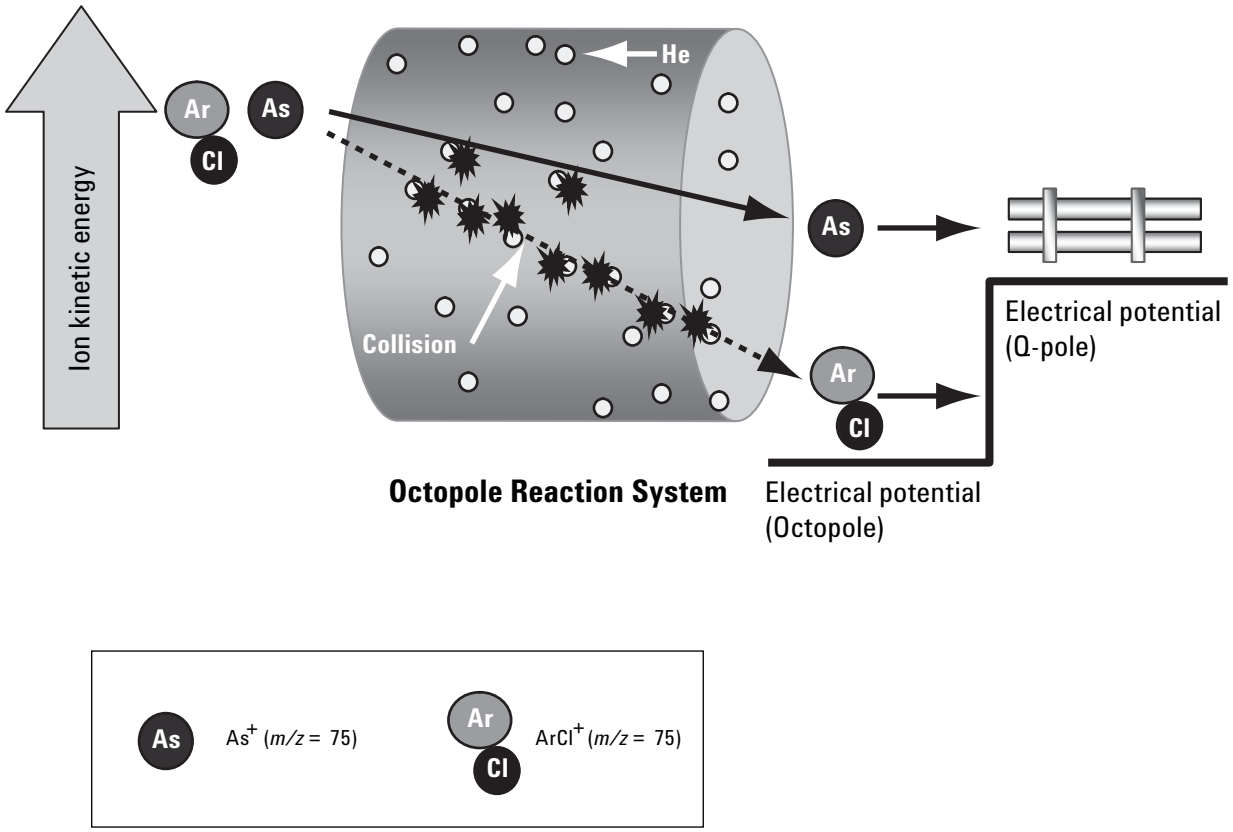

Figure 2. Diagrammatic representation of Kinetic Energy Discrimination after energy-reducing collisions within the Octopole Reaction System cell.

and are transmitted. Since helium is a nonreactive gas, no new interferences are formed in the cell and no analyte signal is lost by reaction, as occurs with any reactive cell gas.

The use of helium collision mode with semiquant conveys all the advantages normally associated with the use of CRC technology in full quant. It also solves the previously critical limitations of semiquant due to unresolved interferences. The advantages include:

- Semiquant is simple, fast, accurate, and interference-free for all analytes in any matrix.
- Helium collision mode allows the use of HCl, H_2SO_4, or other acids in digestion without danger of chlorine- or sulfur-based interferences on elements such as As, Cr, Se, V, Zn, etc.
- Improved stability for elements like Ag, Hg, Sb, Sn, and the Pt group due to the ability to add HCl to samples and standards.
- Ability to select the most abundant isotope for the best sensitivity, or multiple isotopes for absolute data confidence.
- Freedom to use any internal standards.

Table 1. Tune Conditions Used for NIST 1640 Semiquant Analysis in Helium Collision Mode

RF power	1550 W
Sample depth	8.0 mm
Carrier gas flow rate	0.90 L/min
Makeup gas flow rate	0.23 L/min
Sample flow rate	0.4 mL/min
Spray chamber temperature	2 °C
Helium flow rate	5 mL/min
KED	2V

Table 2. Semiquant Acquisition Parameters for NIST 1640

Total run time	**170 seconds**
Acquisition mode	Spectrum - peak hopping
Number of masses	250
Integration time[sec] masses 2 - 260	0.1 sec/point
Number of points per mass	1
Acquisition time	50.9 [sec]
Number of replicates	1
Uptake time	20 sec
Stabilization time	60 sec
Post acquisition rinse	30 sec
Preemptive rinse	On (time = 30 sec)

Experimental

The 7500cx ICP-MS was tuned for the same typical robust plasma conditions that are used in routine quantitative analysis (Table 1). No special tuning is required. Semiquant acquisition parameters are listed in Table 2.

A single calibration standard containing 200 ppb of Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Th, Tl, U, V, and Zn made up in 1% HNO_3/0.5% HCl was used to update the semiquant response factor database for a range of elements across the mass range. Non-calibrated elements are updated by interpolating between calibrated isotopes, which the ChemStation does automatically. Any number of calibration elements may be used, but increasing the number of calibration elements will improve semiquantitative accuracy. Internal standardization was applied using a typical suite of internal standard elements distributed across the mass range.

Results and Discussion

Tables 3 and 4 show the results of a semiquantitative screen of three standard reference materials, NIST 1640 water, LGC 6010 hard drinking water,

Table 3. Results of Helium Collision Mode Semiquant Anaysis NIST 1640 Standard Reference Water

Element	**NIST 1640 certified value**	**SQ conc.**	**Unit**	**Recovery (%)**
9 Be	34.94	33.42	µg/L	95.6
11 B	301.1	335.83	µg/L	111.5
23 Na	29.35	22.25	**mg/L**	75.8
24 Mg	5.819	4.24	**mg/L**	72.9
27 Al	52	48.92	µg/L	94.1
39 K	994	919.17	µg/L	92.5
42 Ca	7.045	5.81	µg/L	82.4
51 V	12.99	12.83	µg/L	98.8
52 Cr	38.6	36.58	µg/L	94.8
55 Mn	121.5	121.67	µg/L	100.1
56 Fe	34.3	30.92	µg/L	90.1
59 Co	20.28	19.75	µg/L	97.4
60 Ni	27.4	25.83	µg/L	94.3
63 Cu	85.2	81.17	µg/L	95.3
66 Zn	53.2	51.83	µg/L	97.4
75 As	26.67	27.75	µg/L	104.0
78 Se	21.96	24.08	µg/L	109.7
88 Sr	124.2	122.50	µg/L	98.6
95 Mo	46.75	46.17	µg/L	98.8
107 Ag	7.62	7.31	µg/L	95.9
111 Cd	22.79	21.50	µg/L	94.3
121 Sb	13.79	12.83	µg/L	93.1
137 Ba	148	139.17	µg/L	94.0
208 Pb	27.89	23.5	µg/L	84.3

Table 3 has been simplified to show only those elements with some reference values, although many other elements were determined in each reference material.

Table 4. Results of Helium Collision Mode Semiquant Analysis of LGC 6010 Hard Drinking Water and LGC 6177 Landfill Leachate Standard Reference Materials

	LGC 6010 hard drinking water			LGC 6177 landfill leachate		
Element	LGC certified value (µg/L)	SQ conc. (µg/L)	Recovery (%)	LGC certified value (µg/L)	SQ conc. (µg/L)	Recovery (%)
10 B	N/A	83	N/A	9,800	6,700	68.4
23 Na	21,900	20,000	91.3	1,750,000	1,500,000	85.7
24 Mg	4,200	3,700	88.1	73,500	62,000	84.4
27 Al	208	160	76.9	N/A	110	N/A
31 P	N/A	670	N/A	11,500	12,000	104.3
39 K	5,100	5,100	100.0	780,000	810,000	103.8
44 Ca	83,200	73,000	87.7	74,800	77,000	102.9
52 Cr	48	51	106.3	180	160	88.9
55 Mn	48	45	93.8	140	130	92.9
56 Fe	236	240	101.7	3,800	3,300	86.8
60 Ni	48	42	87.5	210	170	81.0
66 Zn	542	540	99.6	260	250	96.2
75 As	55	49	89.1	N/A	86	N/A
78 Se	9.5	13	136.8	N/A	< 16.00	N/A
107 Ag	6.2	4.3	69.4	N/A	1.8	N/A
121 Sb	11.9	13	109.2	N/A	5	N/A
137 Ba	116	110	94.8	N/A	770	N/A
208 Pb	95	92	96.8	N/A	17	N/A

Table 4 has been simplified to show only those elements with some reference values, although many other elements were determined in each reference material.

and LGC 6177 landfill leachate. No attempt was made to matrix-match; tune conditions used were as shown in Table 1; and all elements were acquired in helium collision mode. In all cases, for every certified element, the semiquantitative result was within ± 40% of the certified concentration, from as low as 7 ppb for Ag in NIST 1640 to over 1700 ppm for Na in the LGC 6177 landfill leachate.

Conclusions

Semiquant has always been a powerful tool available to the ICP-MS analyst for quickly estimating the concentration of unknown, uncalibrated elements in a variety of simple matrices. However, in complex matrices, polyatomic interferences could render the results for many elements useless. Collision/reaction cell technology, which requires more than one set of conditions for all masses, cannot be used since it would result in deviation from the standard relative response tables upon which semiquant is based. Helium collision mode coupled with kinetic energy discrimination in the Agilent 7500cx can overcome these limitations. By effectively removing polyatomic interferences, rapid, accurate, semiquantitative screening of a wide range of sample types for most analyte elements is possible. In this work, a full mass range, 250 isotope semiquant screen was performed in less than 3 minutes total sample-to-sample time with accuracy comparable to full quantification, for most elements, when measuring three different certified reference materials.

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Printed in the USA
May 4, 2007
5989-6662EN

Achieving Optimum Throughput in ICP-MS Analysis of Environmental Samples with the Agilent 7500ce ICP-MS

Application

Environmental

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Abstract

Throughput enhancements in ICP-MS can be achieved by minimizing sample uptake and rinse-out time through various techniques as well as by reducing data acquisition time. Depending on the application and data quality objectives, significant reductions in average run-to-run time are possible. In this work, we present some practical tips as to how to optimize several areas within the conventional ICP-MS sample introduction and data acquisition systems and make use of new rinse modes within the new Agilent ChemStation to improve productivity. The new methodology is supported by application to the analysis of the entire suite of 21 EPA method 200.8 elements plus 5 mineral elements and internal standards in a mixed run of drinking waters, leachates, and high-matrix wastewaters in approximately 4.5 minutes.

Introduction

High throughput in ICP-MS depends not only on accurate, high-speed data acquisition, but on rapid and complete sample wash-in and wash-out. In fact, as instrument scan speed and data processing speed have improved, it is sample wash-in/wash-out that remains the main limiting factor in achieving minimum run-to-run times and best detection limits, especially for memory-prone elements like Hg, Ag, Sb, Mo, and Tl. Complex, discrete sampling or flow injection techniques have successfully reduced sample uptake and rinse-out times to very low levels. However, until now, little systematic work has been done to efficiently optimize the conventional ICP-MS sample introduction system for highest possible throughput.

The topic of this application note is the maximization of sample throughput through:

- Understanding uptake and rinse out
- Optimization of the conventional sample introduction system on the Agilent 7500 Series ICP-MS for maximum productivity
- Minimization of acquisition time
- Use of intelligent software functions that eliminate wasted time in sample uptake and rinse out

Understanding Uptake and Rinse Out

In theory, minimizing the time it takes to get the sample into and out of the ICP-MS involves only two simple principles, one physical and one chemical.

First, minimize the time required for the sample to flow to the nebulizer. This can be done by minimizing the volume of the flow path and/or maximizing the flow while avoiding mixing at the interface between subsequent samples/blanks as much as possible. At the same time, it is desirable to minimize higher than optimum sample flow to the nebulizer itself to reduce plasma and interface overloading.

Second, minimize chemical interactions such as adsorption/desorption of soluble components in the sample to the sample introduction system. This is especially critical for the analysis of Hg, which must frequently be measured at ultra-trace levels and is very memory prone. These can be addressed by controlling the solution chemistry, sample introduction materials, and the contact surface area and exposure time.

Optimizing the Plumbing

Optimizing the plumbing involves minimizing the total volume of the sample introduction system, including the autosampler probe, peristaltic pump tubing, and sample transfer tubing. The internal diameters of these components should also be minimized in order to reduce the total wetted surface area. The mixing tee where the internal standard and sample are mixed is relocated to the top of the peristaltic pump in order to shorten the distance to the nebulizer as much as possible. The "tails" are clipped from the sample peristaltic pump tubing, leaving only about 1 cm beyond the stops, significantly reducing both the volume and surface area. Of all the polymer components used in the sample introduction system, polyvinyl chloride (PVC), used for the peristaltic pump tubing, is the most prone to causing carryover. Therefore, in order to further reduce the volume and surface area, smaller diameter peripump tubing can be used. In this case, the pump speed must be increased accordingly to maintain proper nebulizer flow. Pump speed correction factors are shown in Table 1. To convert from the standard 1-mm internal diameter (ID) tubing to a smaller size, multiply the normal pump speed used with the 1-mm tubing by the factor shown to maintain the same flow. Switching from 1-mm tubing to 0.64-mm tubing can reduce the sample rinse-out time by as much as 50%.

Table 1. Correction Factors for Various Internal Diameter Peristaltic Pump Tubes Used to Maintain Correct Nebulizer Flow

Peristaltic pump tubing ID (mm)	Correction factor
0.89	1.3
0.76	1.8
0.64	2.55

Minimization of Data Acquisition Time

Significant improvements in ICP-MS quadrupole, detector, and electronic technology have resulted in much better sensitivity and precision than earlier instruments. As a result, shorter integration times can be used while detection limit performance can be maintained or even improved. Total acquisition time is also reduced in Agilent ICP-MS instruments through the use of intelligent, variable quadrupole settling time, which reduces time between mass jumps. In the case of collision/reaction cell instruments such as the Agilent 7500ce or 7500cs, minimization of stabilization time during cell mode switching is also critical. Agilent's Octopole Reaction System (ORS) features a very small-volume cell that minimizes the time needed for gas switching and stabilization. Typically, 15 seconds is sufficient to achieve stable conditions after gas switching.

New Intelligent Software Capabilities (Revision B.03.03)

The ChemStation's new software features have been designed to eliminate wasted time in both sample uptake and rinse out, significantly reducing run times without compromising data quality. Customers with earlier revisions can upgrade to the newest B.03.03 revision by contacting their Agilent sales or service representative.

Pre-emptive Rinse

Pre-emptive Rinse utilizes the volume of the sample introduction tubing (autosampler probe,

sample tubing, and peristaltic pump tubing) to maintain sample flow to the nebulizer *after* the autosampler probe has moved to the rinse position and begun rinsing. On conventional ICP-MS systems, the autosampler probe moves to the rinse position only after acquisition has finished; as much as 30 to 60 seconds can be wasted with the probe sitting in the sample vial waiting for the acquisition to finish. With Pre-emptive Rinse enabled, the autosampler probe moves from the sample to the rinse port at a preset time *before* acquisition has finished, using the sample still in the uptake tubing for the remaining data acquisition. This provides two benefits:

1. No time is wasted while sample flushes from the sample introduction system after acquisition has finished.
2. When the peristaltic pump speed is increased to rinse after a sample, it is pumping rinse solution, not the previous sample. This significantly reduces the total matrix load on the interface since only rinse solution and not sample is introduced at a high rinse flow rate. As a result, interface maintenance is reduced and stability is enhanced.

Intelligent Rinse

Intelligent Rinse is designed to ensure that the absolute minimum amount of time is spent washing out each sample — *independent* of analyte concentration. On systems without Intelligent Rinse, the fixed, postsample rinse time must be long enough to wash out the highest anticipated analyte concentrations, and the most memory-prone analytes, to blank levels. This means that for back-to-back samples of similar concentration or very clean samples, unnecessary time is wasted in rinsing. Intelligent Rinse monitors the background level of up to 10 user-selected elements (or element ratios) to determine when sufficient rinsing has occurred. Since Intelligent Rinse supports internal standard correction (or any other count ratio), it is not necessary to update the background thresholds if the instrument sensitivity changes. Only Agilent Intelligent Rinse provides this level of sophistication. Intelligent Rinse supports up to three distinct rinse steps (for different rinse solutions) in addition to the probe rinse port on the autosampler. Each of these rinse steps can be controlled with respect to rinse time and uptake speed. Any of these rinse steps, including the probe rinse port, can be selected as the Intelligent Rinse step. When using Intelligent Rinse, in many cases, background levels are achieved immediately and the rinse terminates after only a few seconds.

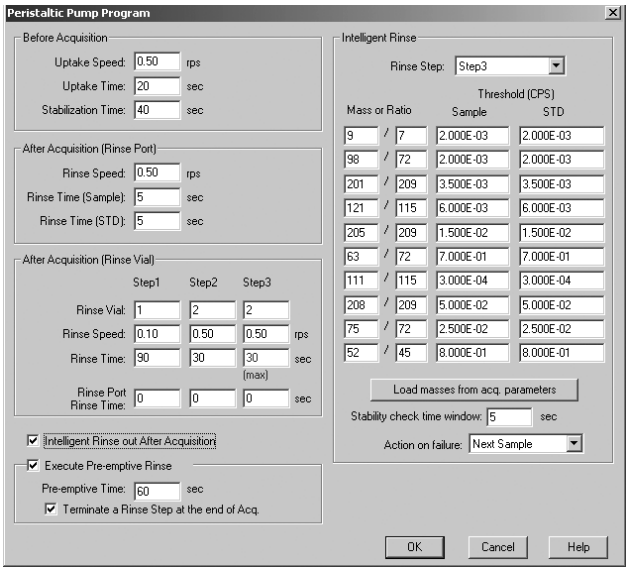

Figure 1. Peristaltic pump program setup panel showing typical settings for both Intelligent and Pre-emptive Rinse functions.

The result is that the shortest possible rinse times that allow complete washout are always achieved. The peristaltic pump control panel that is used to configure Intelligent Rinse is shown in Figure 1.

Putting It All Together

By combining optimized plumbing, Pre-emptive and Intelligent Rinse functions, and streamlined data acquisition parameters, typical average run-to-run times for complex environmental samples — including sticky elements such as Hg — can be reduced by almost 50%.

Rinse Program

The rinse program is shown in Figure 1. Pre-emptive Rinse time is set to 60 seconds, which immediately reduces the run time by a minute and reduces matrix loading on the plasma and interface. By the time the acquisition is finished, the rinse solution (Step 1 in this case) has nearly reached the nebulizer and most of the system is already rinsed. Between each rinse step, a small bubble is introduced into the line, helping to partition the solutions and minimize mixing. Step 2 is the main rinse and the only step using high-speed rinse out. Step 2 is set to 30 seconds, though much shorter times are often sufficient. Step 3 is the Intelligent Rinse step, which is functionally a monitor step. This means that the system will only rinse in Step 3 until the specified background elements reach the set points, or up to a maximum of 30 seconds in this case. At that point, rinsing is

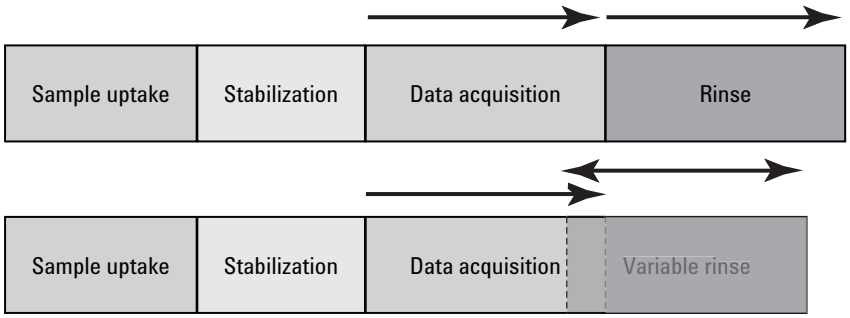

Figure 2. Graphical representation of sample analysis time using conventional uptake and rinse program (top) versus uptake and rinse utilizing Pre-emptive Rinse and Intelligent Rinse (bottom).

Table 2. Comparison of Total Sample Introduced Under Typical Conditions Using Normal Rinse Program Versus Pre-Emptive Rinse. Up to 20% Less Sample Is Introduced Using Pre-Emptive Rinse

	Standard mode	**Pre-emptive rinse**
Sample uptake	1.2 mL/min * 20 sec = 0.4 mL	1.2 mL/min * 20 sec = 0.4 mL
Stabilization	0.4 mL/min * 30 sec = 0.2 mL	0.4 mL/min * 20 sec = 0.13 mL
Sample analysis	0.4 mL/min * 180 sec = 1.2 mL	0.4 mL/min * 180 sec = 1.2 mL
Rinse out	1.2 mL/min * 15 sec = 0.3 mL	0
Total sample	**2.1 mL**	**1.73 mL**

finished and the next sample is introduced. Figure 2 is a graphical representation of the different ways in which Pre-emptive Rinse and Intelligent Rinse reduce sample analysis time.

Streamlined Data Acquisition

Table 3 depicts the additional time savings by optimizing the acquisition integration times, taking into account the high sensitivity provided by the 7500ce[1]. In this case, the entire suite of 21 EPA method 200.8 elements (including Hg, plus the five mineral elements (Na, Mg, K, Ca, and Fe), plus internal standards were analyzed using optimized cell conditions (three modes — H_2, He, and no gas) in 4.5 minutes average sample-to-sample time. Excellent (ppt level) method detection limits (MDLs) were achieved. Carryover, as indicated by the analysis of a blank solution run immediately after a 100 ppb (10,000 ppb for the mineral elements) standard was extremely low. When data quality objectives permit, switching to a single ORS mode (either no gas or He only) can reduce the average analysis time to less than 3 minutes.

[1]Acquisition parameters of 1 point per peak were used, with integration times per point from 0.1 to 0.5 seconds depending on the element and ORS mode.

Table 3. Typical Data Showing Blank Concentration Directly After a 100/10,000 ppb Standard and Calculated Method Detection Limits (3 Sigma) from 10 Separate Replicate Analyses of a 0.1 ppb Standard

1 point/peak acquisition
Average analysis time – 4.5 mins

Sample	**Cal 100.0/ 10000 (ppb)**	**Blank (ppb)**	**MDL (ppb)**
Be/9 [#3]	99.89	0.020	0.0205
Na/23 [#2]	9987	0.088	4.6245
Mg/24 [#2]	9946	1.419	1.7648
Al/27 [#3]	99.61	–0.028	0.0239
K/39 [#2]	9951	0.573	2.0353
Ca/40 [#1]	10020	2.387	1.9820
V/51 [#2]	99.98	–0.010	0.0193
Cr/52 [#2]	99.69	0.003	0.0156
Mn/55 [#2]	99.69	0.015	0.0258
Fe/56 [#1]	9986	2.731	0.6361
Co/59 [#2]	99.63	0.013	0.0092
Ni/60 [#2]	99.43	0.015	0.0100
Cu/63 [#2]	99.26	0.012	0.0292
Zn/66 [#2]	99.59	–0.020	0.0288
As/75 [#2]	100	0.003	0.0286
Se/78 [#1]	99.77	0.019	0.0299
Mo/95 [#3]	101.8	0.018	0.0219
Ag/107 [#3]	99.32	0.021	0.0085
Cd/111 [#3]	99.97	0.012	0.0167
Sb/121 [#3]	100.7	0.026	0.0168
Ba/137 [#3]	99.92	0.009	0.0256
Hg/201 [#3]	2.012	0.004	0.0048
Tl/205 [#3]	100.3	0.021	0.0089
Pb/208 [#3]	100.5	0.012	0.0127
Th/232 [#3]	100.8	0.020	0.0104
U/238 [#3]	101.1	0.015	0.0084

#1 — H_2
#2 — He
#3 — no gas

Conclusions

Faster Sample Runs, Higher Confidence

Sensitivity is maintained, maintenance is reduced, confidence in results is improved, and valuable time is never wasted. Pre-emptive Rinse reduces the total sample-to-sample pre-emptive time by at least 60 seconds, while also exposing the system to less total sample matrix (Table 2). More importantly, the sample matrix is never delivered to the nebulizer at high flow rates, thereby significantly increasing the number of samples that can be run between maintenance intervals.

Intelligent Rinse ensures that, after very clean samples, the rinse thresholds will be met immediately and almost no time will be spent in the final rinse solution, eliminating most of the 30- to 60-second available time. Furthermore, Intelligent Rinse can be counted on to rinse as long as necessary (up to a user-defined limit), ensuring that adequate rinsing will always occur. Rinse time will always be as long as necessary and never any longer. Additionally, the user has the choice of determining which elements need the most complete rinse out. Critical elements, like Hg, that require very low detection limits and are very memory-prone can be analyzed with confidence at ppt levels, even in unknown samples, while the washout threshold of high-level mineral elements such as Na can be set at a much higher level, or ignored if desired.

An illustration of high productivity made possible by the new Agilent ChemStation rinse modes is given by the following example: A 7500ce ORS instrument, analyzing a mixed run of drinking waters, leachates, and high-matrix wastewaters, operating in three modes (H_2, He, and no-gas), covering a full environmental analyte suite including the mineral elements and Hg, has an average sample-to-sample analysis time of 4.5 minutes — without any risk of carryover from unexpectedly high samples.

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Printed in the USA
August 10, 2006
5989-5001EN

Agilent Technologies

Ultra-Trace Analysis of Beryllium in Water and Industrial Hygiene Samples by ICP-MS

Application

Environmental

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Abstract

Exposure to airborne beryllium can lead to adverse health effects in humans. Consequently, many countries have legislation in place to ensure the health and safety of workers and the wider environment. Because even very low concentrations of Be in air constitute a toxic threat to health, analytical methodology that is capable of extremely high-sensitivity measurement is required. Although trace-level analysis of the element is difficult by some ICP-MS instrumentation, the Agilent 7500ce ICP-MS can achieve Be detection limits in the sub-ppt range directly in water and in acid digests of air filters under routine analytical conditions. Long-term precision and accuracy data recorded over 8 hours of continuous analysis of a highly diluted certified reference material (NIST 1640) is presented. Recoveries were greater than 97% and precision was in the order of 1 to 2%. Comparable performance was obtained when measuring spiked membrane filters, indicating that the method is applicable to the ultra-trace analysis of Be in air samples collected on filters.

Introduction

Workplace exposure to beryllium (Be) can pose significant chronic and acute health risks and is receiving increased scrutiny from regulators and industrial hygiene professionals. Be is a metallic element belonging to Group IIA of the periodic table. It has an atomic weight of 9.012 and is monoisotopic. It occurs naturally in the earth's crust at concentrations ranging from 2 to 10 ppm. The average concentration in U.S. soils is about 0.6 ppm, and during the late 1970s and 1980s Be was measured at between 0.03 and 0.4 ng/m^3 in air [1]. In its natural ore state, beryllium is relatively nontoxic. However, all other commercially important Be compounds exhibit significant pulmonary toxicity. Humans are exposed to Be from a number of sources, including food, water, and air. Clinically, the most important exposure pathways are airborne, including smoke from coal combustion, cigarette smoke, and airborne particulates from various Be manufacturing processes. Be use spans numerous industries, including electronics, aerospace, nuclear, and metallurgical. Exposure to airborne Be can lead to pulmonary disease either as Acute Beryllium Disease (ABD) or Chronic Beryllium Disease (CBD), depending on level and duration of exposure. Both can be fatal, and CBD symptoms may only appear after a latency period of up to 25 to 30 years. The U.S. Environmental Protection Agency (EPA) has also classified Be as a "probable human carcinogen" [1]. EPA-developed toxicity values for Be exposure are shown in Table 1. The National Institute for Occupational Safety and Health (NIOSH) exposure limit for Be in air is 0.05 µg/m^3. The limit of detection (LOD) by

Table 1. Chemical Toxicity Values for Beryllium Via Oral and Inhalation Exposure Pathways as Determined by the U.S. EPA [1].

Cancer Risk	Non-Cancer Effect	
Inhalation UR*	Oral RfD*	Inhalation RfC*
2.4 per mg/m^3	0.002 mg/kg-day	0.00002 mg/m^3

*UR – (inhalation unit risk): estimate of number of people per million that likely will get cancer from continuous exposure to Be in air at a concentration of 1mg/m^3.

*RfD – (oral reference dose): An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of a chemical to the human population (including sensitive subpopulations) that is likely to be without risk of deleterious noncancer effects during a lifetime.

*RfC – (inhalation reference concentration): An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure of a chemical to the human population through inhalation (including sensitive subpopulations) that is likely to be without risk of deleterious noncancer effects during a lifetime.

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) according to NIOSH 7301 [2] is 5 ng/filter, which requires air sampling volumes between 1,250 and 2,000 liters. By contrast, the LOD by ICP-MS using the Agilent 7500ce as described in this paper is 50 ppq in solution (0.00005 ng/mL), which is equivalent to 0.00125 ng/filter or a 4000× improvement in sensitivity.

Analytical Challenges

From an analytical standpoint, Be poses several challenges. Because of the toxicity of very low concentrations in air, extremely high sensitivity is desired. Traditional NIOSH and Occupational Safety and Health Administration (OSHA) methods for Be in airborne samples utilize ICP-OES with approximate detection limits of 0.005 µg/filter (NIOSH 7301), which may require a sampling volume of up to 2,000 liters. By using a much more sensitive technique such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), adequate sensitivity can be had at much lower sampling volumes and absolute detection limits can be lowered significantly. While ICP-MS can provide numerous advantages over other techniques in terms of sensitivity and throughput, there are some challenges as well. Be exhibits two characteristics that have traditionally made trace-level ICP-MS analysis difficult. Be is low mass and has a high first ionization potential (9.32 eV). Being low mass (atomic weight = 9), Be ions are subject to scattering in the presence of other higher mass ions (space charge effects), for example other metals in the sample. This can limit the sensitivity by an order of magnitude or more on some ICP-MS instruments. High ionization potential has a similar effect. Since the ICP-MS measures ions, elements that are difficult to ionize like Be have much higher detection limits than elements that ionize easily. Therefore, in order to achieve maximum sensitivity for Be, these two obstacles must be overcome. By specifically designing the plasma and ion optics for maximum ionization and minimum space charge, Agilent Technologies has effectively achieved Be detection limits in the sub ppt range under routine analytical conditions with the 7500 Series instruments. These design characteristics include a unique digitally synthesized 27 MHz RF generator and low flow sample introduction system to ensure maximum plasma temperatures as well as the avoidance of any type of shadow stop in the path of the ion beam that would cause loss of low mass sensitivity.

Instrumentation

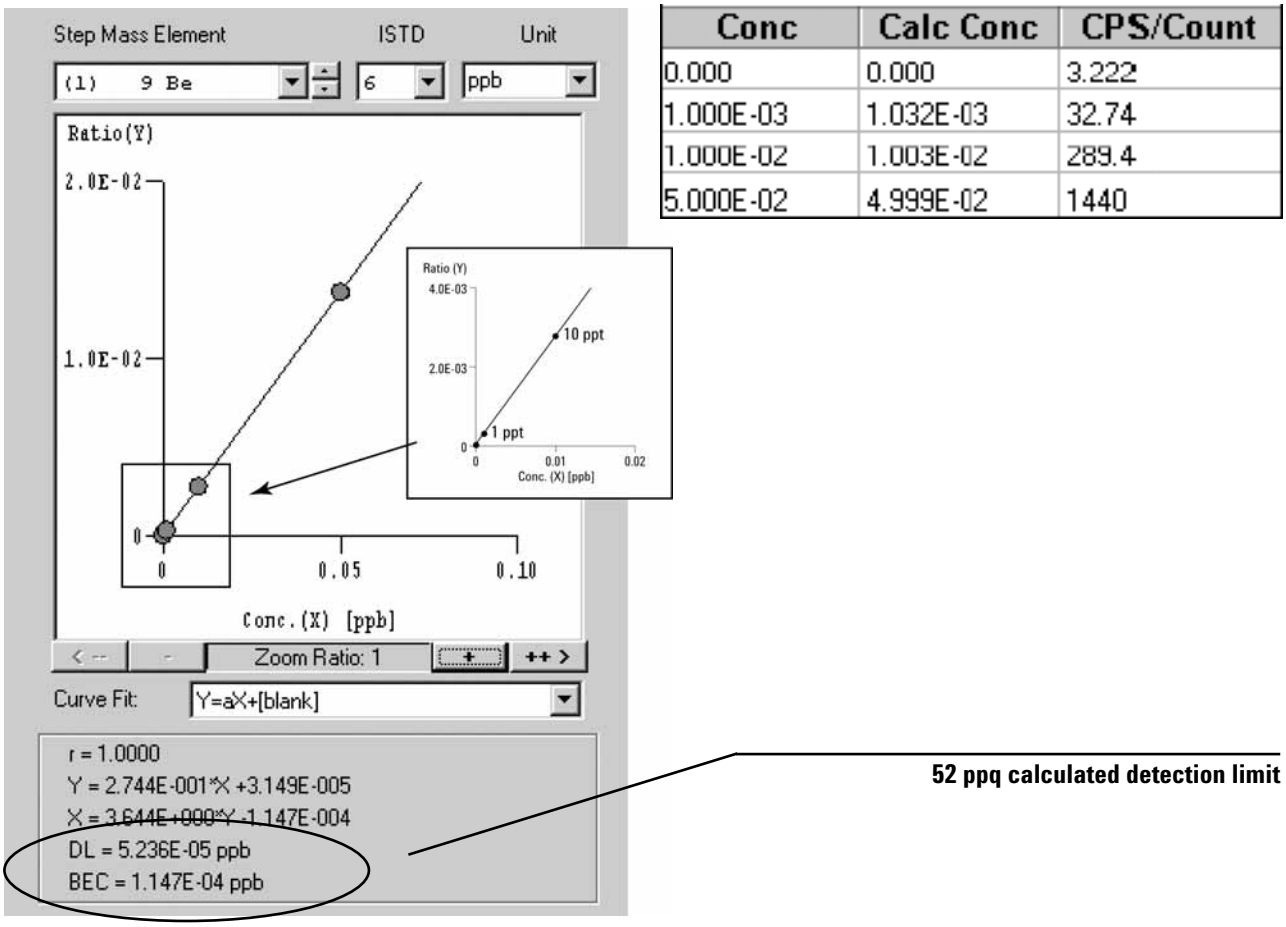
An Agilent 7500ce ICP-MS was used for this work in standard configuration. While the 7500ce is a collision cell instrument, it was used in "no-gas mode"; meaning, the collision cell was unpressurized and no collision/reaction chemistry was employed. The work was done under standard laboratory conditions of cleanliness, no clean room or special apparatus of any type were utilized. Instrument parameters are listed in Table 2.

Table 2. ICP-MS Parameters

Agilent 7500ce Instrument Conditions	
Plasma forward power	1500 W
Carrier gas flow	0.8 L/min
Nebulizer	Glass concentric
Sample flow	400 µL/min
Spray chamber temp	2 ° C
Extraction lens 1	2 V
Extraction lens 2	–110 V
Reaction mode	Off
Isotopes monitored	6, 9, 45
Integration time for ^{9}Be	5 seconds per replicate/3 replicates per analysis
Total run time	3 minutes

Experimental

The initial determination of performance was based on simple calibrations in dilute nitric acid and repeated analyses of diluted certified reference water (NIST 1640) for Be. This is because there are currently no standard reference materials available for Be in air samples. In the initial work, a sequence of 163 separate samples (dilutions of NIST 1640, Figure 2) was performed over 8.5 hours in order to determine the robustness and precision of the method (Figure 3). Calibrations were performed from 1 ppt to 50 ppt as shown in Figure 1.



Conc	Calc Conc	CPS/Count
0.000	0.000	3.222
1.000E-03	1.032E-03	32.74
1.000E-02	1.003E-02	289.4
5.000E-02	4.999E-02	1440

Figure 1. Calibration for Be in dilute nitric acid at 0, 1, 10, and 50 ppt (standard concentrations in the data table at the top right are expressed in ppb). The expanded area shows linearity from 1 to 10 ppt. ^{6}Li was used as the internal standard.

Estimated detection limits based on calibration linearity, response factor, and background were calculated to be 5.2×10^{-5} ppb (52 ppq) or 0.000052 ng/mL, compared with published values of 0.2 ng/mL by ICP-OES (NIOSH 7300). Subsequent work included the analysis of spiked 47 mm diameter cellulose ester membrane filters (0.8 µm pore size) according to the NIOSH 7301 (modified) method. Digestion conditions are outlined in Table 3. The method was slightly modified to use a Hot Block digester and the final diluent was 1% nitric acid. The intent was to determine the suitability and performance of the method to the matrix containing the dissolved filters.

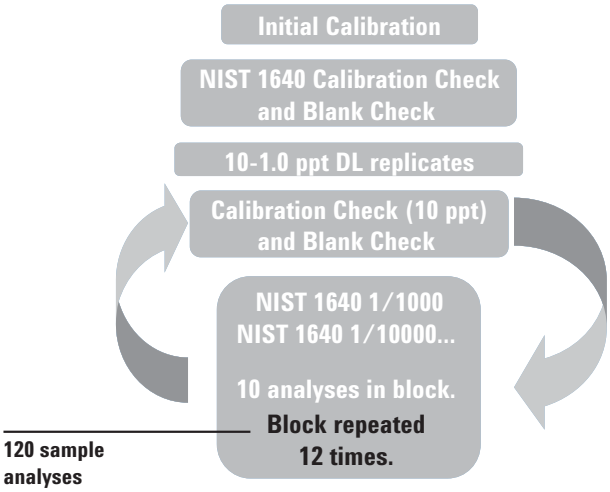


Figure 2. The analytical sequence used to test long-term accuracy and precision through continuous repeat measurements of certified reference material (NIST 1640 water)

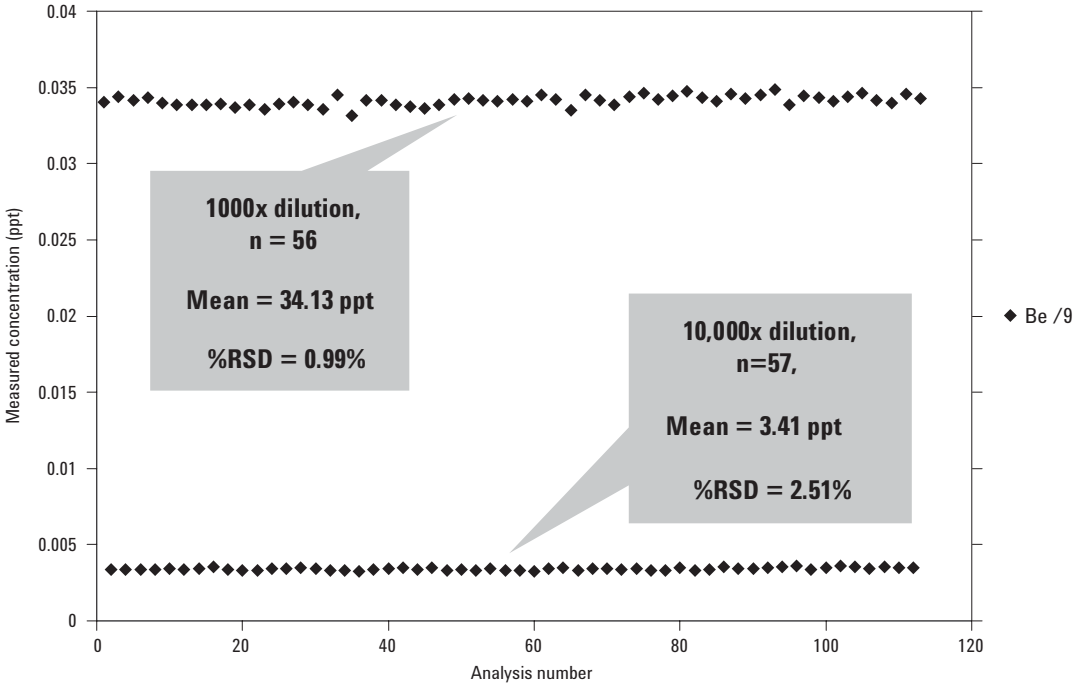


Figure 3. Results of a long-term stability study using repeat analysis of NIST 1640 diluted 1,000 and 10,000 . 1000 and 10,000 samples were analyzed alternately for 8.5 hours. Actual certified concentration in the diluted samples was 34.9 ppt and 3.49 ppt (1000 and 10,000).

Table 3. Modified NIOSH 7301 Digestion Procedure Utilizing Hot Block Digester for Analysis of Be on Filter Samples by ICP-MS.

Digestion Step	Procedure	Notes
1	Place filter into pre-leached 50 mL poly centrifuge tube. Add 5 mL 1:3 HNO_3:HCl. Cover with a plastic watch glass. Let stand 30 min at room temperature.	Start a reagent blank at this step. Some species of Al, Be, Co, Cr, Li, Mo, Sb, W, and Zr may not be completely solubilized by this procedure. Alternative solubilization techniques for these elements can be found elsewhere [2].
2	Heat on hot block (120 °C) until ca. 0.5 mL remains.	Hot block substituted for hot plate.
3	Add 2 mL 1:3 HNO_3 and repeat step 2. Repeat this step until the solution is clear.	PVC filters will not completely dissolve after repeated additions of ashing acid.
4	Remove watch glass and rinse into the digestion tube with distilled water.	
5	Increase the temperature to ~140 °C and take the sample to near dryness (ca. 0.5 mL)	
6	Bring to final volume of 25 mL with 1% nitric acid.	Final solutions ranged from hazy clear to dark amber. Internal standards added at this point. Solutions allowed to settle overnight prior to analysis of supernatant.

Precision and Accuracy in Waters

Precision and accuracy was evaluated by examining the results of the replicate analyses of the 1,000 and 10,000× dilutions of NIST 1640. The certified value for Be in NIST 1640 is 0.03494 ppm. Therefore, after dilution, values were 0.03494 and 0.003494 ppb. During the course of the analytical sequence (Figure 2), NIST 1640 was analyzed 113 times over 8.5 hours, 56 times at 1/1,000 dilution and 57 times at 1/10,000 dilution. The results are shown in Table 4.

Table 4. Analysis of Be in NIST 1640 – Standard Reference Water. Accuracy and Precision of Replicate Measurements of NIST 1640 at 1,000 and 10,000 Dilution Over 8.5 Hours of Continuous Analysis

DF	n	Average measured concentration (ppt)	%RSD	Average % recovery
1,000	56	34.13	0.988	97.67
10,000	57	3.41	2.51	97.49

Precision and Accuracy on Filter Samples

In order to determine the performance of the method for the analysis of Be contained on membrane filter samples according to NIOSH 7301 (modified), replicate filters (10 filter blanks and 10 5-ppt spiked filters) were analyzed. Spike recoveries were performed since no standard reference materials for Be on filters are available. Results are given in Table 5.

Table 5. Summary Results of Replicate Analyses of Spiked Membrane Filters and Blanks for Be

	n	Mean conc. (ppt)	% RSD	% Recovery
Reagent blank	–	–0.08 (n = 2)	–	–
Blank filters	10	0.481	58.8	–
Spiked filters (5 ppt)	10	5.25	4.73	95.3

Conclusions

The Agilent 7500 Series ICP-MS is capable of measuring Be directly in waters and in acid digests of air filters according to NIOSH 7301 at levels up to 4,000 times lower than the published DLs using ICP-OES. By using proprietary Agilent ion optics designed specifically to minimize the effects of space charge on low mass analytes such as Be and very high plasma temperatures, the obstacles to ultra trace determination of Be by ICP-MS have been overcome, allowing measurements at the ppq level (<0.0006 ng/m^3 for a 2,000 L sample). In addition, the long term precision and accuracy, as determined by measuring highly diluted certified reference material (NIST 1640) shows recoveries of greater than 97% and precision on the order of 1 to 2% over more than 8 hours of continuous analysis. Similar performance was obtained when measuring spiked membrane filters, indicating that the method is applicable to the ultra-trace analysis of Be in air samples collected on filters. The very high sensitivity and precision can permit the use of shorter sampling times and/or significantly lower limits of detection.

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Printed in the USA
August 10, 2006
5989-5438EN

Agilent Technologies

Unmatched Removal of Spectral Interferences in ICP-MS Using the Agilent Octopole Reaction System with Helium Collision Mode

Application

Metals Analysis

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Abstract

Many routine laboratories have adopted ICP-MS as their primary technique for metals analysis due to its simple operation as a multi-element analyzer. However, despite its higher performance for the targeted removal of specific interferences, collision/reaction cell (CRC) ICP-MS remains relatively understudied in terms of its multi-element capability. This work demonstrates that the Agilent 7500ce ICP-MS can be operated with a single set of He cell gas conditions, to provide effective interference removal for a range of elements in a challenging and complex sample matrix.

Introduction

ICP-MS is an immensely powerful multi-element analytical technique, but it does suffer from some well-documented spectral interferences, which can be especially problematic when complex and variable samples are analyzed. Most interferences in ICP-MS arise due to an overlap from a molecular (or polyatomic) ion at the same nominal mass as the analyte of interest. Commonly reported interferences can be broadly divided into two groups: those derived from the plasma and aqueous solution (plasma-based), such as ^{40}Ar, $^{40}Ar^{16}O$, and $^{40}Ar^{38}Ar$, and those derived from sample matrix components (matrix-based), such as $^{35}Cl^{16}O$, and $^{32}S^{34}S$. Plasma-based polyatomic ions are both predictable and reasonably constant, regardless of sample matrix, whereas matrix-based polyatomic ions are less predictable and vary with sample matrix components and their relative concentrations.

Recent advances in CRC technology have led to dramatic improvements in the analysis of interfered elements which previously proved difficult or impossible to measure at required levels in certain sample matrices. In a CRC ICP-MS, the cell is typically pressurized with a reactive gas that reacts with the interference (referred to as reaction mode). Attenuation of the interfering species occurs by one of several different processes depending on the gas and the interference. However, in practice, “reaction mode-only” CRCs limit the system to the removal of single interfering ions from single analytes [1–8], using highly reactive gases and specific measurement conditions. Some instruments use “simpler” or less reactive cell gas such as H_2, but its use is limited mainly to plasma-based interferences, as it reacts slowly or not at all with matrix-based interferences which are much more difficult to remove.

Helium (He) Collision Mode

The development of the Agilent Octopole Reaction System (ORS) introduced a new and much more powerful mode of CRC operation – He collision mode – which uses an inert collision gas to remove all polyatomic species based on their size rather

than their relative reactivity with a reaction gas. Since all polyatomics are larger than analyte ions of the same mass, their larger cross-section means that they suffer more collisions with the cell gas and so lose more energy as they progress through the pressurized region. On arrival at the cell exit, the large cross section polyatomic species all have distinctly lower ion energy (due to collisions with the He cell gas) than the analyte ions and so can be prevented from leaving the cell using a stopping voltage, allowing only the analytes to pass through to the analyzer. This separation process is known as kinetic energy discrimination (KED), and this simple yet extremely effective approach offers a number of significant analytical advantages over reaction mode.

Advantages of He Collision Mode:

- In contrast with a reactive cell gas, He is inert - so does not react with the sample matrix - no new interferences are formed in the cell
- As He is inert, it does not react with and cause signal loss for analyte or internal standard ions
- ALL interferences (plasma-based AND matrix-based) are removed or attenuated so multi-element screening or semiquant analysis can be combined with effective interference removal
- Since He collision mode is not interference-specific, multiple interferences can be removed from the same analyte (or different analytes) simultaneously [9, 10]
- No prior knowledge of the sample matrix is required, and no method development is required, in contrast to the extensive, analyte- and matrix-specific method development which is required for any reactive mode of interference removal [11]
- He collision mode can be applied to every sample, every matrix, and the same setup (gas flow rate) is used for every application
- No cell voltages to set up or optimize
- NO interference correction equations are used

Why Can't Other CRC-ICP-MS Use He Collision Mode?

To work properly, He collision mode requires efficient analyte/interference separation by KED, which requires two conditions to be met: first, the energy of all the ions entering the cell must be very tightly controlled. Agilent's unique ShieldTorch interface insures a very narrow ion energy spread of 1 eV: its physically grounded shield plate provides better control of initial ion energy than electrically grounded plasma designs (such as balanced, center-tapped or interlaced coils). Second, in the cell, polyatomic species must experience a sufficiently high number of collisions to differentiate them from the analyte ions at the cell exit. In the Agilent ORS this is achieved by the use of an octopole ion guide – the only implementation of an octopole cell in ICP-MS. There are two key benefits to the use of an octopole cell:

- Octopoles have a small internal diameter. As a result, the cell entrance and exit apertures are small – so the cell operates at relatively higher pressure compared to quadrupole or hexapole cells which increases ion/gas collisions.
- Octopoles also have better focusing efficiency than hexapole and quadrupole ion guides. The ion beam is tightly focused, which insures good ion transmission and high sensitivity at its higher cell operating pressure.

Only the Agilent ORS combines the ShieldTorch interface with an octopole cell and so only the Agilent ORS can effectively use He collision mode.

Testing He Collision Mode – a Worst Case Scenario

A synthetic sample matrix was prepared to give rise to multiple interferences across a range of common analytes and test the ability of He collision mode to remove all overlapping polyatomic species. A standard solution was prepared, containing 1% HNO_3, 1% HCl and 1% H_2SO_4 (all UpA UltraPure Reagents, Romil, Cambridge, UK), 1% Butan-1-ol (SpS Super Purity, Romil, Cambridge, UK) and 100 mg/L (ppm) each of Na and Ca (both prepared from 10,000 mg/L Spex CertiPrep Assurance single element standards), to simulate a very complex natural sample matrix. Table 1 summarizes the potential polyatomic species in this sample matrix, illustrating that practically every element in the mid-mass region (from 50 to 80 amu) suffers from multiple interferences. This makes the accurate determination of these elements in complex sample matrices extremely challenging for conventional ICP-MS, as the complex nature of the multiple interferences means mathematical corrections will be unreliable. This also illustrates why reactive cell gases are unsuitable for the multi-element analysis of complex samples; no single reaction gas can be effective for a range of

polyatomic ions, each of which will have different reactivity with any given reactive cell gas. However, every interference shown in Table 1 is a polyatomic ion and can therefore be attenuated effectively using a single set of He collision mode conditions. Two sets of spectra were acquired to show the ability of the He collision mode to remove multiple interferences; one in no-gas mode and the second with He added to the cell. No data correction or background subtraction was applied. Finally, a 5-ppb multi-element spike was added to the matrix and spectra acquired to confirm the recovery of all analytes and check for correct isotopic fit.

Table 1. Principal Polyatomic Interferences from an Aqueous Matrix Containing N, S, Cl, C, Na, and Ca

Isotope	Principal interfering species
^{51}V	$^{35}Cl^{16}O$, $^{37}Cl^{14}N$
^{52}Cr	$^{36}Ar^{16}O$, $^{40}Ar^{12}C$, $^{35}Cl^{16}OH$, $^{37}Cl^{14}NH$
^{53}Cr	$^{36}Ar^{16}OH$, $^{40}Ar^{13}C$, $^{37}Cl^{16}O$, $^{35}Cl^{18}O$, $^{40}Ar^{12}CH$
^{54}Fe	$^{40}Ar^{14}N$, $^{40}Ca^{14}N$
^{55}Mn	$^{37}Cl^{18}O$, $^{23}Na^{32}S$
^{56}Fe	$^{40}Ar^{16}O$, $^{40}Ca^{16}O$
^{57}Fe	$^{40}Ar^{16}OH$, $^{40}Ca^{16}OH$
^{58}Ni	$^{40}Ar^{18}O$, $^{40}Ca^{18}O$, $^{23}Na^{35}Cl$
^{59}Co	$^{40}Ar^{18}OH$, $^{43}Ca^{16}O$
^{60}Ni	$^{44}Ca^{16}O$, $^{23}Na^{37}Cl$
^{61}Ni	$^{44}Ca^{16}OH$, $^{38}Ar^{23}Na$, $^{23}Na^{37}ClH$
^{63}Cu	$^{40}Ar^{23}Na$, $^{12}C^{16}O^{35}Cl$, $^{12}C^{14}N^{37}Cl$
^{64}Zn	$^{32}S^{16}O_2$, $^{32}S_2$, $^{36}Ar^{12}C^{16}O$, $^{38}Ar^{12}C^{14}N$, $^{48}Ca^{16}O$
^{65}Cu	$^{32}S^{16}O_2H$, $^{32}S_2H$, $^{14}N^{16}O^{35}Cl$, $^{48}Ca^{16}OH$
^{66}Zn	$^{34}S^{16}O_2$, $^{32}S^{34}S$, $^{33}S_2$, $^{48}Ca^{18}O$
^{67}Zn	$^{32}S^{34}SH$, $^{33}S_2H$, $^{48}Ca^{18}OH$, $^{14}N^{16}O^{37}Cl$, $^{16}O_2{}^{35}Cl$
^{68}Zn	$^{32}S^{18}O_2$, $^{34}S_2$
^{69}Ga	$^{32}S^{18}O_2H$, $^{34}S_2H$, $^{16}O_2{}^{37}Cl$
^{70}Zn	$^{34}S^{18}O_2$, $^{35}Cl_2$
^{71}Ga	$^{34}S^{18}O_2H$
^{72}Ge	$^{40}Ar^{32}S$, $^{35}Cl^{37}Cl$, $^{40}Ar^{16}O_2$
^{73}Ge	$^{40}Ar^{33}S$, $^{35}Cl^{37}ClH$, $^{40}Ar^{16}O_2H$
^{74}Ge	$^{40}Ar^{34}S$, $^{37}Cl_2$
^{75}As	$^{40}Ar^{34}SH$, $^{40}Ar^{35}Cl$,$^{40}Ca^{35}Cl$
^{77}Se	$^{40}Ar^{37}Cl$, $^{40}Ca^{37}Cl$
^{78}Se	$^{40}Ar^{38}Ar$
^{80}Se	$^{40}Ar_2$, $^{40}Ca_2$, $^{40}Ar^{40}Ca$

Instrumentation

An Agilent 7500ce ICP-MS was optimized using the typical tuning conditions for high and variable sample matrices (plasma conditions optimized as usual for ~0.8% CeO/Ce). No attempt was made to optimize any parameter for the targeted removal of any specific interference. 5.5 mL/min He gas (only) was added to the cell for the collision mode measurements.

Comparison of Spectra

The background spectrum obtained in no-gas mode is shown in Figure 1a, together with the same spectrum (same mass range and intensity scale) under He collision mode conditions, in Figure 1b. From Figure 1a, it is clear that the normal background components of the argon plasma gas and aqueous sample solution (Ar, O, H), together with the additional components of the synthetic sample matrix (HNO_3, HCl, H_2SO_4, butanol, Ca and Na), lead to the formation of several high intensity background peaks in the no-gas mode spectrum, notably $^{40}Ar^{16}O^+$ and $^{40}Ar_2{}^+$ from the plasma, but also $^{40}Ar^{12}C^+$, $^{32}S_2{}^+$, $^{35}Cl^{16}O^+$, etc, from the matrix. These high intensity background peaks show why several interfered elements (^{56}Fe, ^{78}Se and ^{80}Se, ^{52}Cr in a carbon matrix, ^{64}Zn in a sulfur matrix) have traditionally been considered as difficult elements for ICP-MS.

When helium is added to the cell (He collision mode conditions) all of these high intensity background peaks are removed from the spectrum, (Figure 1b – same sample, same intensity scale as Figure 1a) demonstrating the effectiveness and the universal applicability of He collision mode. Figures 2a and 2b are the same two spectra as in Figure 1, but with the vertical scale expanded 100x. Many more, lower intensity, matrix-derived polyatomic species are now observed. These interferences, though present at lower levels than the plasma-based polyatomic ions, have the potential to cause more serious errors in routine sample analysis, as their presence and intensity is dependent on matrix composition, which, in routine laboratories, may be variable and unknown. At this expanded scale, it is clear that the use of He collision mode has reduced the background

species to very low levels, including the high intensity plasma-based species ArO^+ and Ar_2^+. The only peaks clearly visible in He collision mode (Figure 2b) on this scale are Fe and Zn (the peak template confirms the Zn isotopic pattern at *m/z* 64, 66, and 68), due to trace level contamination present in the matrix components. By contrast, in no-gas mode (Figure 2a), almost every isotope of every element in this mass region has an overlap from at least one matrix-derived polyatomic interference.

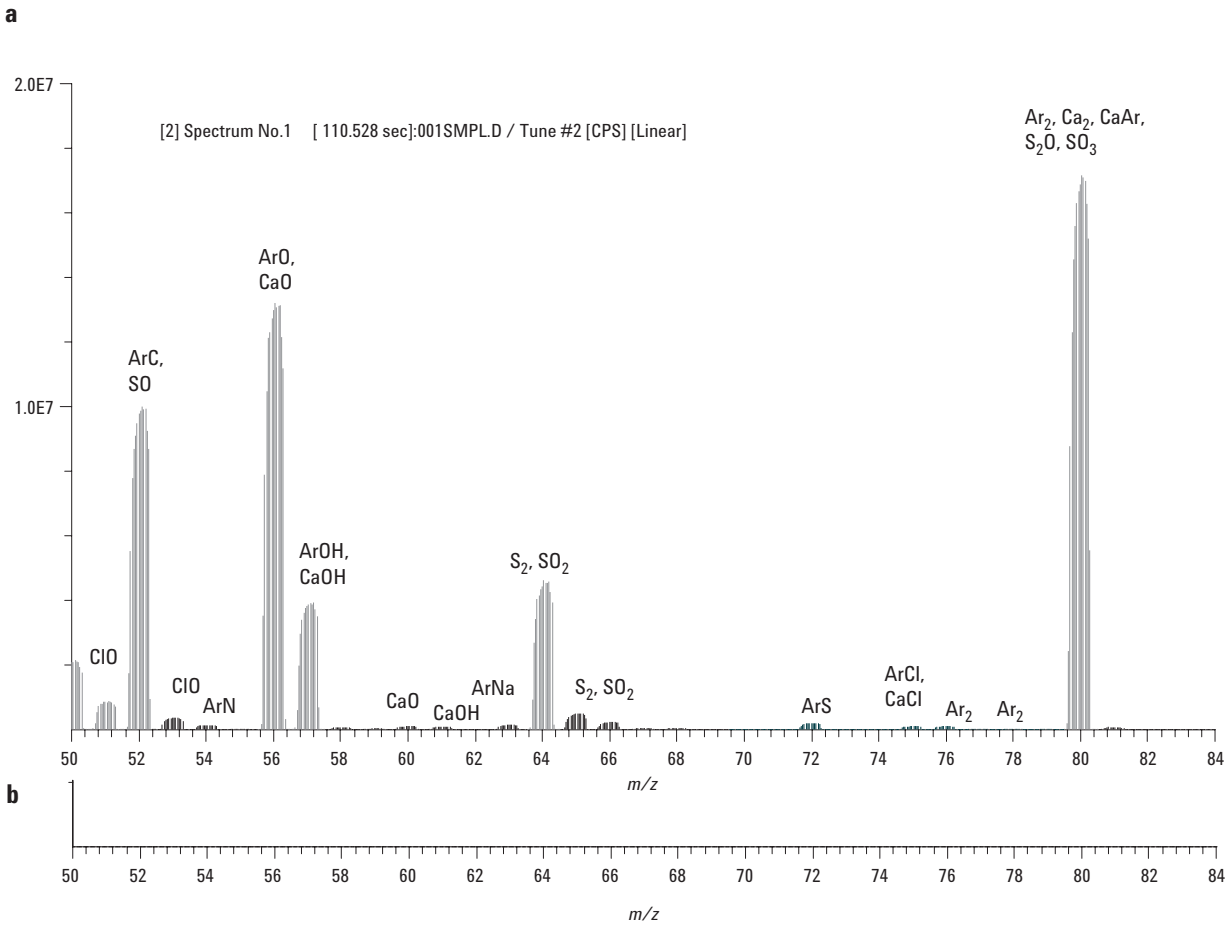

Figure 1. High intensity interfering polyatomic ions from complex matrix sample (see text for composition) in (a) no-gas mode and (b) He collision gas mode, on same intensity scale (2.0E7).

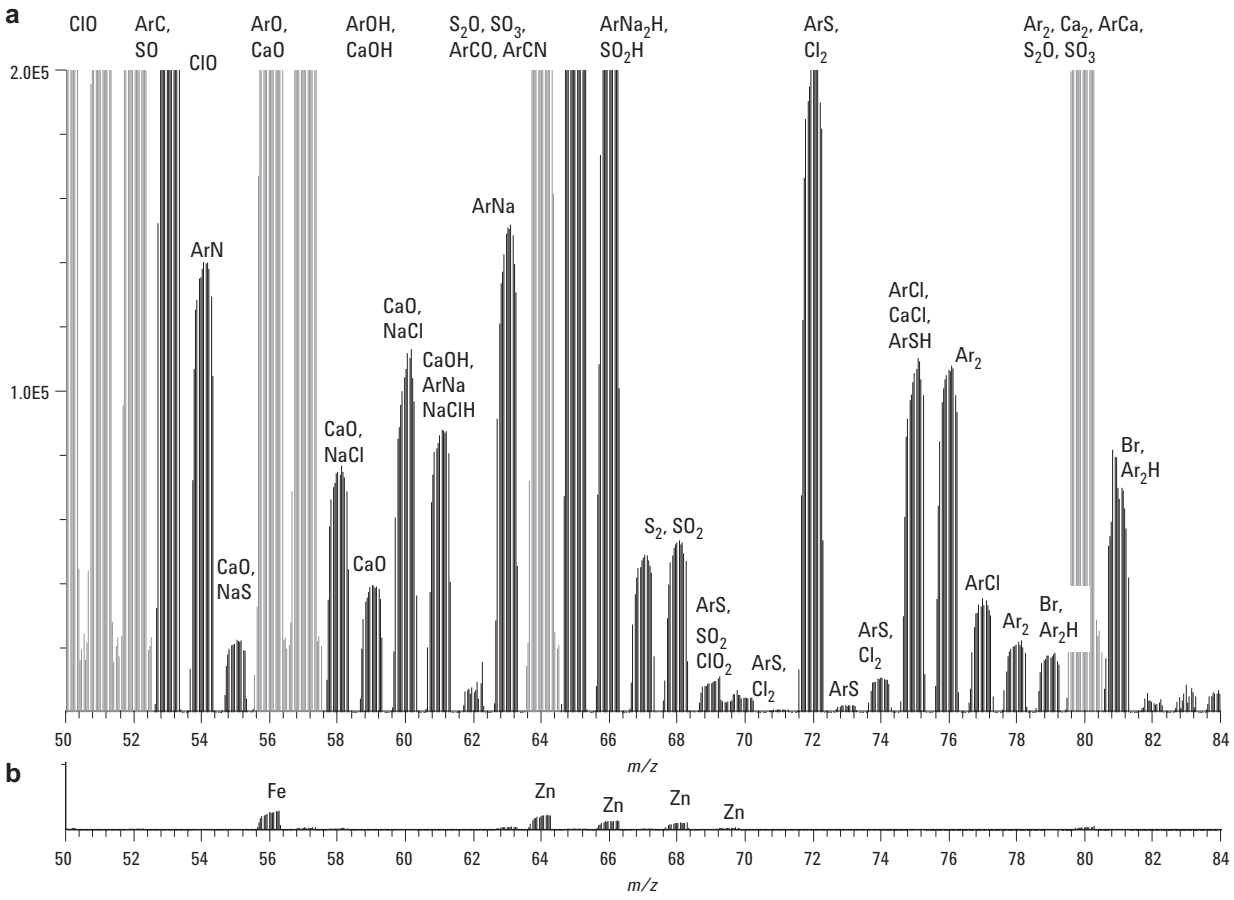

Figure 2. Low intensity interfering polyatomic ions from complex matrix sample in (a) no-gas mode and (b) He collision gas mode on same intensity scale (2.0E5), which is expanded 100x compared to Figure 1.

Measurement of Analytes in the Presence of the Sample Matrix

Having demonstrated the effective reduction of both plasma-based and matrix-based polyatomic ions using a single set of He collision mode cell conditions (Figures 1b and 2b), a second sample was analyzed. This time the sample consisted of the same multi-component matrix, but was spiked with a 5-ppb multi-element standard. Data was acquired in He collision mode to ensure that the same cell conditions used for interference removal also gave sufficient analyte sensitivity to permit the measurement of the previously interfered trace elements in this mass range. The spike consisted of 5 ppb each of V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ge, As and Se, all of which had at least one analytically useful isotope which suffered a polyatomic overlap in no-gas mode in this matrix.

Spectra obtained in He collision mode for the blank (unspiked) matrix and the spiked matrix are compared in Figures 3a and 3b respectively. Note that these spectra are shown on an intensity scale that is a further 4x lower than that used for Figures 2a and 2b, allowing the presence of the contaminant elements (Fe, Ni, Cu, Zn) to be confirmed from their isotopic templates (Figure 3b). The spectrum shown in Figure 3a clearly illustrates the capability of He collision mode to perform multi-element measurements at the low ppb level in this most complex and challenging sample matrix. Good isotopic fit is shown for every analyte. The only residual interferences observed were the plasma-based species ArOH and Ar_2 at mass 57 and 80 respectively. The Ar_2 signal at mass 80 is equivalent to ~5 µg/L Se. However, the polyatomic interferences on the other Se isotopes at *m/z* 77, 78, and 82 were removed completely, allowing Se determination at any of these isotopes (^{76}Se would also be available, but is overlapped by ^{76}Ge which was in the spike mix).

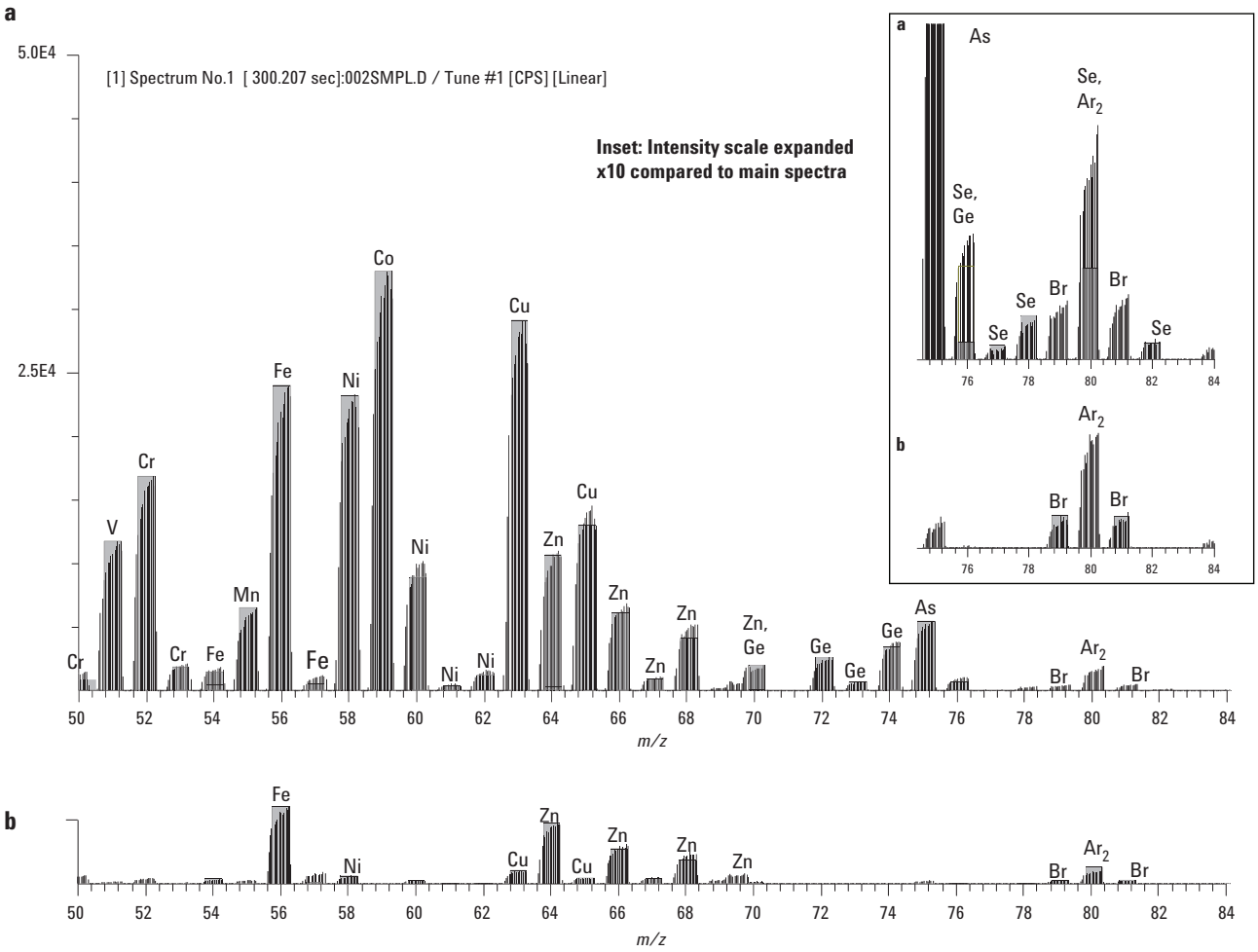

Figure 3. Complex matrix sample in He collision mode, (a) spiked at 5 ppb with V, Cr, Fe, Mn, Ni, Co, Cu, Zn, Ge, As, and Se and (b) unspiked. Intensity scale is 5.0E4 (5.0E3 for inset spectra).

Conclusions

The ability to remove ALL polyatomic interferences under a single set of conditions means that He mode is effectively universal – being suitable for any isotope of any element in any sample matrix. The use of He collision mode provides a unique new mode of operation, in which ALL the isotopes of each analyte become accessible. This, in turn, means that major isotopes that could not previously be used due to interferences (for example: ^{52}Cr in a carbon matrix, ^{56}Fe in any aqueous sample, ^{63}Cu in a sodium matrix, and ^{64}Zn in a sulfate matrix) - now become available. This is a great advantage to the analyst since, if desired, results can be verified by measuring many elements at both the preferred isotope AND at a second, "qualifier" isotope. Since both isotopes are free from polyatomic interference when measured using He collision mode, the use of two independent measurements gives a valuable confirmation of the reported result.

A further benefit of this powerful mode of analysis concerns sample preparation. In normal (non-CRC) ICP-MS, the choice of dilution media was limited mostly to nitric acid. Hydrochloric and sulfuric acid could not be used because of the problems of chloride or sulfur-based matrix interferences. Analysts can now choose the most appropriate digestion technique for the sample, secure in the knowledge that any new polyatomic interferences will be removed under the existing, standard He mode conditions.

The use of He collision mode on the 7500ce was demonstrated to provide effective removal of all polyatomic interferences under a single set of conditions, thereby enabling accurate multi-element analysis in complex and unknown samples. The use of an inert cell gas insures that there is no loss of analyte signal by reaction and that no new interfering species are generated, in contrast to the use of a reactive cell gas.

Since no analytes are lost by reaction and no new interferences are formed, uninterfered elements (and internal standards) can be measured under the same conditions as potentially interfered elements, and the use of a single set of cell conditions for all analytes allows multi-element analysis of transient signals (such as those derived from chromatography or laser ablation sample introduction), as well as semiquantitative screening analysis.

He collision mode is suitable for all analytes that suffer from polyatomic ion interferences and the cell conditions do not need to be set up specifically for each analyte, so the same cell conditions can be applied to new analyte suites, without requiring method development. Furthermore, since the He mode conditions are not set up specifically for the removal of individual interferences, identical cell conditions can be used for highly variable or completely unknown sample matrices, which greatly simplifies operation in a routine laboratory. The ORS enables ICP-MS to be used for the trace multi-element measurement of the most complex, real world sample matrices with no method development and with complete confidence.

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Printed in the USA
March 23, 2006
5989-4905EN

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Determination of Methyl Mercury in Water and Soil by HPLC-ICP-MS

Application

Environmental

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Abstract

This application note describes a method based on high-performance liquid chromatography (HPLC) coupled to inductively-coupled plasma mass spectrometry (ICP-MS) for the separation and determination of methyl and ethyl mercury. Because the different chemical forms of mercury exhibit different toxicities, separating elemental mercury from the alkylated forms provides vital information on the actual risk posed by a sample. The HPLC-ICP-MS method is applied to the analysis of water and soil samples. The method detection limits in water for MeHg, EtHg, and Hg^{2+} are better than 10 ng/L, and recoveries between 80% and 120% were obtained for the Hg species extracted from the soil samples.

Introduction

Heavy metals are among the most significant pollutants in natural waters. Within this group of pollutants, mercury (Hg) is of particular concern because of its toxicity and accumulative nature in the food chain. It is found throughout the ecosystem in trace amounts in air, water, soil, and living organisms. The different physical and chemical forms of this trace element have significantly different properties [1]. It is well known that the toxicity of Hg is highly dependent on its chemical form, with inorganic and organic Hg species presenting different toxicities. Methylmercury (MeHg) is the most commonly occurring organo-mercury compound in environmental and biological materials and the most toxic Hg species, whereas ethylmercury (EtHg) and phenylmercury are rarely present in the environment. MeHg is 10–100 times more toxic than inorganic Hg compounds [2, 3], and certain levels of MeHg exposure in humans can lead to neurological problems [4]. Because of its high-lipid solubility, MeHg penetrates the blood-brain barrier and readily diffuses into cell membranes [5]. Fetuses are particularly vulnerable because of their rapid brain development. The main source of Hg is air emissions from power generation and other industrial activities. Once in the environment, biological activity will typically methylate Hg to either MeHg, or less commonly, di-methyl mercury. Because fish and other seafood products are the main source of MeHg in the human diet, pregnant women are advised to limit the consumption of certain fish. Recently, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended the Provisional Tolerable Weekly Intakes (PTWI) of MeHg be reduced to 1.6-µg/kg body weight per week, down from 3.3-µg/kg body weight per week. See Table 1.

Table 1. Regulated levels (PTWI) of MeHg in Foods

Authority	MeHg (µg/kg body weight)
Food and Agriculture Organization of the United Nations (FAO)	1.6
World Health Organization (WHO)	1.6

PTWI = Provisional Tolerable Weekly Intakes.

In China, the permitted level for MeHg in the "discharge standard of pollutants for municipal wastewater treatment plant of China (GB 18918-2002)" is "undetectable", that is, below the detection limit (DL) of the recommended method (10 ng/L) [6].

There are several means of determining total Hg in environmental samples, but the simultaneous determination of inorganic and organic Hg is difficult. This is because the typical concentration of MeHg is much lower than for inorganic Hg. The most common methods of Hg speciation are gas chromatography (GC) or HPLC coupled to a Hg-specific detector (fluorescence, photometry, or other elemental detector). The low concentration of Hg in natural waters leads to the need for processing very large sample volumes. A preconcentration step is also required because the target reporting limit is often below the sensitivity of the detector used.

In China, the recommended method for MeHg measurement (GB/T14204-93) uses GC with an electron capture detector (ECD). Limitations of the method include:

- Method detection limit (MDL) of 10 ng/L, despite a complicated enrichment procedure
- Method is not element specific
- Method suffers from interferences, leading to false positive results or low recoveries

The objective of this study was to develop a sensitive and specific MeHg analysis method by combining HPLC with ICP-MS – see Figure 1.

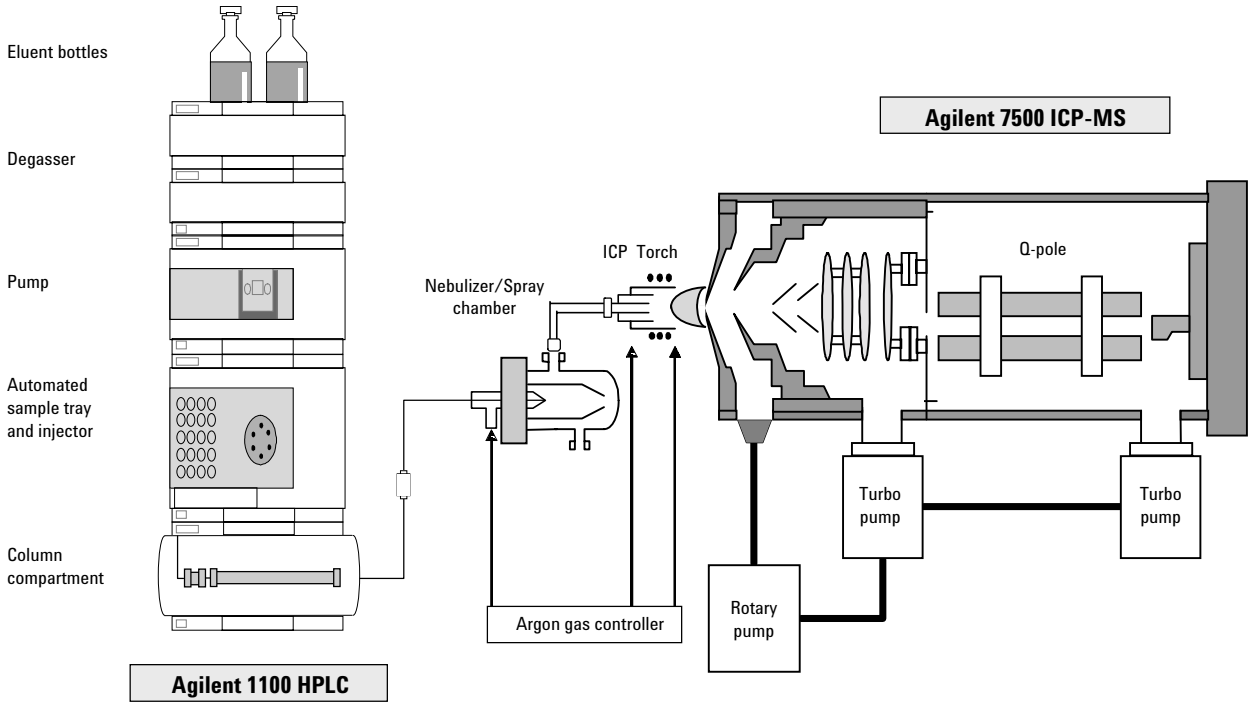

Figure 1. Agilent 1100 HPLC schematic.

Instrumentation

An Agilent 1100 HPLC was interfaced to an Agilent 7500a via the Agilent HPLC-ICP-MS interface. PEEK 20 µL, 100 µL, and 1000 µL sampling loops were selected. The operating parameters of the HPLC and ICP-MS are listed in Table 2a and 2b.

Table 2a. Working Parameters of HPLC

Column	ZORBAX Eclipse XDB-C18, 2.1-mm id × 50 mm, 5 µm
Mobile Phase	0.06-mol/L ammonium acetate, 5% v/v methanol, 0.1% 2-mercaptoethanol, pH = 6.8
Flow rate	0.4 mL/min
Injection volume	100 µL

Table 2b. Working parameters of Agilent 7500a ICP-MS

RF power	1550 W
Nebulizer	PFA concentric 100 µL/min
Spray chamber	Quartz, Scott double pass, chilled to –5 °C
Torch	Quartz one piece Fassel type, 2.5-mm injector
Sampling depth	4.5 mm
Carrier gas flow rate	0.75 L/min
Make-up gas flow rate	0.40 L/min

Stability and Sensitivity

To monitor the stability of the instrument, 1.0 µg/L bismuth (Bi) was added to the methanol eluent as an internal standard (ISTD). The 7500a was tuned for maximum sensitivity by optimizing the Bi signal. During the testing period of 10 hours, the RSD of the ISTD was less than 5%. Because the drift was minimal, there was no need for an ISTD correction. No Bi or any other ISTD was used for the actual analyses.

HPLC Column

For best results, precondition the HPLC column (ZORBAX Eclipse XDB-C18, 2.1 mm id × 50 mm, 5 µm) by pumping HPLC grade methanol at 0.4 mL/min for at least 2 hours, and then condition with eluent (same flow rate) for at least half an hour. Without this conditioning procedure, the inorganic Hg will be affected by contamination in the system leading to poor recovery or peak splitting.

Results and Discussion

Chromatographic Separation Using Standard Solutions

Using the operating conditions stated in Table 2a and Table 2b, a mixed Hg species standard in pure water was injected into the HPLC. The resulting total ion chromatogram (TIC) showed good separation of target species. See Figure 2.

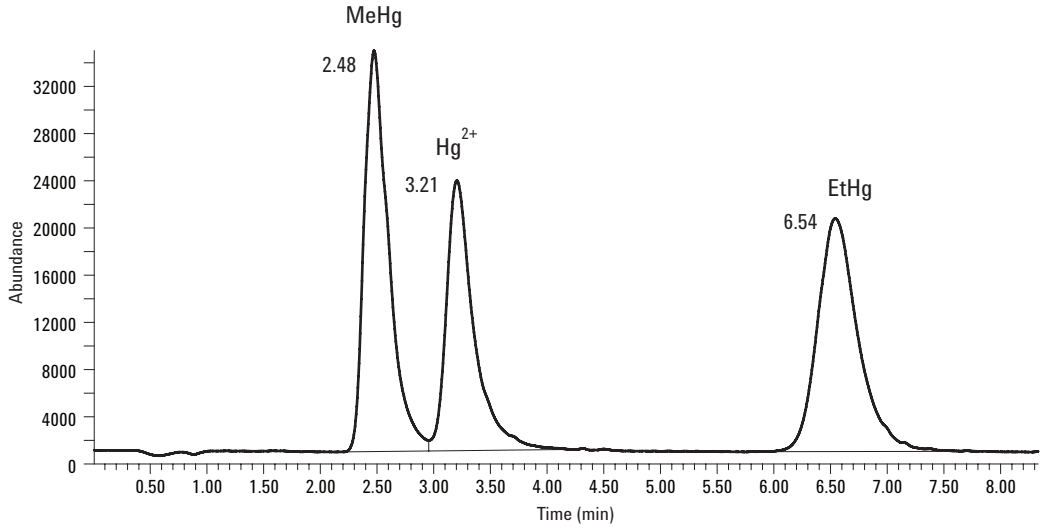

Figure 2. HPLC-ICP-MS TIC of three mixed Hg species standards in pure water: MeHg 2.48 min, Hg^{2+} 3.21 min, and EtHg 6.54 min, 100-µL loop. 1.0 ppb each.

Table 3. Integration Results of Hg Species Measurement by HPLC-ICP-MS

RT (min)	Species	Hg Conc. (µg/L)	Area
2.48	MeHg	1.0	5.09E+06
3.21	Hg^{2+}	0.8	3.87E+06
6.54	EtHg	1.0	4.91E+06

Efficiency of Detection

Because 1.0 µg/L of each Hg species was analyzed, the peak areas for the three species are similar (see Table 3). The slight difference is probably due to the purity of the standards or the error introduced during preparation of the standards.

One of the advantages of an HPLC-ICP-MS system is the ability of the argon plasma to decompose and ionize an element, irrespective of the chemical structure of the species. This independence of signal to original structure is called compound independence, and allows a calibration to be constructed based upon Hg molar concentration (Compound Independent Calibration or CIC). Reported results for all species identified will be fairly accurate, even when the compound is unidentified (unknown species).

Linearity

When the sample was diluted 100 times (10 ng/L for MeHg and EtHg, 8 ng/L for Hg^{2+}), the Hg species could still be measured, as shown in Figure 3. The retention times (RTs) also remained fairly stable. The minor peak with a RT of 1.35 min was caused by column contamination. When the column was cleaned with methanol, the peak disappeared.

The chromatogram in Figure 3 shows that the MDLs for the Hg species are better than 10 ng/L. If the eluent contamination problem can be solved by using higher purity reagents, sub ng/L MDLs will be achievable.

A series of calibration standards was prepared from 10 ng/L to 100 µg/L by diluting the mixed Hg species stock solution (1.0-µg/mL Hg for MeHg and EtHg, 0.8 µg/mL Hg for Hg^{2+} in pure water). A 20-µL injection loop was used throughout, except for the 10-ng/L data, which was obtained using a 100-µL loop. The peak areas were integrated for the different concentration levels of three mixed Hg species. The linear range of the calibration curves (Table 4 and Figure 4) for Hg speciation by the HPLC-ICP-MS method was at least 4 orders. This range covers expected real sample levels, and so the method is appropriate for direct determination of water samples without the application of complicated preconcentration procedures.

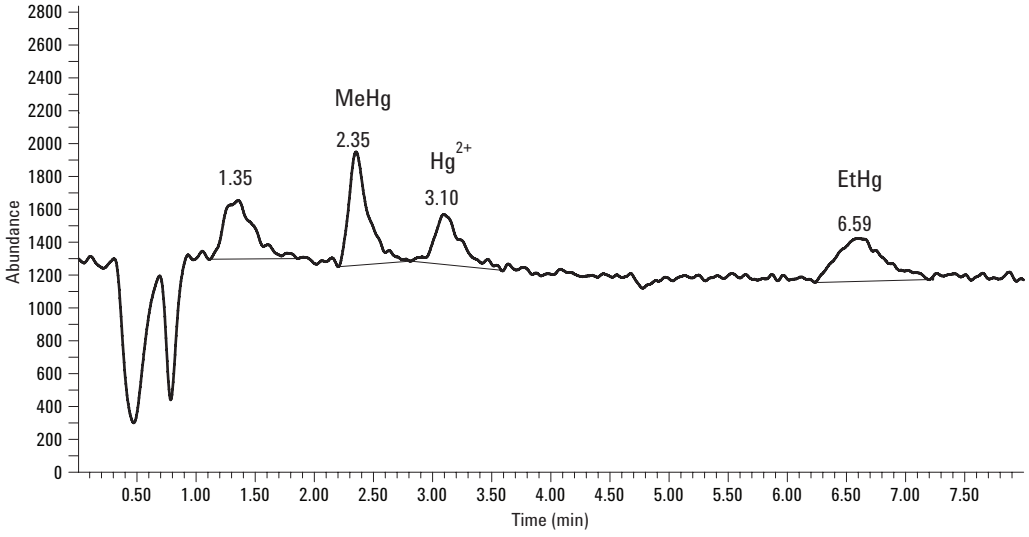

Figure 3. The HPLC-ICP-MS TIC of 10-ng/L Hg species standards in pure water. (Contaminant 1.35 min, MeHg 2.35 min, Hg^{2+} 3.10 min, and EtHg 6.59 min, 100-µL loop)

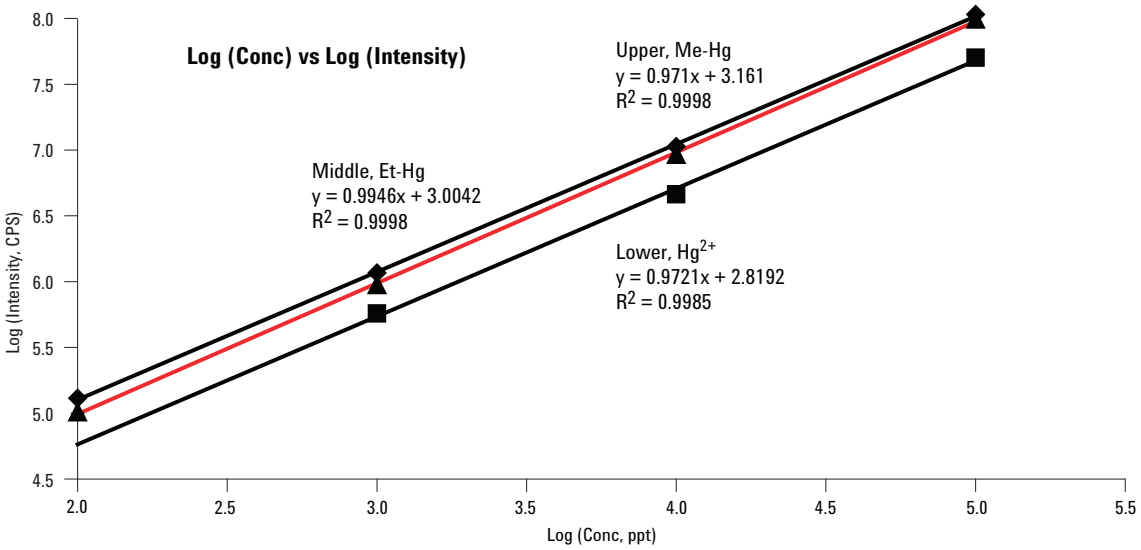

Figure 4. Calibration curves for MeHg, Hg^{2+}, and EtHg.

Table 4. Integration Peak Areas for Different Concentration Hg Species by HPLC-ICP-MS (Hg^{2+} Concentrations were 80% of the Shown Values)

Conc.(ng/L)	MeHg	Hg^{2+}	EtHg
100000	1.07E+08	5.03E+07	9.83E+07
10000	1.06E+07	4.61E+06	9.24E+06
1000	1.17E+06	5.72E+05	9.44E+05
100	1.30E+05	1.23E+05	1.02E+05
10	5.86E+04	3.55E+04	5.86E+04

Chromatographic Separation of Hg Species in 3% NaCl

In order to test the ability of the method for high matrix sample analysis, the stock Hg species solution was diluted into 3% NaCl (w/v in water) to obtain 100 ng/L MeHg, EtHg, and Hg^{2+}. The solution was filtered through a 0.45-µm membrane before analysis. A 20-µL injection loop was used for the measurement. The ^{202}Hg ion chromatogram was overlaid with the corresponding ion chromatogram of the pure water diluted solution at the same concentration, as shown in Figure 5. The peak areas of the Hg species in 3% NaCl were also integrated and the recoveries were between 90% and 110% relative to standards in pure water. This demonstrates that the method is suitable for even high matrix samples such as seawater.

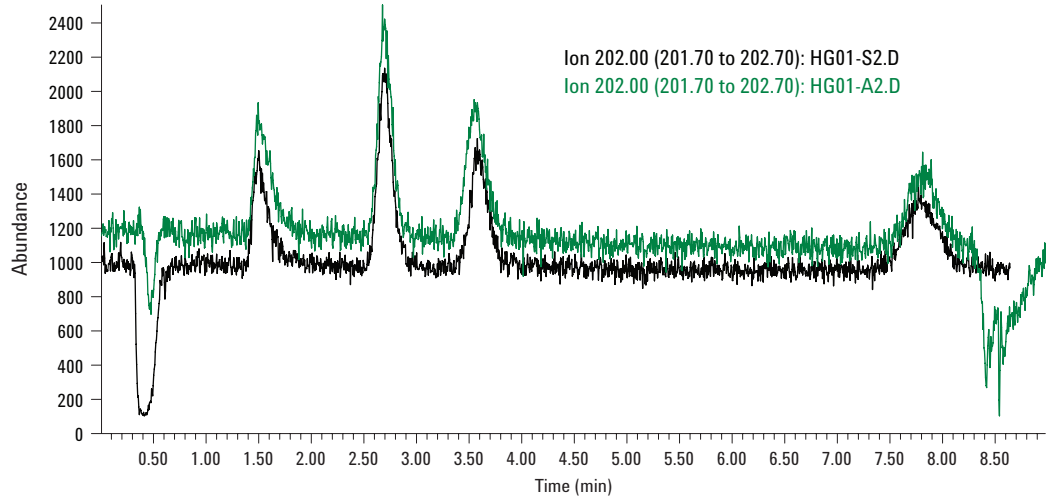

Figure 5. Overlaid HPLC-ICP-MS ion chromatograms for 100-ng/L Hg species standards in pure water (upper) and in 3% NaCl (w/v, lower) (20-µL loop).

Application to Soil Samples

When the HPLC-ICP-MS method is applied to solid samples such as tissues, soils, or sediments, sample preparation is necessary. The extraction of Hg species from the solid samples is a crucial step due to the presence of inorganic Hg in environmental samples at low levels. The Hg species, especially MeHg, are easy to lose or transform to other species. To prevent the possible destruction of the MeHg species in a digestion procedure, a number of methods have been reported, including extraction of the compound from samples using dilute hydrochloric acid or chelating agents [7]. Published methods for the extraction of both inorganic and organo-mercury compounds are time-consuming, labor-intensive, and require large amounts of high-purity solvents or special reagents. In the present work, a simple extraction method based on diluted hydrochloric acid was used. The spike recoveries of the soil samples were between 80% and 120%. Further testing of the method and the MeHg containing reference soil sample are planned.

Hg Species Extraction Method

1. Weigh 1.00 g soil sample into a 20-mL plastic centrifuge tube.
2. Spike 0 to 90 µL of a 100 ng/L mixed Hg species standard solution into the soil samples. Shake to mix.
3. Add 9.0-mL 7.6% HCl (w/v) and 1.0-mL 10% 2-mercaptoethanol to each tube. Place samples in an ultrasonic bath for 30 minutes to assist the extraction.
4. Centrifuge the samples at 3000 rpm for 5 minutes to partition the particulate matter.
5. Transfer 2.0 mL of the upper (clear) solution in to a 50-mL clean PET bottle. Add 15.0 mL of pure water.
6. Use 10% ammonia solution to adjust the pH of the solution to pH 6.8.
7. Add pure water to the solution until the final solution weight is 20.0 g.
8. Filter the solution through a 0.45-µm membrane before HPLC-ICP-MS measurement.

The HPLC-ICP-MS TIC for the soil sample extraction by 7.6% HCl is shown in Figure 6. The soil sample was spiked with 90-ng (as Hg) mixed Hg species standard. The peak height of Hg^{2+} in the spectrum is higher than the other two peaks because of the presence of inorganic Hg in the soil. Analysis of the unspiked soil sample showed an insignificant level of MeHg and EtHg. The measured results for the spike recovery test are shown in Table 5.

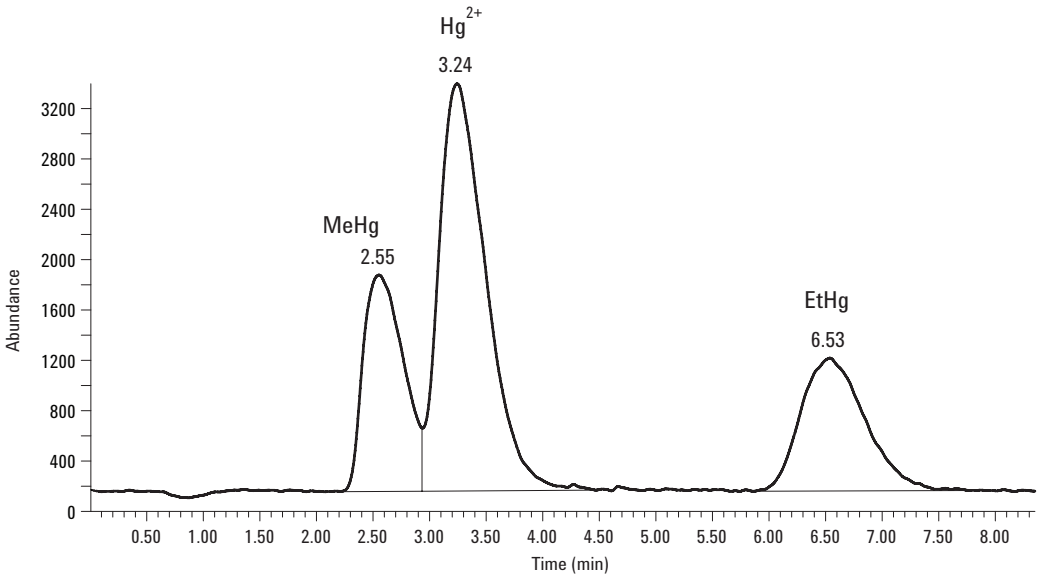

Figure 6. HPLC-ICP-MS TIC for soil sample, S-A-03, extracted by 7.6% HCl, spiked with 0.9-ng (as Hg) mixed Hg species standard. MeHg 2.55 min, Hg^{2+} 3.24 min and EtHg 6.53 min, 100-µL loop.

Table 5. Spike Recoveries of Hg Species in Soil Samples by HPLC-ICP-MS

Sample	Hg-Species	True Value (pg)	Measured value (pg)	Recovery (%)
S-BLK-1	MeHg	NA	2	NA
	Hg^{2+}	61	63	103
	EtHg	NA	+	NA
S-BLK-2	MeHg	NA	9	NA
	Hg^{2+}	61	65	107
	EtHg	NA	+	NA
S-A-03	MeHg	90	85	95
	Hg^{2+}	151	185	122
	EtHg	90	82	91
S-A-04	MeHg	90	80	89
	Hg^{2+}	151	181	120
	EtHg	90	75	83
S1-1	MeHg	36	34	94
	Hg^{2+}	97	88	91
	EtHg	36	28	77
S1-2	MeHg	36	37	104
	Hg^{2+}	97	105	108
	EtHg	36	35	97
S1-3	MeHg	36	41	113
	Hg^{2+}	97	98	101
	EtHg	36	43	120

NA Not applicable
+ No measurements made

The spike recoveries were all between ~80% and 120%, confirming the suitability of the sample preparation procedure for soil sample analysis by HPLC-ICP-MS.

Conclusions

HPLC-ICP-MS is appropriate for water samples analysis, even when the matrix in the water sample is high. The MDLs for MeHg, EtHg, and Hg^{2+} are better than 10 ng/L and meet current regulatory requirements. When the method is applied to soil samples, Hg species extraction by 7.6% HCl is appropriate, with recoveries between 80% and 120%.

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Printed in the USA
September 6, 2005
5989-3572EN

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Ion Chromatography (IC) ICP-MS for Chromium Speciation in Natural Samples

Application

Environmental

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Abstract

Trace measurements of the element chromium (Cr) are of interest in a wide range of applications and matrices. In the environment, Cr exists in two different oxidation states, the trivalent Cr(III) cation and hexavalent Cr(VI) anion. In mammals, Cr(III) is an essential element involved in the regulation of glucose; however, the element in its hexavalent form demonstrates mutagenic and carcinogenic effects at relatively low levels. Because of this duality, total Cr measurements do not provide sufficient information to establish potential toxicity. In order to assess the potential toxicity of the Cr level in a sample, it is the Cr(VI) concentration that must be measured, rather than the total Cr concentration. A new method was developed to couple Ion Chromatography to Octopole Reaction Cell ICP-MS (inductively coupled plasma mass spectrometry), to give a simple and reliable method for the separation and measurement of Cr(III) and Cr(VI), and so provide an accurate indication of the toxicity of the Cr level in a sample. This method has the merit of being applicable to high matrix samples, such as hard drinking water, due to the optimization of the sample preparation method and the chromatography. Also, the ICP-MS method provides excellent signal to noise, as a result of the removal of potentially interfering background species in the reaction cell, allowing the accurate determination of toxicologically useful levels of Cr(VI), at concentrations below 0.1 µg/L.

Introduction

The measurement of chromium toxicity is a requirement across a wide range of sample types, including drinking water, foodstuffs, and clinical samples (the latter used primarily to assess occupational exposure). However, it is the hexavalent form of Cr - Cr(VI) that is the toxic form, while the trivalent form - Cr(III) is an essential element for human nutrition. Methods to establish the potential toxicity of Cr must therefore determine the concentration of Cr(VI), rather than simply total Cr.

Two common approaches are used to address the issue: First, if the total Cr level measured is below the toxic level for Cr(VI), then it is reasonable to state that the Cr level will not be toxic, even if all of the Cr is present as Cr(VI). However, this approach can lead to a large number of false positives if samples contain a high concentration of Cr(III), so a more accurate approach is to separate and measure the Cr(VI) itself or, ideally, separate and measure both forms of Cr, giving an indication of the level of total Cr AND the level of toxic

Cr(VI), from a single analysis.

Separating and detecting individual forms or species of elements is usually a straightforward analytical challenge, but Cr is an unusual case in this respect. This is because the common forms of Cr in natural samples such as water are chromate (CrO_4^{2-}) for Cr(VI) and chromic ion (Cr^{3+}) for Cr(III). Chromate is an anion and the chromic ion is cationic, so a single ion exchange method will not work for both forms under the same conditions. A further problem is that Cr(III) is the most stable oxidation state in samples such as water, whereas Cr(VI) ions are strong oxidizing agents and are readily reduced to Cr(III) in the presence of acid or organic matter. Consequently great care must be taken during sample collection, storage and preparation, to ensure that the Cr species distribution present in the original sample is maintained up to the point of analysis.

Experimental

The method described in this application note used an optimized sample stabilization method, in which the samples were incubated at 40 °C with EDTA, which forms a complex with the Cr(III), allowing a single chromatographic method to be used to separate the Cr(III)EDTA complex and the Cr(VI). The reaction to form the Cr(III)EDTA complex is dependent on the incubation time and temperature, with complete conversion occurring after less than 1 hour at 60 °C or 3 hours at 40 °C. Complete conversion did not occur even after 7 hours incubation at room temperature.

Note that a relatively high concentration of EDTA is required for this method to be applicable to natural water samples, since other ions, such as Ca and Mg, which are commonly present at 10's or 100's mg/L in hard drinking water for example, would compete with the Cr(III) to form EDTA complexes, leading to low and matrix dependent Cr(III) recovery.

The combination of the separation of the Cr species using ion chromatography (IC), together with analysis of the separated species using ICP-MS, offers an ideal analytical method, as it permits the individual Cr species to be separated using a simple, low cost IC configuration. ICP-MS detection also allows the separated Cr species to be measured at extremely low concentrations, providing accurate assessment of exposure levels, even for natural or background Cr concentrations.

ICP-MS has excellent sensitivity and so is a good detector for many trace elements. The introduction of collision/reaction cells (CRC's) for ICP-MS allows Cr to be measured even more accurately and with better sensitivity, using the main isotope at mass 52, with removal of the primary matrix-based interferences ArC and ClOH. The sample preparation method, column type and chromatographic conditions used for Cr speciation are shown in Table 1. Note that, in addition to the stabilization of the samples with Na EDTA, EDTA was also added to the mobile phase, to stabilize the Cr(III) complex during separation. In addition, it was found that the use of pH 7 was essential for species stabilization and

Table 1. Chromatographic Conditions for Cr Speciation

Cr column	Agilent part number G3268A, 30 mm × 4.6-mm id
Mobile phase	5 mM EDTA (2Na), pH 7 adjust by NaOH
Flow rate	1.2 mL/min
Column temperature	Ambient
Injection volume	50~500 µL
Sample preparation	
Reaction temperature	40 °C
Incubation time	3 h
EDTA concentration	5~15 mM pH 7 adjust by NaOH

optimum chromatographic separation.

The IC configuration used for the work presented in this note is illustrated in Figure 1. Note that the nonmetal IC pump (Metrohm 818 IC Pump was used to deliver the mobile phase, but the sample loop was filled and switched using the optional Integrated Sample Introduction System (ISIS) of the Agilent 7500ce ICP-MS. While this configuration maintains the high precision and relatively high pressure of the IC pump, it provides a much simpler and lower-cost alternative to a complete IC or HPLC system,

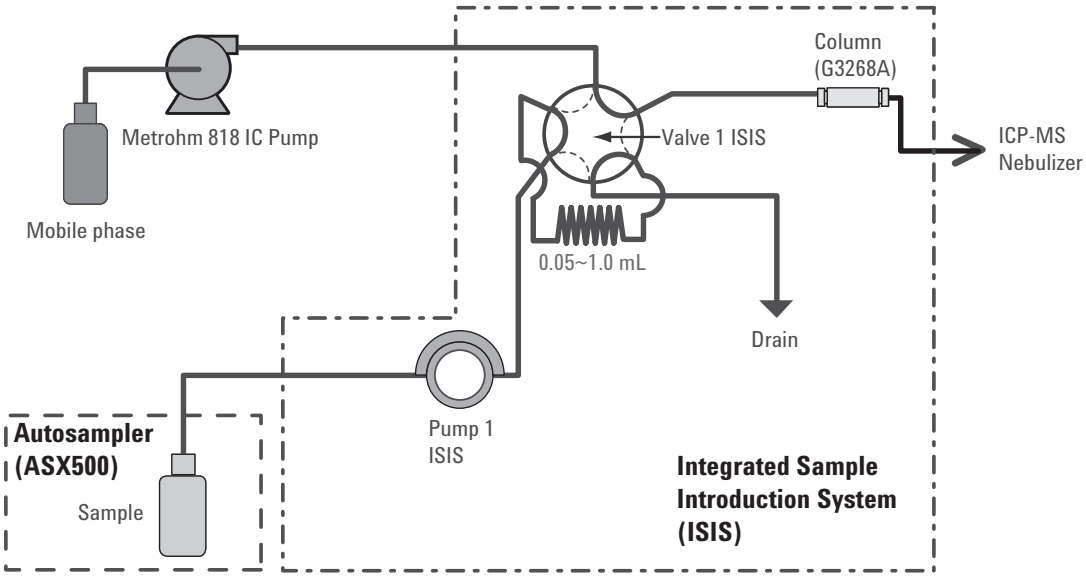


Figure 1. IC-ICP-MS configuration used for Cr speciation.

since only the IC pump module is required in addition to the ICP-MS system.

Results and Discussion

Under the conditions described above, with ICP-MS detection using the Agilent 7500ce in H_2 cell gas mode to remove the ArC and ClOH interferences on Cr at mass 52, detection limits (DLs) of <20 ng/L were obtained for the individual Cr species, as shown in Table 2. Many international regulations for hexavalent Cr specify a maximum allowable concentration of 1 µg/L, with a required DL of one-tenth of this level (100 ng/L), and even the small sample volume injection of 100 µL easily meets these

Table 2. DLs for Cr Species by IC-ICP-MS

	RT/min		Peak height/counts		Peak area/counts		DL (ng/L)*	
Inject/µL	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
50	0.79	2.09	8548	4261	1082295	914804	69.5	139.4
100	0.79	2.09	13688	7173	1704312	1525147	43.4	82.8
250	0.85	2.21	33967	20830	4939876	4546219	17.5	28.5
500	0.97	2.39	44870	37502	10268086	9398651	13.2	15.8

*Detection limits calculated as three times the peak-to-peak signal-to-noise as measured on standard chromatograms.

requirements. However, increasing the injection volume to 500 µL allowed the DLs to be reduced to 13.2 ng/L for Cr(III) and 15.8 ng/L for Cr(VI).

For a simple standard solution, these conditions give an excellent signal to noise for both Cr species, as illustrated in Figure 2. This chromatogram shows the separation of the two Cr species each at a concentration of 0.1 µg/L (ppb), using an injection

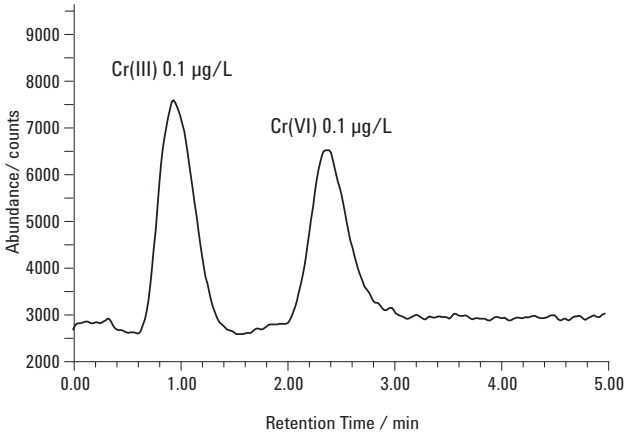


Figure 2. Separation and detection of Cr(III) and Cr(VI) at a concentration of 0.1 µg/L each species.

volume of 500 µL. Clearly the peaks are easily detected above the background and the baseline separation of the two species in a total time of about 3 minutes is also illustrated.

Using a series of synthetic standard solutions at low concentrations, a calibration was created for each of the two Cr species. Quantification was based on

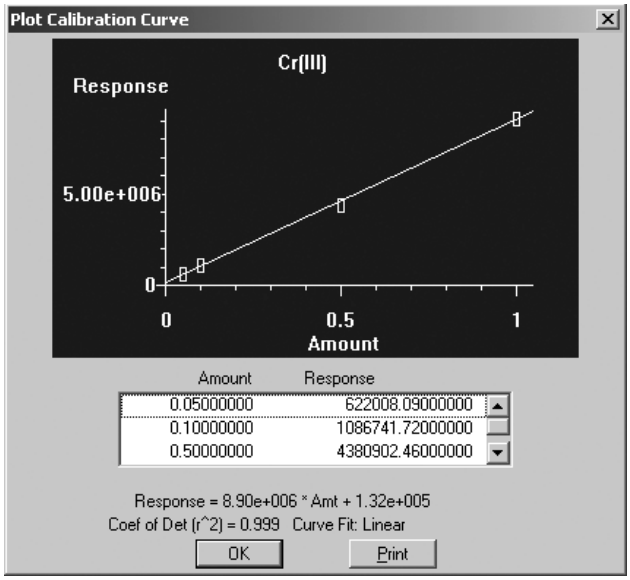


Figure 3. Calibration for Cr(III) - Standard concentrations 0.05, 0.1, 0.5 and 1.0 µg/L.

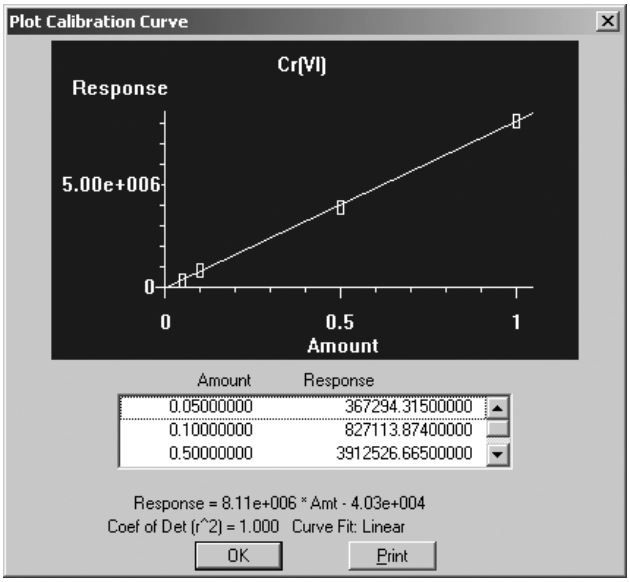


Figure 4. Calibration for Cr(VI) - Standard concentrations 0.05, 0.1, 0.5 and 1.0 µg/L.

peak area. The calibrations obtained for Cr(III) and Cr(VI) are illustrated in Figures 3 and 4, respectively, each showing excellent sensitivity and linearity.

In addition to sensitivity, species stability, chromatographic separation and calibration linearity, for the method to be suitable for routine analysis, it is essential that it provides acceptable long-term stability. In chromatographic analysis, stability is governed by two factors, RT stability and peak area stability. The data in Table 3 illustrates both of these parameters and indicates that the stability of the method is certainly acceptable for routine operation.

Routine Analysis

Of more interest for the routine analysis of chromium species (or specifically hexavalent Cr) in natural water samples is the maintenance of this excellent sensitivity, stability and chromatographic separation in samples that contain a high concentration of other ions. In order to test the suitability of the method for these real-world sample types, the method was applied to the determination of both Cr species in both spiked and unspiked mineral water samples.

The first sample evaluated was a leading French mineral water referred to in this study as mineral water A. Figure 5 shows the chromatogram of the two Cr species in the unspiked and spiked samples of mineral water A. The major element composition of the water is also shown in the table inset in Figure 5, indicating the typical drinking water com-

Table 3. Stability of RT and Peak Area for Multiple 500 µL Injections of 0.5 µg/L Each Cr Species

	RT/min		Peak height/counts		Peak area/counts	
Number	**Cr(III)-EDTA**	**Cr(VI)**	**Cr(III)-EDTA**	**Cr(VI)**	**Cr(III)-EDTA**	**Cr(VI)**
1	0.969	2.338	23514	18437	5331427	4621752
2	0.969	2.338	22642	18784	5280683	4758462
3	0.969	2.338	22832	18615	5220349	4742259
4	0.952	2.338	24104	19944	5470760	4800723
5	0.969	2.372	22797	19203	5287094	4726640
6	0.969	2.405	23830	19328	5498172	4760285
7	0.985	2.338	23971	19479	5481984	4824934
8	0.969	2.338	23393	19675	5474510	4883193
9	0.969	2.355	23070	20097	5355106	4892160
10	0.969	2.372	23826	19896	5428247	4886400
Avg	0.97	2.38	23398	19346	5382833	4789681
STD	0.008	0.014	534.45	581.88	100413.18	85782.42
RSD%	**0.80**	**0.57**	**2.28**	**3.01**	**1.87**	**1.79**

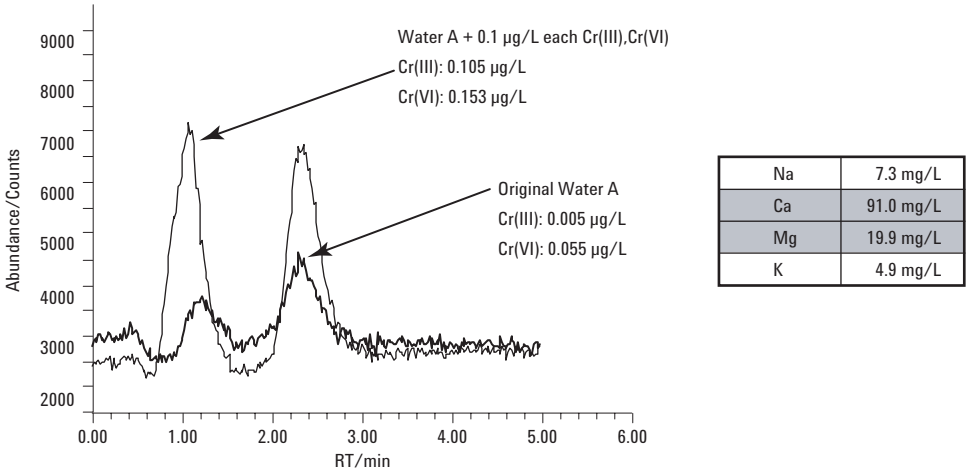


Figure 5. Major element composition (mg/L) and chromatogram for spiked and unspiked mineral water A.

position of about 100 mg/L Ca and between 5 mg/L and 20 mg/L of the other major elements K, Mg and Na.

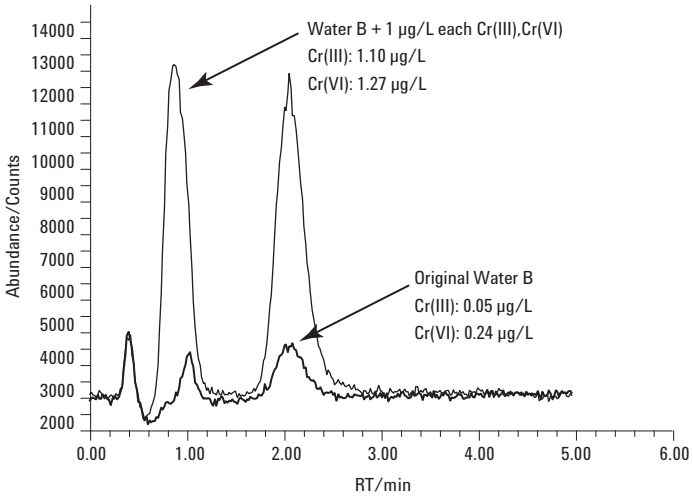
The spike recovery data for mineral water A is shown in Table 4, indicating the very low level at which the Cr species were quantified (0.005 µg/L

Table 4. Spike Recovery Data for 0.1 µg/L Spikes of Cr(III) and Cr(VI) in Mineral Water A

		Found (µg/L)		
Element	**Original mineral water A**	**Spike added**	**Spike found**	**Recovery (%)**
Cr(III)	0.005	0.10	0.105	100.0
Cr(VI)	0.055	0.10	0.150	95.0

and 0.055 µg/L for Cr(III) and Cr(VI), respectively), and the excellent spike recovery accuracy for the low concentration spikes in this sample - better than 5% error in both cases.

The second mineral water sample analyzed was another French mineral water, referred to as mineral water B, which has among the highest levels of calcium and sulfates of any commonly available mineral water (over 450 mg/L Ca and more than 1000 mg/L sulfates). As for the mineral water A sample, mineral water B was analyzed with and



Na	9.1 mg/L
Ca	486.0 mg/L
Mg	84.0 mg/L
K	3.2 mg/L

Figure 6. Major element composition (mg/L) and chromatogram for spiked and unspiked mineral water B.

without a spike of the two Cr species and the spike recovery was assessed. The results for the measured chromatograms are shown in Figure 6, while the spike recovery data are shown in Table 5.

As shown for the mineral sample A, the major element composition of the mineral water is shown as an inset in the chromatogram, illustrating the very high mineral levels in mineral water B. Despite these high major element levels, the optimized sample preparation and chromatographic method gave good chromatographic separation and excellent spike recovery results for both Cr species. A higher spike level was used for mineral water B, due to the higher baseline (unspiked) concentration for the Cr species in this sample.

The ability to recover low concentration spikes for both Cr species in such a high matrix sample indicates the effectiveness of the optimized method for sample stabilization, which ensures that a high enough concentration of EDTA is available for complete complexation of the Cr(III) species, even in the presence of a high level of competing ions.

Furthermore the accurate recovery of low concentration spikes of both species indicates that potential problems of species interconversion (reduction of Cr(VI) to Cr(III)) was avoided through the selection of an appropriate pH for the samples and the mobile phase, together with the use of EDTA in the mobile phase as well as for sample stabilization. See Table 5.

Conclusions

A new method for the stabilization and analysis of Cr(III) and Cr(IV) in natural, high matrix water samples was developed and optimized with DLs in the region of 0.05 µg/L for 100-µL injections, or 0.015 µg/L for larger, 500-µL injections.

Reliable and stable separation of the Cr(III) and Cr(VI) species was achieved in a method taking approximately 3 minutes per sample and the separation and accurate quantification of the two species could be maintained even in the presence of a high concentration of competing ions, such as >500 mg/L mineral elements in the highly mineralized water.

Accurate and interference-free determination of Cr at the low concentrations (0.1 µg/L) required by international regulations was made possible by the simple and consistent operation of the Agilent 7500ce in reaction mode, using H_2 as a cell gas. This mode of operation does not preclude the simultaneous analysis of other analytes of interest, such as As, in contrast to the use of highly reactive cell gases such as CH_4 or NH_3.

Table 5. Spike Recovery Data for 1.0 µg/L Spikes of Cr(III) and Cr(VI) in a Highly Mineralized Water (B)

		Found (µg/L)		
Element	**Original mineral water B**	**Spike added**	**Spike found**	**Recovery (%)**
Cr(III)	0.05	1.0	1.10	105
Cr(VI)	0.24	1.0	1.27	102

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Printed in the USA
April 27, 2005
5989-2481EN

Agilent Technologies

A Comparison of the Relative Cost and Productivity of Traditional Metals Analysis Techniques Versus ICP-MS in High Throughput Commercial Laboratories

Application

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Abstract

A financial model was developed to help the metals laboratory using graphite furnace atomic absorption and inductively coupled plasma optical emission spectroscopy calculate the potential savings by switching to inductively coupled plasma mass spectrometry. Results based on several typical laboratory examples are presented.

Introduction

The past 5 years have seen significant growth in the use of inductively coupled plasma mass spectrometry (ICP-MS) for the analysis of trace metals in many applications in the environmental, semiconductor, geological, and health sciences industries. This growth is driven by three factors. First is the need for increasingly lower limits of detection for many metals in many applications. Second is the significantly improved performance, reliability, and ease of use of modern ICP-MS instruments. And third is economics.

Traditionally, most elemental analysis has been performed by either atomic absorption (AA) or optical emission spectroscopy (OES). Generally, the ultratrace (sub-ppb) elements were measured by graphite furnace atomic absorption (GFAA), a highly sensitive single-element technique. The trace and minor (ppb to ppm) elements were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES), which is less sensitive but capable of simultaneous multi-element analysis.

As the need for sub-ppb detection limits extends to more elements in more samples, ICP-OES becomes less useful and the reliance on GFAA increases. However, GFAA, while sensitive, is slow, expensive to operate, and has limited dynamic range. Because GFAA is much slower than ICP-OES, many routine labs have a dedicated GFAA instrument for each analyte that is required to be measured by GFAA - multiple GFAAs working with one ICP-OES. Furthermore, the analysis of mercury will add the need for a third technique, either cold vapor AA or atomic fluorescence. However, in the interest of simplicity, a separate mercury analyzer was not considered in the examples used. Each of these techniques may require separate sample handling and preparation, as well as separate analysis, data processing and archival, significantly increasing the cost per sample.

The subject of this application note is to evaluate the productivity and cost effectiveness of ICP-MS as a routine, highly sensitive, multi-element technique where a single ICP-MS instrument has the potential to replace an ICP-OES, multiple GFAAs, and a mercury analyzer for most routine elemental analyses. The analytical applicability of ICP-MS to many types of samples is already well established. More recently, the introduction of the Octopole Reaction System on the 7500 Series ICP-MS instruments from Agilent has removed the final performance barriers that have prevented ICP-MS being proposed as a complete replacement for GFAA and ICP-OES.

Methods

To facilitate this study, a spreadsheet-based sample cost comparison model was developed in Excel. This tool allows the user to provide detailed parameters related to numbers and types of samples, as well as associated costs of sample preparation, instrumentation, and analysis. Output is simply cost of analysis per sample. Also reported are the total time required for sample analysis per month, the number of analysts required, and the number of instruments. The model compares the results for GFAA, ICP-OES, and ICP-MS. While it will allow almost any values to be entered for most parameters, the results presented here are based on values obtained from several commercial laboratories doing these analyses. No model can exactly predict the results for all situations and still be simple enough to be useful. Therefore, in the interest of simplicity, a number of assumptions were made in the design of the model and in the example data entered. We feel that the assumptions are realistic and do not impart significant bias on the results. The tool is easy to use and can allow a laboratory to quickly and simply evaluate the cost effectiveness of the three techniques based on laboratory-specific information.

Assumptions

- GFAA system costs US$30K
- ICP-OES system costs US$100K
- ICP-MS system costs US$180K
- Cost of funds (finance) is 6%
- General facilities costs, such as laboratory space, utilities etc., are ignored since they are difficult to estimate and do not significantly affect the results in most cases.
- An instrument operator can keep a modern, automated GFAA, ICP-OES, or ICP-MS running for two shifts (16 hours) per day. When analysis times exceed 16 hours per day for any technique, additional instrumentation and operators will be required. Instruments are added in increments of one; operators are added in fractions since it is assumed that they can be shared with other tasks in the laboratory and cost calculations are based only on the portion of time the operator spends on the specific analysis.
- GFAA is a single element technique. Instruments with multiple lamps still perform a single analysis at a time. Typical analysis time is 90 seconds per element and each element requires two replicate analyses (burns).
- ICP-OES and ICP-MS are multi-element techniques and the number of elements does not significantly effect the analysis time. This is not strictly true, but the assumption is reasonable for the sake of simplicity.
- GFAA will use pressurized argon and the consumption is 40 hours of use per cylinder ($100).
- GFAA graphite tubes and platforms cost $50 per set and last for 100 burns.
- ICP-MS and ICP-OES will use liquid argon and the typical consumption is 3 weeks of use per dewar ($250).
- ICP-MS detectors last typically for 3 years and the cost per year is amortized based on 3-year lifetime.

Results

Several typical laboratory scenarios were evaluated by varying the current instrument complement of the laboratory, and by varying the current and anticipated number of samples to be analyzed per month. Also examined was the effect of the number of elements that must be analyzed by GFAA (in the case of laboratories without ICP-MS) to meet required DLs.

Scenario 1

Laboratory currently has one GFAA plus one ICP-OES, which are paid for. ICP-MS must be purchased and amortized over 3 years. See Table 1.

Table 1. Scenario 1

Samples/ month	GFAA elements	# GFAA required	Cost/sample GFAA + ICP-OES	# ICP-MS required	Cost/ sample ICP-MS	Savings/ month
400	8	1	$41	1	$30	$4,536
1000	8	2	$33	1	$15	$18,196
5000	8	9	$31	2	$9	$112,968

Scenario 2

Laboratory currently has two GFAA plus one ICP-OES, which are paid for. ICP-MS must be purchased and amortized over 3 years. See Table 2.

Table 2. Scenario 2

Samples/ month	GFAA elements	# GFAA required	Cost/sample GFAA + ICP-OES	# ICP-MS required	Cost/ sample ICP-MS	Savings/ month
400	8	1	$41	1	$30	$4,536
1000	8	2	$32	1	$15	$17,283
5000	8	9	$31	2	$9	$112,055

Scenario 3

Laboratory currently has no instrumentation and must decide on purchasing GFAA plus ICP-OES versus ICP-MS. See Table 3.

Table 3. Scenario 3

Samples/ month	GFAA elements	# GFAA required	Cost/sample GFAA + ICP-OES	# ICP-MS required	Cost/ sample ICP-MS	Savings/ month
400	8	1	$51	1	$30	$8,491
1000	8	2	$37	1	$15	$22,151
5000	8	9	$32	2	$9	$116,923

Scenario 4

Comparison of costs per sample as a function of number of GFAA elements. (All instruments must be purchased.) See Table 4.

Table 4. Scenario 4

Samples/ month	GFAA elements	# GFAA required	Cost/sample GFAA + ICP-OES	# ICP-MS required	Cost/ sample ICP-MS	Savings/ month
1000	2	1	$24	1	$14	$9,601
1000	4	1	$28	1	$14	$12,751
1000	8	2	$38	1	$14	$22,151
1000	10	3	$42	1	$14	$27,490

Discussion

In all cases, even when the laboratory already owns two graphite furnaces and one ICP-OES (a common configuration) and must purchase the ICP-MS, the cost per sample is lower for ICP-MS. This is mainly due to the high cost of consumables for GFAA plus the fact that GFAA and ICP-OES requires two separate sample prep steps. Additionally, as the number of samples increases from a conservative number of 400 per month to 1000 and 5000 per month, the differential becomes much greater. This is caused by rapidly increasing labor costs for GFAA, as well as the much higher sample capacity of ICP-MS, lower consumables costs, and requirements for only a single sample prep.

Return on Investment for ICP-MS

A simple return on investment (ROI) can be calculated from the above tables. In this case, the cost per month of the new ICP-MS system is approximately US $5500.00 (assuming purchase price of US$180K financed for 3 years at 6%). Figure 1 shows the payback times for a laboratory that already owns two GFAAs and one ICP-OES as a function of the sample load. The y-axis represents the accumulated monthly savings of using ICP-MS versus GFAA + ICP-OES for three different sample loads compared to the unpaid balance on the ICP-MS instrument. As can be seen, the accumulated savings of ICP-MS is equal to the payoff amount after just 4 months when analyzing 2000 samples per month. Even when analyzing as few as 400 samples per month, the accumulated savings is sufficient to pay off the ICP-MS instrument in around 20 months. In this case, eight furnace elements are assumed. Other assumptions are as above.

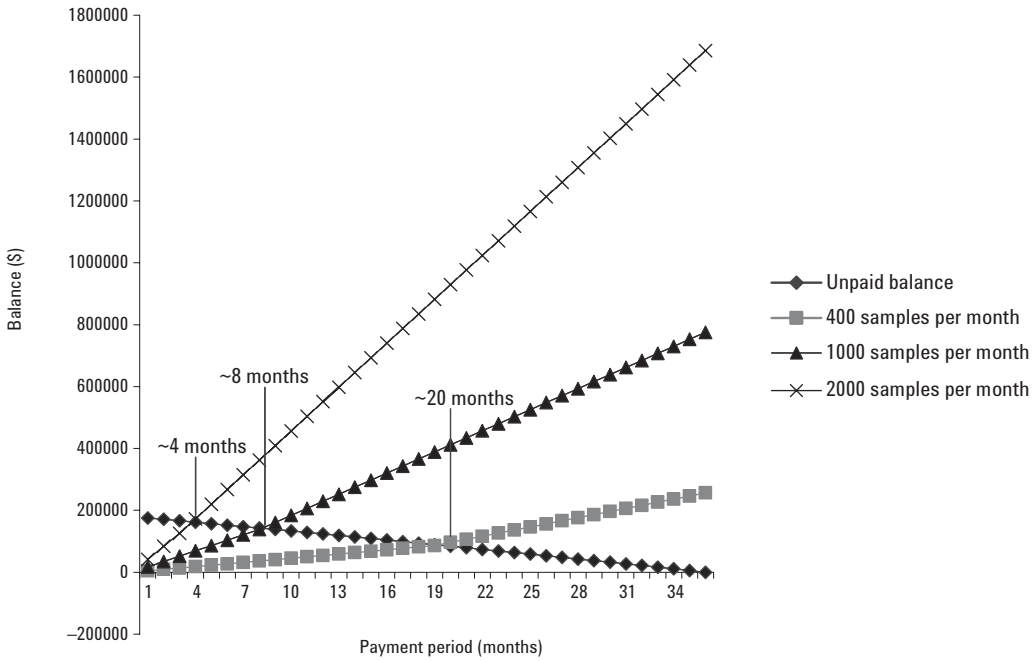

Figure 1. Cumulative return on investment of ICP-MS purchase for three sample levels plotted against the monthly unpaid balance on the ICP-MS. In this case, it is assumed that the accumulated revenue will be used to pay off the loan when the balance equals the residual loan amount. At that point, the net monthly revenue is increased by the loan amount. In this example, laboratories running 2000 samples per month will be able to pay off the ICP-MS in about 4 months, 1000 sample laboratories in about 8 months, and 400 sample laboratories in about 20 months. At the end of 36 months (the original loan period), net revenue exceeds $200K for the 400 sample lab, $750K for the 1000 sample lab, and $1.7 million for the 2000 sample lab.

Conclusions

For almost any metals laboratory, analyzing at least 100 samples per week (400 per month) and using a combination of GFAA and ICP-OES for the analysis, converting to ICP-MS will save money. Depending on the number of samples, the payback for the ICP-MS can be as short as a few months. The cost advantages are not reduced significantly, even if the laboratory already owns its GFAA and ICP-OES instruments. They are also not significantly affected by the number of GFAA elements. As Scenario 4 shows, for the laboratory analyzing at least 1000 samples per month with only two elements by GFAA, the cost savings of switching to ICP-MS is approximately $10,000 per month. Add to this the increased confidence in results obtained by ICP-MS, the ability to analyze all analyte elements at GFAA (or better) DLs, and the robustness and simplicity of operation of modern ICP-MS instruments, and the choice becomes simple. The productivity of ICP-MS in a high-volume laboratory can quickly pay off the purchase price and increase laboratory profitability significantly.

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Printed in the USA
January 17, 2005
5989-1585EN

Agilent Technologies

Analysis of High Matrix Environmental Samples with the Agilent 7500ce ICP-MS with Enhanced ORS Technology

Part 3 of a 3 part series on Environmental Analysis

Application

Environmental

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Abstract

The Agilent 7500ce ICP-MS was designed and optimized specifically to analyze unknown, high matrix samples. The 7500ce uses enhanced Octopole Reaction System technology for removal of interferences and improved ion optics for greater sensitivity than previous ORS instruments. This application note describes the performance of the instrument when analyzing various, high-matrix samples.

Introduction

This application note represents *Part Three* of the three part series of environmental application notes based on the Agilent 7500ce ICP-MS (inductively coupled plasma mass spectrometer). It examines its suitability for the routine analysis of trace metals in unknown high-matrix samples.

- Part one of this series details the theory of operation of the 7500ce ORS ICP-MS system and the related hardware and software [1].
- Part two is a drinking water application note demonstrating the ability of the Agilent 7500ce ICP-MS system to measure trace elements in drinking water substantially below regulated levels under challenging real-world conditions [2].

The experimental setup, instrument conditions, and sample sequence are described in *Part Two* [2]. The data for both application notes was acquired in a single 15.5 h sequence of samples including drinking waters, ground waters, synthetic seawaters, soil digests, and EPA interference check samples (ICS-A, ICS-AB). A single optimization, calibration and method were used for all samples as described in *Part Two*. Calibrations were not matrix-matched, and octopole reaction system (ORS) conditions were not optimized for a particular analyte or matrix. No mathematical interference correction equations were used. No re-optimizations, recalibrations or maintenance were performed during the sequence of samples. A graphic representation of the analytical sequence is displayed in Figure 1.

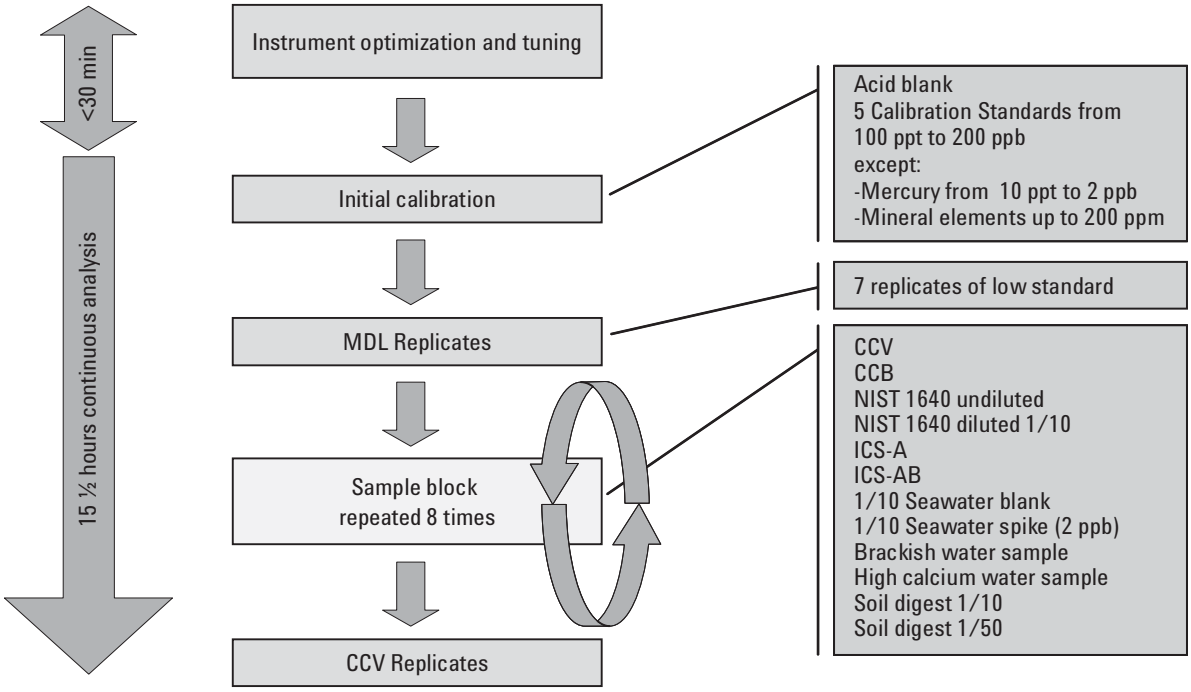

Figure 1. Analytical sequence.

Analytical Challenges

Since the inception of ICP-MS, numerous difficult challenges have slowed its complete adoption over the more traditional techniques of graphite furnace atomic absorption (GFAA) and ICP optical emission spectroscopy (ICP-OES) in the environmental monitoring industry. In the analysis of high matrix samples including soils, sludges, industrial wastes, and even food samples by ICP-MS, the principal obstacles have been overcoming interferences and improving stability. Numerous approaches* have had incomplete success at resolving these problems. More recently, the use of collision/reaction cells (CRCs) to remove interferences has had good success [3]. However, CRCs alone cannot completely eliminate the detrimental effects of high matrix samples on the ICP-MS instrument. This is because in addition to the formation of polyatomic interferences, high matrix samples can have other negative effects on the plasma, interface, and mass spectrometer of the ICP-MS. These include ionization suppression, reduced ion transport efficiency, and matrix deposition in the interface, ion optics, and mass spectrometer that can affect sensitivity, and stability. In order to overcome these obstacles, the ideal environmental ICP-MS must have excellent matrix tolerance, the ability to remove interferences, high sensitivity, and wide dynamic range. It must possess these attributes for a variety of unknown and varied matrices, for all analytes using a simple, universal set of conditions. The Agilent 7500ce was designed specifically to address these challenges. A new ion optic and a highly efficient on-axis ORS easily and effectively eliminate polyatomic interferences. Robust plasma conditions due to the use of high RF power (1500-1600 W), efficient RF coupling, and a cooled, low-flow sample introduction system minimize the effects of matrix on the ICP-MS interface. Hardware and software details are covered in *Part One* of this series.

*Techniques used to control the effects of sample matrix on the ICP-MS have included the use of mathematical interference equations, aerosol desolvation, high efficiency nebulizers, various means of controlling plasma temperature and secondary ionization in the interface and even high-resolution mass spectrometry. None were completely successful at eliminating interferences and other matrix effects.

In summary, improvements in ion optic and octopole design, created specifically for the environmental laboratory have resulted in an ICP-MS instrument with unprecedented sensitivity, matrix tolerance and stability [1]. By using a highly efficient octopole reaction cell and careful control of ion energy, most polyatomic interferences can be removed under a single set of generic conditions using helium-only collision mode with kinetic energy discrimination. A few argon-based polyatomics are more efficiently removed using pure hydrogen in reaction mode.

Experimental

Detailed experimental conditions are discussed elsewhere [2]. Instrumental conditions are outlined in Table 1. This work was designed to replicate the workload in a typical environmental laboratory where sample matrices vary widely and are frequently unknown. Under these conditions, it is not practical to matrix-match calibrations to multiple sample matrices. It is also not practical to depend on matrix-specific or analyte-specific reaction cell conditions. The data shown in this note were all generated using a single set of calibration standards in 1% HNO_3/0.5% HCl. Calibration was performed once only at the beginning of the sequence and not repeated or updated during the sequence. No attempt at matrix matching either the calibration standards or CRC conditions was made. No mathematical interference corrections were employed and all analytes were measured at their elemental masses.** The instrument was tuned for robust plasma conditions*** resulting in sensitivity of approximately 50 million cps/ppm at mid-mass with background less than 5 cps, CeO^+/Ce^+ less than 1% and Ce^{++}/Ce^+ less than 1.5%. The samples included a natural water certified reference material (CRM), NIST 1640, a 1/10 diluted synthetic seawater and low-level spike, as well as various ground waters and soil samples. In addition, the US Environmental Protection Agency (EPA) ICS-A and ICS-AB were used to simulate a challenging high matrix reference material. This was done due to the lack of suitable, prepared CRMs for high matrix samples. Rather than introduce extraction efficiency into recovery calculations of nonprepared samples, it was decided to use a well-characterized sample designed to simulate a difficult waste sample digestate. ICS-A contains high concentrations of elements known to cause interferences in ICP-MS. It is intended to test the ability of the ICP-MS system to compensate for both spectral and nonspectral interferences. ICS-A also contains sufficient total dissolved solids (TDS) to test the robustness of the ICP-MS interface and ion optics to salt buildup. ICS-AB is a spiked ICS-A sample intended to test the ability of the system to accurately detect low-level analyte elements in this challenging matrix. The composition of ICS-A and ICS-AB are listed in Table 2. Table 3 depicts the ORS mode each element was acquired in. Details of hardware and reagents are described elsewhere [1]. All are typical of a routine commercial environmental laboratory. The accuracy and precision of the repeat analyses of each sample type over the entire sequence were monitored.

**Some CRC ICP-MS systems depend on the use of reactive gases to deliberately form polyatomic species of certain analyte elements. In this way the element is "shifted away" from the interference to another mass. However, the rate of formation of the polyatomic species can be concentration and matrix dependent resulting in potentially inaccurate results in variable or unknown matrices.

***Robust plasma conditions are defined as those promoting the most complete atomization and ionization of analyte and matrix components, minimizing polyatomic interferences and the deposition of salts on the interface and mass spectrometer. The generally accepted measure of plasma robustness is the ratio of CeO^+/Ce^+ when Ce is introduced. The ratio should be as low as possible, ideally less than 1%, indicating excellent breakdown of metal oxides (and therefore, other matrix interferences) in the plasma.

Table 1. Instrument Conditions Used for All Samples for Maximum Plasma Robustness and Polyatomic Interference Removal. No Analyte-Specific Settings Were Required.

Instrument parameter	Normal mode	Hydrogen mode	Helium mode
RF Power	1500 W	<Same	<Same as H_2
Sample depth	8 mm	<Same	<Same as H_2
Carrier gas	0.85 L/min	<Same	<Same as H_2
Makeup gas	0.2 L/min	<Same	<Same as H_2
Spray chamber temp	2 °C	<Same	<Same as H_2
Extract 1	0 V	<Same	<Same as H_2
Extract 2	–160 V	<Same	<Same as H_2
Omega bias	–24 V	<Same	<Same as H_2
Omega lens	–0.6 V	<Same	<Same as H_2
Cell entrance	–30 V	<Same	<Same as H_2
QP focus	3 V	–11 V	<Same as H_2
Cell exit	–30 V	–44 V	<Same as H_2
Octopole bias	–7 V	–18 V	<Same as H_2
QP bias	–3.5 V	–14.5 V	<Same as H_2
Cell gas flow	0	3.0 mL/min H_2	4.5 mL/min He

Table 2. Composition of EPA Interference Check Samples, ICS-A and ICS-AB

Solution component	Comment	Solution A concentration mg/L	Solution AB concentration mg/L
Al	Possible interference with Ni as AlCl	100	100
Ca	Interferes with Fe as CaO	300	300
Fe	Can interfere with Zn and Se as FeN and FeOH	250	250
Mg	Interferes with Ca, Ni, and Cu as MgCl	100	100
Na	Interferes with Cu as ArNa	250	250
P	Interferes with Cu and Ti as PO2 and PO	100	100
K	Easily ionized, suppresses Hg, As, Se, Zn, Cd, etc.	100	100
S	Interferes with Ti as SO, SOH	100	100
C	Interferes with Cr as ArC	200	200
Cl	Interferes with As, Se, Cr, Co, Cu, Ba, etc. as various chlorides	2000	2000
Mo	Interferes with Cd as MO	2	2
Ti		2	2
As		0	0.02
Cd		0	0.02
Cr		0	0.02
Co		0	0.02
Cu		0	0.02
Mn		0	0.02
Hg		0	0.02
Ni		0	0.02
Se		0	0.02
Ag		0	0.02
V		0	0.02
Zn		0	0.02

Table 3. Summary of Analyte Masses, Analytical Conditions and Method Detection Limits in Both Screening Mode and Full Quantitative Mode for Regulated Elements

Analyte	Isotope	ORS mode (typical)*	Integration time (s)	Calibration range (ppb)	MDL screening (ppt)**	MDL Tri-Mode (ppt)†
Calcium (Ca)	40	H2	0.3	50–200,000	-	16.2
Iron (Fe)	56	H2	0.3	50–200,000	31.6	19.9
Selenium (Se)	78	H2	1.5	0.5–100	117.2	16.3
Sodium (Na)	23	He	0.3	50–200,000	55.2	55.2
Magnesium (Mg)	24	He	0.3	50–200,000	24.6	24.6
Potassium (K)	39	He	0.3	50–200,000	785.8	785.8
Vanadium (V)	51	He	1.5	0.5–100	32.6	32.6
Chromium (Cr)	52	He	1.5	0.5–100	27.1	27.1
Nickel (Ni)	60	He	1.5	0.5–100	25.6	25.6
Copper (Cu)	63	He	1.5	0.5–100	12.7	12.7
Arsenic (As)	75	He	1.5	0.5–100	45.2	45.2
Beryllium (Be)	9	Norm	0.3	0.5–100	113.2	26.5
Boron (B)	10	Norm	0.3	0.5–100	125.7	35.1
Aluminum (Al)	27	Norm	0.3	0.5–100	131.4	23.7
Manganese (Mn)	55	Norm	0.3	0.5–100	26.8	16.2
Cobalt (Co)	59	Norm	0.3	0.5–100	28.1	18.0
Zinc (Zn)	66	Norm	0.3	0.5–100	33.7	24.3
Molybdenum(Mo)	95	Norm	0.3	0.5–100	22.4	20.4
Silver (Ag)	107	Norm	0.3	0.5–100	18.2	15.4
Cadmium (Cd)	111	Norm	0.3	0.5–100	45.3	27.9
Tin (Sn)	118	Norm	0.3	0.5–100	51.2	14.0
Antimony (Sb)	121	Norm	0.3	0.5–100	51.2	13.7
Barium (Ba)	137	Norm	0.3	0.5–100	32.6	15.7
Mercury (Hg)	202	Norm	3.0	0.01–2.0	13.6	7.3
Thallium (Tl)	205	Norm	0.3	0.5–100	29.7	13.0
Lead (Pb)	208††	Norm	0.3	0.5–100	30.8	10.4
Thorium (Th)	232	Norm	0.3	0.5–100	27.5	12.0
Uranium (U)	238	Norm	0.3	0.5–100	29.3	10.2
Useful ISTDs						
^{6}Lithium (Li)	6	Norm	0.3	50 ppb		
Scandium (Sc)	45	All	0.3	50 ppb		
Germanium (Ge)	70,74	All	0.3	50 ppb		
Indium (In)	115	Norm	0.3	50 ppb		
Terbium (Tb)	159	Norm	0.3	50 ppb		
Platinum (Pt)	195	Norm	0.3	50 ppb		
Bismuth (Bi)	209	Norm	0.3	50 ppb		

*Typical ORS mode selected for best overall performance for most common matrices.

**Screening protocol uses He collision mode only for rapid screening where optimum sensitivity is not required for all elements, MDLs calculated according to EPA 200.8 requirements

†Method detection limits calculated according to EPA 200.8 requirements. Three sigma of seven replicate analyses of a fortified blank at 3-5 times the estimated MDL. MDLs are reported in ng/L (ppt) for ease of presentation

††Lead is measured as the sum of isotopes 206, 207, and 208 to eliminate error due to variable isotope ratios.

Results and Discussion

Analysis of Spiked Sea Water

In addition to the water CRM described in detail in *Part Two* [2], and the high TDS ground water samples, the sequence included replicate analyses of spiked synthetic seawater samples. The synthetic seawater consisted of 0.3% high purity sodium chloride solution (SPEX Certiprep) to simulate 1/10 diluted seawater. The synthetic seawater was spiked with 2 ppb of the trace elements and 200 ppb of Mg. Spike recoveries were calculated for all elements and are shown in Figure 2 and Table 5. Saline waters are a particularly challenging matrix due to potential Ar, Na, and Cl-based interferences on Cu, As, Se, V, and Ni (Table 4). Significant suppression of high ionization potential elements such as Zn, Cd, and Hg can also limit the sensitivity for these elements. The maximization of plasma temperature and use of well-matched internal standards (ISTD) is necessary to avoid this suppression. Typical recoveries (Table 5) are 90% or greater for most elements with the exception of Ag, which has limited solubility in chloride solutions. Long-term stability as measured by %RSD of eight replicate analyses over the 15.5-hour sequence is excellent, indicating no cumulative effects of long-term exposure to high TDS samples on the analytical accuracy, even at low (2 ppb) concentrations.

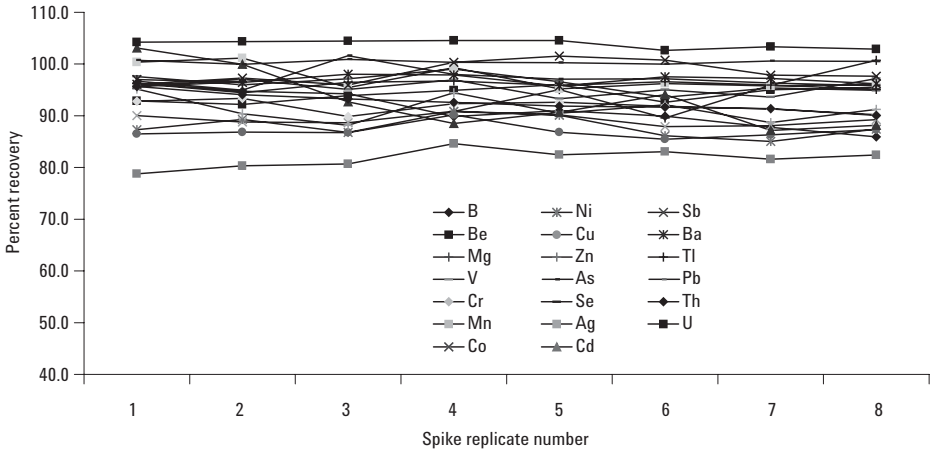

Figure 2. Spike recoveries in 1/10 synthetic seawater for eight replicate spikes at 2 ppb for trace elements and 200 ppb for magnesium measured over 15.5-hour sequence.

Table 4. Possible Polyatomic Interferences in Typical Environmental Samples and the ORS Mode Used to Eliminate Them

Analyte Isotope	Principal Interferences	Corrective ORS Mode
^{24}Mg	$^{12}C^{12}C$	He
^{27}Al	$^{12}C^{14}N^{1}H$	He
^{40}Ca	^{40}Ar	H_2
^{51}V	$^{35}Cl^{16}O$	He
^{52}Cr	$^{40}Ar^{12}C$, $^{35}Cl^{16}O^{1}H$, $^{36}Ar^{16}O$	He
^{55}Mn	$^{40}Ar^{14}N^{1}H$, $^{38}Ar^{17}O$	He
^{56}Fe	$^{40}Ar^{16}O$, $^{40}Ca^{16}O$	H_2 or He
^{60}Ni	$^{44}Ca^{16}O$, $^{23}Na^{37}Cl$, $^{43}Ca^{16}O^{1}H$, ArS	He
$^{(63,65)}Cu$	$^{40}Ar^{23}Na$, SO_2	He
$^{(64,66,68)}Zn$	SO_2, ArS	
^{75}As	$^{40}Ar^{35}Cl$, $^{40}Ca^{35}Cl$	He
$^{(78,80)}Se$	$^{40}Ar^{38}Ar$, SO_3	H_2

Table 5. Spike Recoveries and %RSDs for Eight Replicate Analyses of 1/10 Synthetic Seawater over a 15.5-Hour Period

Element	B	Be	Mg	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Ag	Cd	Sb	Ba	Tl	Pb	Th	U
Recovery % (mean)	91	94	95	96	92	97	89	88	87	91	97	95	82	93	98	97	96	100	93	104
%RSD	4.0	1.5	3.9	1.8	1.4	2.5	1.2	2.3	1.6	2.7	2.1	1.6	2.2	6.2	2.2	0.8	0.8	0.3	1.9	0.8

Analysis of EPA ICS-A and ICS-AB

Of the samples analyzed, the ICS-A and ICS-AB samples were the most demanding. A total of 16 analyses of these samples was performed over the course of the sequence. Under routine conditions, a laboratory in the US analyzing waste samples would be required by EPA method 6020 to analyze a single ICS pair with each sequence or every 12 hours of sample analysis. Because of the difficulty of this analysis, no control limits are specified for recovery of analytes in the ICS-AB spiked solution, corrective action being left to the judgment of the laboratory QA manager [4]. In this work, all elements showed excellent recovery, most between 90%–105%, over the entire sequence (Figure 3). No mathematical interference correction equations were used and all analytes were measured at their elemental masses. No reslope or recalibration was performed by the ChemStation. There is no evidence of drift from the beginning to the end of the sequence. Examination of the results of ICS-A in Figure 4 shows very low levels of analytes (<1 ppb), even though no interference correction equations were used and most elements were acquired in the generic He collision mode. Previous determinations of this standard using multiple isotopes per element have shown that most of the "interferences" are actually low-level contaminants.

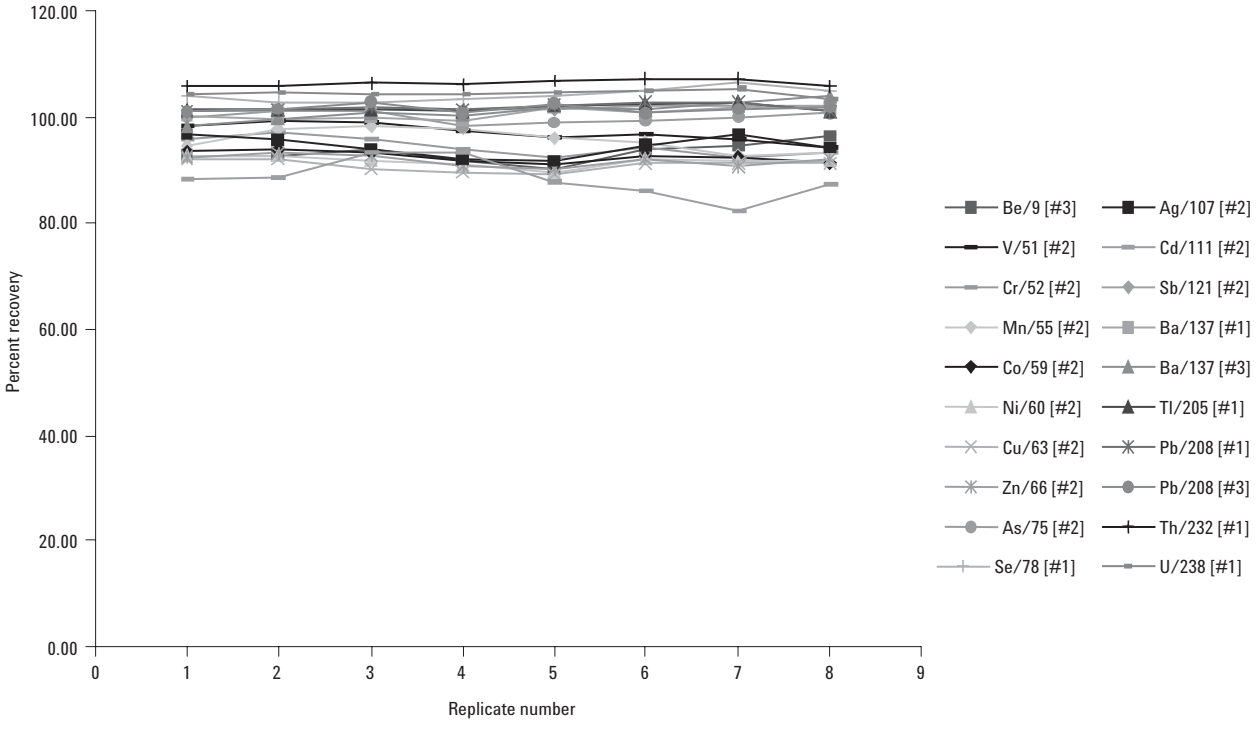

Figure 3. Recovery of analytes in EPA ICS-AB mix. Analytes (B) are spiked into ICS-A at 20-ppb each. Eight replicate analyses distributed over 15.5 hours.

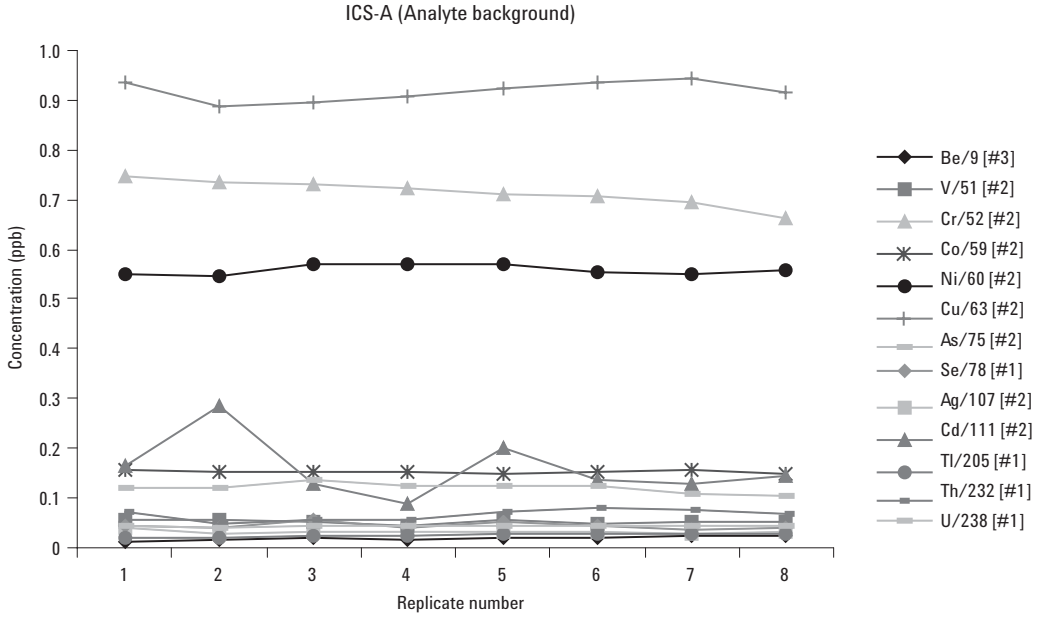

Figure 4. Measurement of apparent analyte concentrations in ICS-A replicates in ppb. No interference correction equations were used. Inspection of multiple isotopes indicates that apparent interferences were due to contaminants rather than interferences.

ISTD Response

All environmental ICP-MS methods recommend or require the use of ISTDs to correct for both mass-dependent long-term drift of instrument response as well as suppression or enhancement within a particular sample. These nonspectroscopic matrix effects are common in ICP-MS, particularly in older design instruments and when analyzing high matrix samples. EPA methods use ISTD responses in samples and the ICS solutions to monitor these effects. By setting control limits on ISTD response, the sensitivity of the instrument can be monitored and controlled. If the ISTD response falls outside the recommended control limits, the sample must be diluted to reduce matrix effects and reanalyzed. The control limits vary with the method and sample type. Figure 5 provides ISTD recoveries relative to the calibration blank for all ISTD elements in all samples of the sequence. EPA method 200.8, a drinking water method, mandates the strictest limits (60%–125%) over the course of the samples run. The EPA method for waste analysis, EPA 6020, in its most recent version, 6020a, specifies only a lower recovery limit of >30%. This is based on the knowledge that waste samples will typically display more severe nonspectroscopic interferences than clean drinking-water samples. The interference check solutions are an excellent indicator of the instruments ability to tolerate such interferences. Figure 5 illustrates the ISTD recoveries for the ISTDs, ^{6}Li, Sc, Ge, In, Tb and Bi in all three ORS modes for all samples of the sequence. The cyclic appearance is due to the repeated nature of the samples. (eight replicate analyses of sample group). The lowest recoveries in each block are for ICS-A and ICS-AB (approximately 80%) which are well within even the acceptable range for drinking water (60%–125%) and do not approach the lower EPA limit for waste samples. It is important to note that the ISTD response recovers immediately after each ICS sample indicating an absence of residual matrix effects. The small amount of gradual drift seen near the end of the 15.5-hour sequence would be corrected automatically by the ChemStation via periodic recalibration if necessary, though most analytical sequences do not approach the duration or difficulty of this one. The plots in Figure 5 show that the average sensitivity of the instrument has not changed from the beginning of the sequence to the end. The relatively minor divergence in ISTD responses is due to a slight shift in mass response toward greater high mass sensitivity at the cost of low mass sensitivity as a result of conditioning the interface with high TDS samples. Since high mass sensitivity is generally more critical, this shift is usually acceptable, even desirable. However, a simple adjustment in the extraction lens voltage is all that is required to return the system to the original condition if necessary.

Calibration Stability

Good laboratory practices require the monitoring of calibration accuracy for all analytes over the course of the sequence. This is normally accomplished by periodically analyzing a midpoint calibration standard as an unknown and comparing the result with the known value. Typically control limits of ±10 percent are set for acceptance of the continuing calibration verification (CCV) result. If the CCV sample results fall outside the 10 percent limit for any element, then sample results for that element will be inaccurate. If this occurs, the system must be recalibrated and any samples analyzed under the out-of-control conditions must be reanalyzed. CCV recoveries for all analytes over 13 replicate analyses are shown in Figure 6. In no case did any analyte recovery fall outside the ±10 percent limit. Had this occurred, the ChemStation would have automatically determined the degree of the failure, resloped or recalibrated the method as needed and rerun any out-of-control samples. This was, however, unnecessary, as stated earlier.

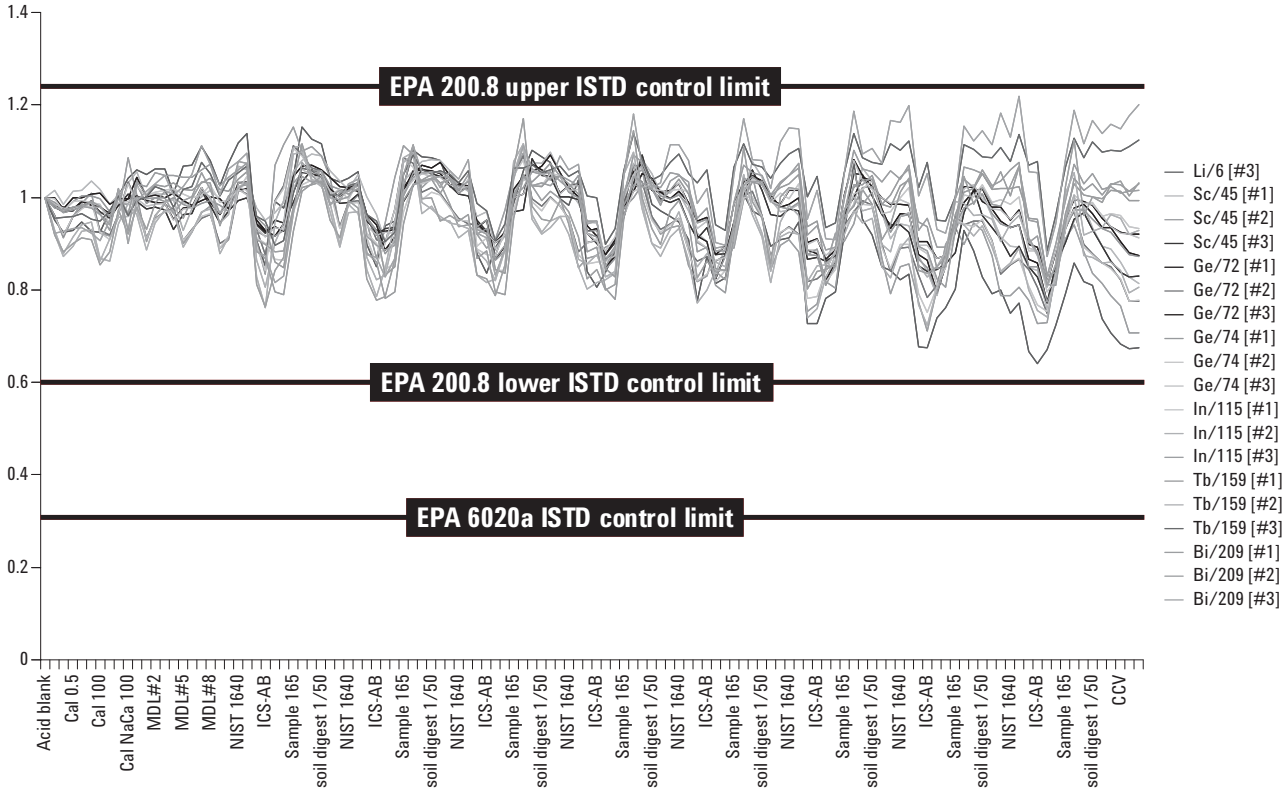

Figure 5. ISTD recoveries for all ISTD used in three ORS modes (mode indicated by number to the right of IS mass, 1 = hydrogen, 2 = helium, 3 = normal). Control limits for EPA methods 200.8 (Drinking Waters) are 60%–125% relative to the calibration blank, EPA 6020a (Wastes) has only a lower control limit at 30% relative to the calibration blank.

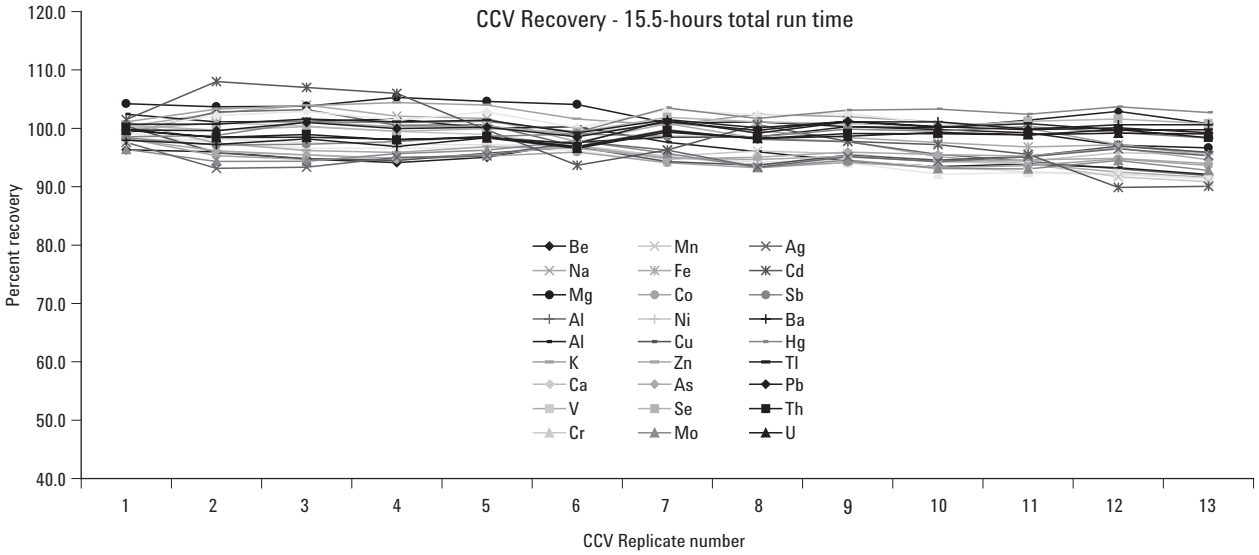

Figure 6. Results of 13 separate analyses of the CCV sample over the 15.5-hour sample sequence. All analyte elements are reported. Acceptable control limits according to US EPA method 6020 ±10%. At no time did any element fall outside the 10% control limits.

Conclusions

The Agilent 7500ce ICP-MS was designed specifically to meet the demanding requirements of environmental laboratories worldwide that must adhere to rigorous regulatory requirements while analyzing a wide range of difficult and unknown sample types with the highest sample throughput. Using ORS technology operating predominantly in He-only collision mode, the 7500ce is easy to set up and operate and delivers unprecedented performance in a wide range of unknown sample types.

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3. E. McCurdy and G. Woods (2004) "The Application of collision/reaction cell inductively coupled plasma mass spectrometry to multi-element analysis in variable sample matrices, using He as a non-reactive cell gas," *JAAS*, **19** (3).
4. US EPA Method 6020a, revision 1, January 1998.

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Printed in the USA
April 28, 2004
5989-0915EN

Real World Analysis of Trace Metals in Drinking Water Using the Agilent 7500ce ICP-MS with Enhanced ORS Technology

Part 2 of a 3 part series on Environmental Analysis

Application

Environmental

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Abstract

ICP-MS can be used as a powerful screening tool for the presence of toxic elements and chemicals in the environment. The biggest challenge in environmental ICP-MS is obtaining precise, accurate measurement in the large range of concentrations encountered in waters. Adding to this problem are interferences on important metals including As, Se, Cr, V, and Fe. Most developed countries have implemented programs and regulations to ensure the quality of public water systems, but without accurate, precise measurement of water samples, these requirements are difficult to meet. The Agilent 7500ce ICP-MS is specifically designed to meet these demanding regulatory requirements of environmental laboratories worldwide by analyzing a wide range of difficult and unknown sample types in the shortest amount of time. Using advanced ORS technology based on the use of simple gases, the Agilent 7500ce makes it possible to analyze multiple sample types over a wide range of analyte and matrix concentrations at well-below regulated concentrations, all in a single sequence.

Introduction

This application note represents part two of the three part series of environmental application notes based on the Agilent 7500ce ICP-MS.

- Part one details the theory of operation of the 7500ce octopole reaction system (ORS) inductively coupled plasma mass spectrometry (ICP-MS) system and the hardware and software advances incorporated into this new instrument
- Part three is an application note covering the analysis of various high matrix environmental samples using the Agilent 7500ce ICP-MS.

Recent developments in both hardware and software have resulted in the new benchmark for environmental ICP-MS, the Agilent 7500ce. The 7500ce has taken the proven 7500c ORS technology to new levels of performance in sensitivity, stability, and ease of use. Since the inception of ICP-MS there have been a number of difficult challenges that have slowed its complete adoption over the more traditional techniques of graphite furnace atomic absorption (GFAA) and ICP optical emission spectroscopy (ICP-OES) in the environmental monitoring industry. The primary difficulties have been the very large range of concentrations encountered in waters and the potential for difficult-to-resolve interferences on critical elements such as As, Se, Cr, V, and Fe from common matrix components.

Virtually all developed countries have adopted programs and regulations to monitor and maintain the quality of public water systems. In the US,

water quality is regulated by the United States Environmental Protection Agency (USEPA), as mandated by the Safe Drinking Water Act of 1974. In the European Union, drinking water is regulated by the Council Directive 98/83/EC of 3rd Nov., 1998 on the Quality of Water Intended for Human Consumption. In Japan, the quality of drinking water is regulated by the Japan Water Supply Act, dating from 1957. Most other developed countries have adopted drinking water quality standards based on World Health Organization (WHO) Standards, Guidelines for Drinking Water Quality, 1996, 1998, or on the USEPA standards. See Table 1. While these guidelines as they pertain to trace metals vary somewhat in their lists of regulated metals and concentrations, they are fundamentally similar. They all require accurate, precise measurement of multiple toxic metals in drinking waters at the lowest practical limits of quantification. The purpose of this application note is to demonstrate that the sensitivity, accuracy, and precision requirements for the analysis of trace metals in drinking water worldwide can be met or exceeded by a single, robust technique using the Agilent 7500ce environmental ICP-MS. Additionally, as regulated limits continue to decrease and the requirements for monitoring ultratrace levels of metals in ambient waters become more important, the Agilent 7500ce ICP-MS has the capability to meet future needs as well. For details of worldwide regulatory requirements, see Agilent Application Note 5988-8902EN, "Meeting Worldwide Requirements for Trace Metals in Drinking Water using the Agilent 7500c ICP-MS" [1].

Table 1. Elements Regulated Worldwide in Drinking Water, their Maximum Allowable Concentrations and the Agilent 7500ce Method Detection Limits (MDLs) for Those Elements

Analyte	Isotope	WHO standard (µg/L)	EC Directive 98/83/EC (µg/L)	Japan drinking water standard (µg/L)	USEPA Primary MCL (µg/L)	Agilent 7500ce MDLs†† (µg/L)***
Aluminum (Al)	27	-	200	200	20-200*	0.027
Antimony (Sb)	121	5†	5	2**	6	0.015
Arsenic (As)	75	10†	10	10	10	0.028
Barium (Ba)	137	700	-	-	2000	0.014
Beryllium (Be)	9	-	-	-	4	0.027
Boron (B)	10	500†	1000	1000	-	0.035
Cadmium (Cd)	111	3	5	10	5	0.009
Chromium (Cr)	52	50†	50	50 as Cr^{6+}	100	0.022
Copper (Cu)	63	2000†	2000	1000	1300	0.013
Iron (Fe)	56	-	200	300	300*	0.020
Lead (Pb)	208	10	10	10	15	0.008
Manganese (Mn)	55	50†	50	50	50*	0.016
Mercury (Hg)	202	1	1	0.5	2	0.007
Molybdenum(Mo)	95	70	-	10	-	0.020
Nickel (Ni)	60	20†	20	(10)**	-	0.026
Selenium (Se)	78	10	10	10	50	0.016
Silver (Ag)	107	-	-	-	10*	0.015
Sodium (Na)	23	-	200 ppm	200 ppm	-	0.018
Thallium (Tl)	205	-	-	-	2	0.011
Uranium (U)	238	2†	-	(2)**	30	0.006
Zinc (Zn)	66	-	-	1000	5000*	0.021

*Secondary standard
†Provisional guideline value
**Guideline
††MDLs determined according to USEPA criteria as described elsewhere in this document
***Regulatory concentrations converted to micrograms per liter (ppb) for ease of comparison

Analytical Challenges

Regulatory levels for trace metals in drinking water are periodically revised downward as our understanding of their toxicity and our ability to measure them at lower concentrations improves. Additionally, the need to monitor even lower ambient levels of trace metals in difficult matrices like seawater is becoming more important. The challenge has been to extend the dynamic range of analysis to cover the ranges of the traditionally used techniques from GFAA through ICP-OES and to remove polyatomic interferences while avoiding unwanted matrix effects. Polyatomic ions formed in the plasma and interface of the ICP-MS are the main sources of interferences. See Table 2 for a list of polyatomic interferences on various environmentally important analytes.

Table 2. Possible Polyatomic Interferences in Typical Environmental Samples and the ORS Mode Used to Eliminate Them

Analyte isotope	Principal interferences	Corrective ORS mode
^{24}Mg	$^{12}C^{12}C$	He
^{27}Al	$^{12}C^{14}N^{1}H$	He
^{40}Ca	^{40}Ar	H2
^{51}V	$^{35}Cl^{16}O$	He
^{52}Cr	$^{40}Ar^{12}C$, $^{35}Cl^{16}O^{1}H$, $^{36}Ar^{16}O$	He
^{55}Mn	$^{40}Ar^{14}N^{1}H$, $^{38}Ar^{17}O$	He
^{56}Fe	$^{40}Ar^{16}O$, $^{40}Ca^{16}O$	H_2 or He
^{60}Ni	$^{44}Ca^{16}O$, $^{23}Na^{37}Cl$, $^{43}Ca^{16}O^{1}H$	He
^{63}Cu	$^{40}Ar^{23}Na$	He
^{75}As	$^{40}Ar^{35}Cl$	He
^{78}Se	$^{40}Ar^{38}Ar$	H_2

Clean drinking water samples typically require interference control for only a few elements. As, Se, Ca, V, and Fe can be problems depending on the matrix and required detection limit (DL). While it is possible to address interferences on these elements with the use of mathematical corrections, in some matrices the results can be unpredictable, resulting in elevated DLs. As a result, these elements are frequently analyzed by another technique such as ICP-OES or GFAA. The use of the ORS not only eliminates the need for complex and sometimes unreliable mathematical corrections, it extends the dynamic range in both directions allowing lower DLs for many elements and higher maximum concentrations for others. Furthermore, many “drinking water” samples can be quite high in dissolved minerals that can cause additional interferences. Since most commercial environmental laboratories do not have the luxury of limiting their samples to a single, well-defined type, it is important for the ICP-MS system to be able to measure multiple, unknown matrix samples under a single set of conditions. The purpose of this work is to show that drinking waters can be analyzed with high accuracy, precision, and sensitivity, even when analyzed together, in large numbers, with much more complex unknown samples.

ORS - Matrix Independent Analytical Quality

The Agilent 7500ce ICP-MS uses collision/reaction cell (CRC) technology in the form of the ORS to remove polyatomic interferences. The use of CRC technology to reduce interferences in ICP-MS is well documented [2,3]. Figure 1 illustrates the efficiency of interference removal in a synthetic matrix blank containing carbon (1% methanol), chloride (1% HCl), and nitrogen (1% HNO_3). Under normal (no gas in cell) conditions, this matrix would cause severe interferences on several analyte elements (Table 2). An excellent test of the efficiency of interference removal can be seen in these low-level calibration plots. When interferences are present, the response curve is offset in the y direction by the magnitude of the interference, increasing the background equivalent concentration (BEC) and the DL. When the interference is removed, the calibration curve intersects the y-axis at a point much nearer to zero with a correspondingly lower BEC and DL.
Figure 1 depicts sub-ppb calibration curves for chromium and vanadium in 1% each methanol, HCl and HNO_3, with and without the use of helium collision mode.

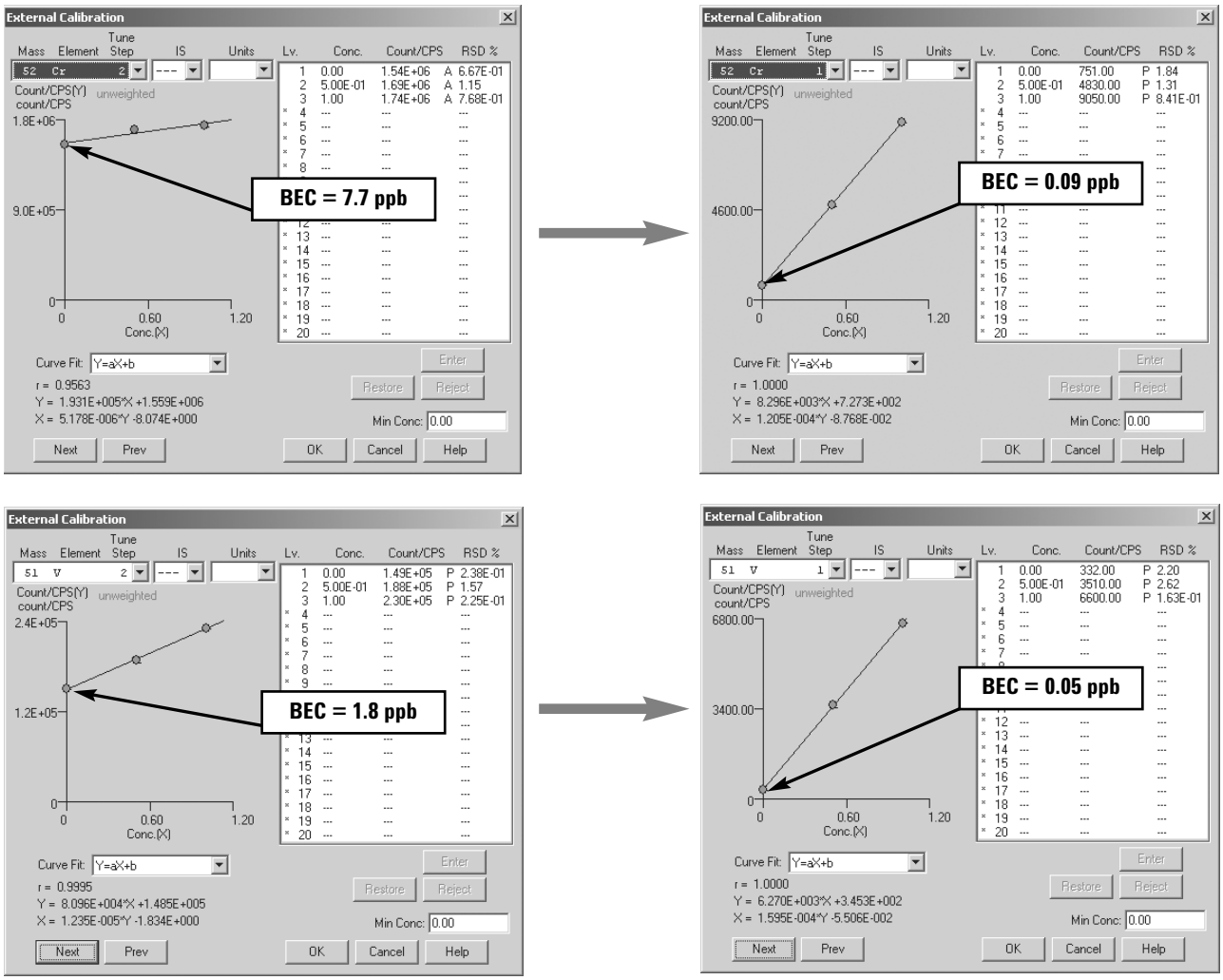

Figure 1: Calibration plots of ^{52}chromium and ^{51}vanadium in 1% nitric, 1% hydrochloric, 1% methanol showing effects of interferences from ArC^+ and ClO^+ in normal mode on the left and after removal of interferences by the ORS using He on the right.

Experimental

All work was performed using a standard Agilent 7500ce ICP-MS system with standard MicroMist glass concentric nebulizer. Laboratory conditions were typical of a commercial environmental laboratory. The instrument was tuned for robust plasma conditions (Table 3) yielding sensitivity of approximately 50 million cps/ppm at mid-mass with background less than 5 cps, CeO^+/Ce^+ less than 1% and Ce^{++}/Ce less than 1.5%. The instrument automatically groups elements into the selected ORS mode and switches between modes while scanning each sample. No additional optimization is required for specific analytes or matrices. Since the instrument conditions are mostly the same for all three modes, switching is rapid and precise. Table 4 depicts the mode each element was acquired in. A Cetac ASX-510HS high speed autosampler was used. All water was ASTM type 1, 18MΩ/cm (MilliQ), and acids were Seastar semiconductor grade. Tracemetal grade acids are more commonly used but can contain contaminants at the very low DLs presented in this work. If low ppt MDLs are not required for all analytes, tracemetal grade acids are sufficient.

In this work, an extended sequence of samples simulating the workload in a typical environmental laboratory was analyzed repeatedly for more than 15 ½ hours. The samples included high dissolved solids water samples, 1/10 diluted seawater samples and spikes, soil digests, interference check solutions (ICS-A and ICS-AB), standard reference materials and periodic calibration check samples. The system was calibrated once at the beginning of the sequence and not recalibrated or resloped during the entire sequence. The bulk of the sequence consisted of eight repeats of the sample block shown in Figure 2. The accuracy and precision of the repeat analyses of each sample type over the entire sequence were monitored.

Table 3. Instrumental Conditions Used in this Work and Typically Used for Environmental Samples of All Types

Instrument parameter	Normal mode	Hydrogen mode	Helium mode
RF Power	1500 W	<Same	<Same as H_2
Sample depth	8 mm	<Same	<Same as H_2
Carrier gas	0.85 L/min	<Same	<Same as H_2
Spray chamber temp	2° C.	<Same	<Same as H_2
Extract 1	0 V	<Same	<Same as H_2
Extract 2	–160 V	<Same	<Same as H_2
Omega bias	–24 V	<Same	<Same as H_2
Omega lens	–0.6 V	<Same	<Same as H_2
Cell entrance	–30 V	<Same	<Same as H_2
QP focus	3 V	–11 V	<Same as H_2
Cell exit	–30 V	–44 V	<Same as H_2
Octopole bias	–7 V	–18 V	<Same as H_2
QP bias	–3.5 V	–14.5 V	<Same as H_2
Cell gas flow	0	3.0 mL/min H_2	4.5 mL/min He

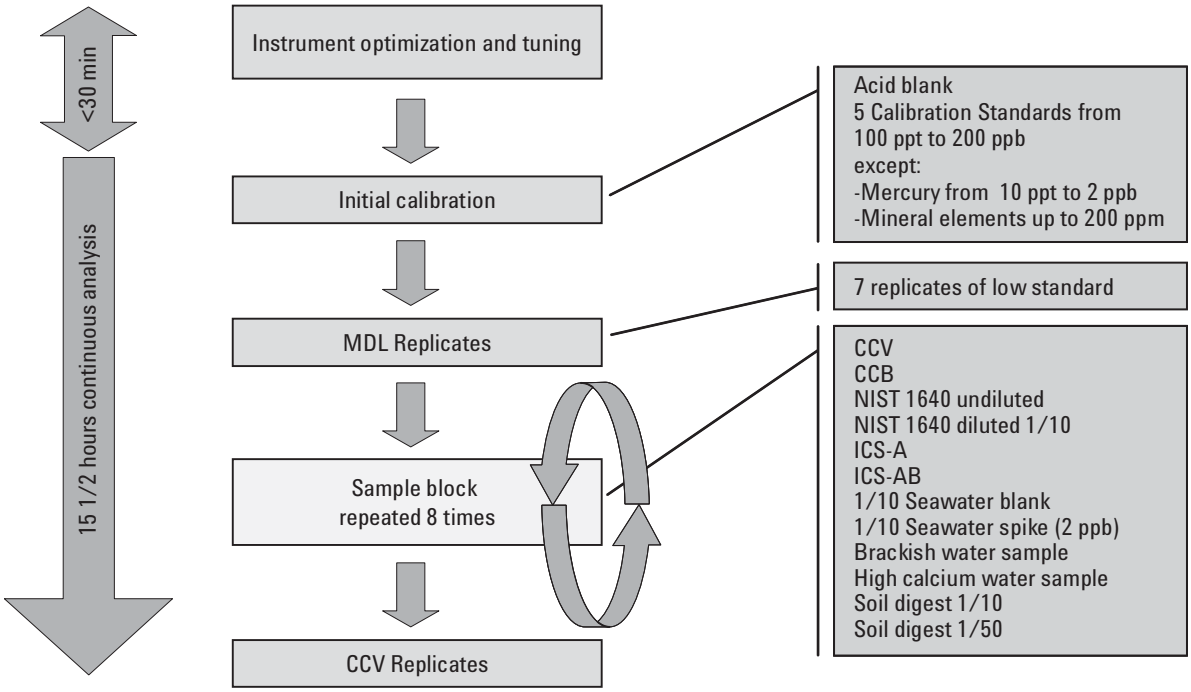

Figure 2. Analytical sequence.

Table 4. Summary of Analyte Masses, Analytical Conditions, and Measured DLs in Both Screening Mode and Full Quantitative Mode for Regulated Elements

MDL Analyte	MDL Isotope	ORS Mode (typical)*	Integration time (s)	Calibration range (ppb)	MDL He screening (ppt)†	MDL Tri-Mode (ppt)**
Calcium (Ca)	40	H_2	0.3	50 - 200,000	-	16.2
Iron (Fe)	56	H_2	0.3	50 - 200,000	31.6	19.9
Selenium (Se)	78	H_2	1.5	0.5-100	117.2	16.3
Sodium (Na)	23	He	0.3	50 - 200,000	55.2	55.2
Magnesium (Mg)	24	He	0.3	50 - 200,000	24.6	24.6
Potassium (K)	39	He	0.3	50 - 200,000	785.8	785.8
Vanadium (V)	51	He	1.5	0.5-100	32.6	32.6
Chromium (Cr)	52	He	1.5	0.5-100	27.1	27.1
Nickel (Ni)	60	He	1.5	0.5-100	25.6	25.6
Copper (Cu)	63	He	1.5	0.5-100	12.7	12.7
Arsenic (As)	75	He	1.5	0.5-100	45.2	45.2
Beryllium (Be)	9	Norm	0.3	0.5-100	113.2	26.5
Boron (B)	10	Norm	0.3	0.5-100	125.7	35.1
Aluminum (Al)	27	Norm	0.3	0.5-100	131.4	23.7
Manganese (Mn)	55	Norm	0.3	0.5-100	26.8	16.2
Cobalt (Co)	59	Norm	0.3	0.5-100	28.1	18.0
Zinc (Zn)	66	Norm	0.3	0.5-100	33.7	24.3
Molybdenum(Mo)	95	Norm	0.3	0.5-100	22.4	20.4
Silver (Ag)	107	Norm	0.3	0.5-100	18.2	15.4
Cadmium (Cd)	111	Norm	0.3	0.5-100	45.3	27.9
Tin (Sn)	118	Norm	0.3	0.5-100	51.2	14.0
Antimony (Sb)	121	Norm	0.3	0.5-100	51.2	13.7
Barium (Ba)	137	Norm	0.3	0.5-100	32.6	15.7
Mercury (Hg)	202	Norm	3.0	0.01-2.0	13.6	7.3
Thallium (Tl)	205	Norm	0.3	0.5-100	29.7	13.0
Lead (Pb)	208††	Norm	0.3	0.5-100	30.8	10.4
Thorium (Th)	232	Norm	0.3	0.5-100	27.5	12.0
Uranium (U)	238	Norm	0.3	0.5-100	29.3	10.2
Useful ISTDs						
^{6}Lithium (Li)	6	Norm	0.3	50 ppb		
Scandium (Sc)	45	All	0.3	50 ppb		
Germanium (Ge)	70,74	All	0.3	50 ppb		
Indium (In)	115	Norm	0.3	50 ppb		
Terbium (Tb)	159	Norm	0.3	50 ppb		
Platinum (Pt)	195	Norm	0.3	50 ppb		
Bismuth (Bi)	209	Norm	0.3	50 ppb		

* Typical ORS mode selected for best overall performance for most common matrices.

† Screening protocol uses He collision mode only for rapid screening where optimum sensitivity is not required for all elements, MDL calculated according to EPA 200.8 requirements.

** MDLs calculated according to EPA 200.8 requirements. 3-sigma of seven replicate analyses of a fortified blank at 3–5 times the estimated MDL. MDLs are reported in ng/L (ppt) for ease of presentation.

†† Lead is measured as the sum of isotopes 206, 207, and 208 to eliminate error due to variable isotope ratios.

Results and Discussion

Typical calibration plots are shown in Figure 3. By using the ORS to eliminate polyatomic interferences for some analytes and to attenuate excessive signal for others, the practical dynamic range of the analysis extends from low part per trillion levels (ppt) for elements such as Se (hydrogen reaction mode), As, V, and Cu (helium collision mode) and Hg (normal mode) to 1000s of parts per million (ppm) for high concentration elements like sodium (helium collision mode). This is accomplished in a single analysis using automatic switching of ORS conditions through three modes without the need for complex “on the fly” resolution changes or detector gain changes. No interference correction equations were required and all analytes were measured at their elemental mass. Table 4 summarizes the ORS conditions, calibration ranges and method detection limits for this work. Note that two sets of MDLs are reported; Screening and Tri-Mode. Depending on the data quality objectives of the analytical project, the appropriate ORS mode(s) can be selected. In Screening Mode, all elements are analyzed under a single set of helium collision conditions. Since He mode depends on kinetic energy discrimination to reduce polyatomic interferences, it is independent of analyte or matrix and can be used to effectively analyze most elements under a variety of matrix conditions. In many cases, the best possible MDLs are achieved under He conditions. These cases include analytes for which there are multiple interferences at the analyte mass and cases where the interference is unpredictable or unknown. For some elements, however, it is possible to achieve better sensitivity or interference reduction through the use of one of the other ORS modes, hydrogen or normal (no gas). Typically, for the best overall performance in unknown environmental samples, Tri-Mode is used. This mode combines the best possible sensitivity with the best possible interference removal for all analytes. All data in this work was acquired under Tri-Mode conditions, in other words, optimized for maximum performance with variable, unknown matrices.

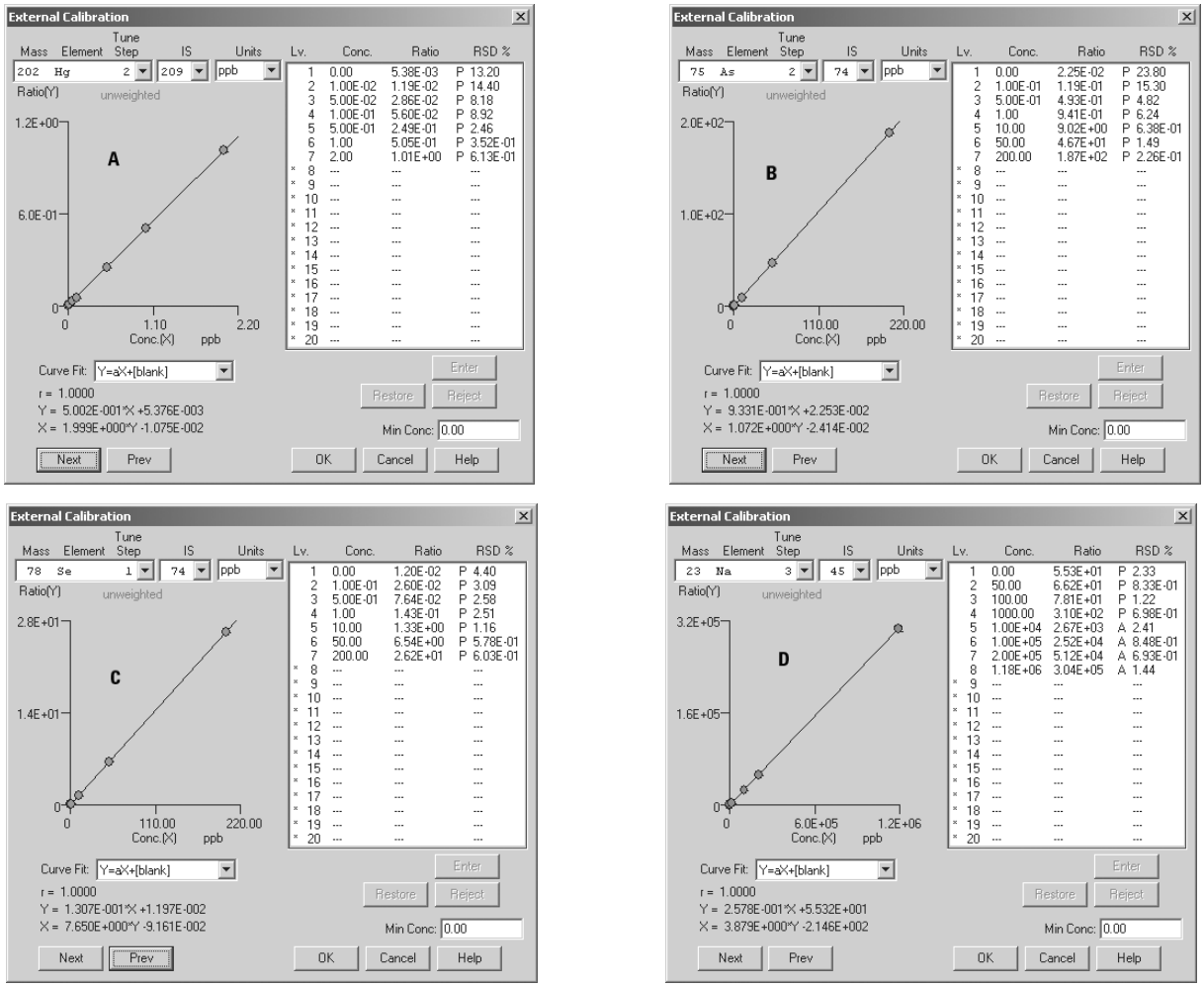

Figure 3: Calibration curves (A) mercury 10 ppt - 2-ppb normal mode, (B) arsenic 100 ppt - 200-ppb helium mode, (C) selenium 100 ppt - 200-ppb hydrogen mode, (D) sodium 50 ppb - 1180-ppm helium mode.

Certified Reference Materials

NIST 1640 certified reference water was analyzed repeatedly throughout the sequence, both neat (without any additional acidification or matrix matching) and diluted 1/10 into 1% nitric/0.5% hydrochloric acid. Figure 4 shows the mean recoveries and %RSDs for all certified analytes in both the undiluted and diluted samples. Note that there is no difference in either the recoveries or % RSDs between the neat and diluted samples even though they differ significantly in matrix and concentration.

Continuing Calibration Verification

USEPA methods and good laboratory practices mandate that calibration validity be checked periodically and updated if necessary. This was accomplished by measuring the continuing calibration verification (CCV) and continuing calibration blank (CCB) after every 10 analytical samples according to USEPA method 200.8. The CCV sample is typically a midpoint calibration standard. The CCB is equivalent to the calibration blank. Method 200.8 requires that the calibration check results be within ±10% of the actual value in order for the calibration to be in control. If any element falls outside the ±10% limit, the system must be recalibrated before proceeding with additional analyses.

Figure 5 depicts the results of the 13 replicate CCV analyses during the 15.5 h sequence (one for each ten sample analyses and five more at the end of the sequence. At no time during the sequence did any element exceed the ±10% limit. No recalibrations or adjustments to the initial calibration were performed.

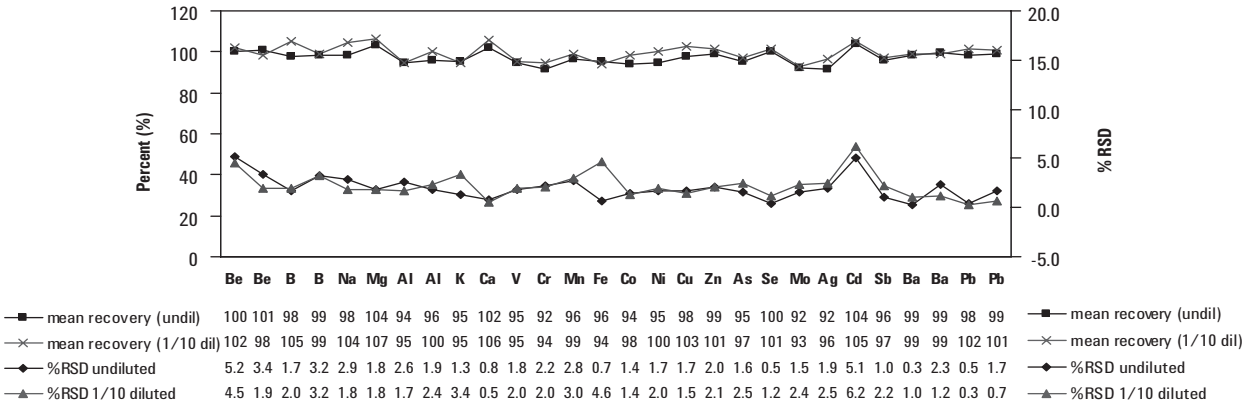

Figure 4: Mean recovery and percent relative standard deviation (%RSD) for eight replicates each NIST 1640 (neat) and NIST 1640 (diluted 1/10) acquired over 15.5 h using a single calibration.

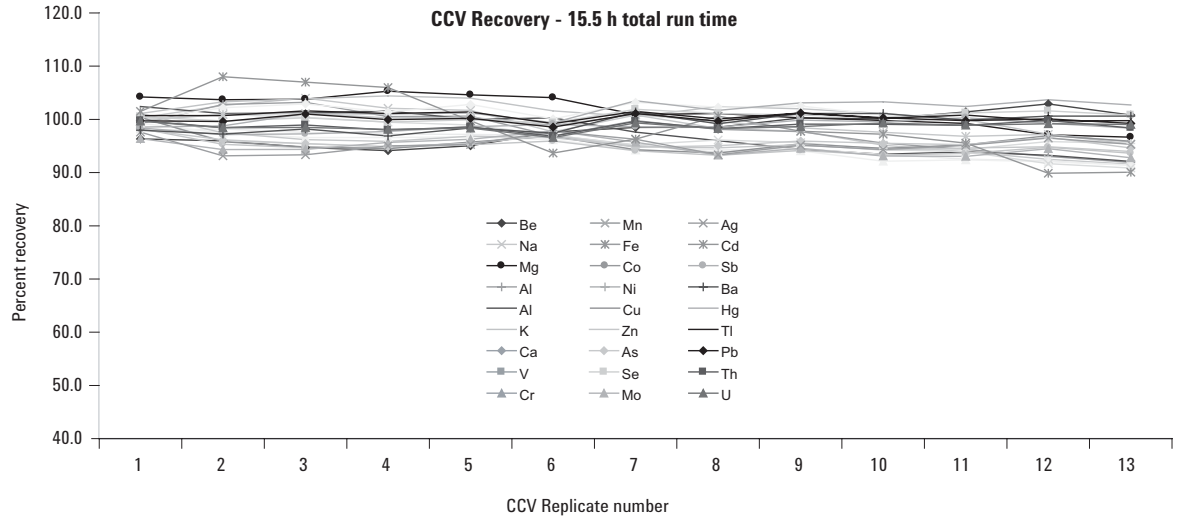

Figure 5. Results of 13 separate analyses of the CCV sample over the 15.5 h sample sequence. All analyte elements are reported. Acceptable control limits according to USEPA method 200.8 are ±10%. At no time did any element fall outside the 10% control limits.

Summary

Improvements in ion optic and octopole design, created specifically for the environmental laboratory, have resulted in an ICP-MS instrument with unprecedented sensitivity, matrix tolerance, and stability. This work was designed to replicate the workload in a typical environmental laboratory where sample matrices are not always under the control of the analyst and are frequently unknown. Under these conditions, it is not practical to matrix-match calibrations to multiple, unknown sample matrices. It is also not practical to depend on complex matrix or analyte specific reaction cell conditions. The data shown in this note were all generated using a single set of calibration standards in 1% nitric acid / 0.5% HCl. Calibration was performed once only at the beginning of the sequence and not repeated or updated during the sequence. No attempt at matrix matching either the calibration standards or CRC conditions was made. No mathematical interference corrections were employed and all analytes were measured at their elemental masses. Sample matrices varied from a clean drinking water CRM, both acidified and unacidified, through high total dissolved solids (TDS) ground waters. Also included were simulated waste samples designed to detect potential interference problems (EPA ICS-A and ICS-AB) as well as typical soil digests and 1/10 spiked simulated seawater. The results of these samples will be discussed in detail in part three of this series. In all cases, recovery of expected values was within expected limits.

In summary, it is now possible, using a single ICP-MS instrument to analyze multiple sample types, over a wide range of analyte and matrix concentrations at well-below regulated concentrations in a single sequence.

Conclusions

The Agilent 7500ce ICP-MS was designed specifically to meet the demanding requirements of environmental laboratories worldwide that must adhere to rigorous regulatory requirements while analyzing a wide range of difficult and unknown sample types in the shortest amount of time. Using advanced ORS technology based on the use of simple gases, the 7500ce is easy to set up and operate, and delivers unprecedented DLs in a wide range of unknown sample types.

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Printed in the USA
March 31, 2004
5989-0870EN

Agilent Technologies

Fast and Accurate Determination of Arsenobetaine (AsB) in Fish Tissues Using HPLC-ICP-MS

Application

Foods

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Abstract

A high performance liquid chromatography-inductively coupled plasma mass spectrometry method was developed for the fast and accurate analysis of arsenobetaine in fish samples extracted by accelerated solvent extraction. The combined extraction and analysis approach was validated using certified reference materials for arsenobetaine in fish and during a European intercomparison exercise with a blind sample. Up to six species of arsenic can be separated and quantified in the extracts within a 10-minisocratic elution. The method was optimized so as to minimize time-consuming sample preparation steps and to allow for automated extraction and analysis of large sample batches. A comparison of standard addition and external calibration showed no significant difference in the results obtained, which indicates that the liquid chromatography-inductively coupled plasma mass spectrometry method is not influenced by severe matrix effects. The extraction procedure could process up to 24 samples in an automated manner while the robustness of the developed high performance liquid chromatography-inductively coupled plasma mass spectrometry approach is highlighted by the capability to run more than 50 injections per sequence which equates to a total run-time of more than 12 hours. The method can therefore be used to rapidly and accurately assess the proportion of nontoxic arsenobetaine in fish samples with high total arsenic content during toxicological screening studies.

Introduction

The element Arsenic (As) has long been thought of as poisonous and highly toxic. However, it has since been shown that the toxicity of As is largely dependent on the form or “species” the arsenic is in. Arsenic is ubiquitous in the environment due to natural and anthropogenic sources, and the relative contribution of these factors is estimated as roughly 60% and 40% respectively. In the environment, As behaves in similar ways to the Group V elements nitrogen (N) and phosphorus (P). As a result of these similarities, arsenic gets taken into the biochemical pathways of N and P. This results in the formation of compounds such as arsenobetaine (AsB) in fish and arseno-sugars, which are found in marine algae. The toxicity of the inorganic As-species (such as arsenite, As(III) and arsenate, As(V)) is far greater than the organic forms, such as monomethylarsonic and dimethylarsinic acid (MMAA and DMA) and AsB. The International Agency for Research on Cancer (IARC) has classified inorganic arsenic as a human carcinogen, whereas AsB, the predominant form of As in most marine organisms [1], is considered nontoxic to humans. Although AsB is the major form of As in many marine organisms, it is not present in all fish species [2]; therefore, an evaluation of the proportion of AsB to the total As determined can give a useful and rapid estimate of the toxicological significance of a sample. In order to determine the toxicity of seafood, the determination of the total As alone is of limited value, and the different species of As have to be extracted, separated, and determined. Fast, reliable, and practical methods are therefore required that can provide speciation information for the screening of large sample batches.

Aims and Objectives

The aim of this study was to develop a semi-automated analytical method for the extraction and determination of As-species in fish tissues. Requirements for high sample throughput analysis were the automation of the extraction procedure as well as a fully automated separation and detection method capable of analyzing large sample batches (up to 50 injections per run) during overnight runs. In order to streamline the analytical procedure, an attempt was made to develop a method with a minimal number of sample preparation steps. It was intended that the method should be established using calibration by external calibration curves, rather than the lengthy alternative of standard additions. The use of an isocratic liquid chromatography (LC) elution can be favorable in terms of time-efficiency during the liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS) analysis because it negates the need for column re-equilibration between injections.

Calibration Standards

The following standards were obtained from Fluka (Sigma-Aldrich, Gillingham, UK): di-sodium hydrogen arsenate heptahydrate ($AsHNa_2O_4.7H_2O$) ≥98.5%, sodium (meta)arsenite ($AsNaO_2$) ≥99.0%, and cacodylic acid (dimethylarsinic acid, DMA, $C_2H_2AsO_2$) ≥99.0%; monomethylarsonic acid disodium salt (MMAA, $CH_3AsNa_2O_3$) >98% was obtained from Argus Chemicals (Vernio, Italy). Arsenobetaine (AsB, $C_5H_{11}AsO_2$) was obtained from BCR (Brussels, Belgium) as a solution of AsB in water at 1031 ±6 (95% C.I.) mg/kg (BCR 626).

Extraction

Accelerated solvent extraction (ASE) has been used previously for As-speciation [3, 4] and was chosen as the sample preparation method because it allows for the automated extraction and online filtration of up to 24 samples. In addition, the extraction solution is collected in glass vials, which negates further sample preparation steps such as filtration or centrifuging.

The samples were extracted using a Dionex ASE 200 accelerated solvent extractor. Sample sizes from 0.1–0.3 g were weighed accurately into 11-mL stainless steel extraction cells fitted with filter papers and PTFE liners. The extraction program was set up as shown in Table 1.

Table 1. Extraction Conditions Used for ASE

Instrument	Dionex ASE 200
Preheat	2 min
Heat	5 min
Extraction steps	5 × 2 min
Temperature	100 °C
Pressure	1500 psi
Solvent	Methanol

HPLC-ICP-MS Methodology

The HPLC-ICP-MS instrumentation consisted of an Agilent Technologies 1100 HPLC system coupled to an Agilent Technologies 7500i ICP-MS fitted with a second roughing pump, which enhances sensitivity by increasing ion transmission across the interface. The HPLC system comprised a quaternary pump module, a vacuum degasser, a temperature controlled autosampler, and column compartment. The ICP-MS instrument was tuned for sensitivity, reduced oxides, and doubly charged species prior to connection to the liquid chromatograph by performing a standard instrument tune using a 10 ng/g solution of Li, Y, Ce, and Th in 1% HNO_3. The pulse to analog (P/A) factor was adjusted on a daily basis using a solution containing ~50 ng/g Li, Mg, Mn, Cu, As, Gd, Y, Cd, Pb, and Ba. After this optimization, a 50 ng/g solution of As in 1% HNO_3 was used to specifically optimize the sensitivity for arsenic. The ICP-MS nebulizer was then connected to the HPLC-column using a length of PEEK tubing (yellow, 1/16-inch od, 0.007-inch id). See Table 2 for the ICP-MS conditions used.

Table 2. ICP-MS Conditions Used for HPLC-ICP-MS Determination of As-Species

RF Power	1430–1550 W
RF Matching	1.89–1.92 V
Sampling depth	4.0–4.8 mm
Carrier gas flow	0.89–0.93 L/ min
Make up gas flow	0.10–0.14 L/ min
Optional gas	Oxygen at 5%
Spray-chamber temperature	0 °C
Cones	Platinum
Isotopes monitored	^{75}As ^{103}Rh ^{77}Se ($^{40}Ar^{37}Cl$) to monitor Cl interferences ^{53}Cr ($^{40}Ar^{13}C$) to monitor C interferences
Other parameters	Injector diameter: 2.4 mm Nebulizer 100 µL/min PFA, Two interface pumps used

In order to develop a rapid chromatographic separation of the main As-species in fish tissues, an anion exchange column (Hamilton PRP X-100) was chosen in combination with an isocratic elution profile. Several mobile phases were tested and the best separation of AsB and As(III) as well as DMA and MMAA was achieved within 10 min using 2.2-mM NH_4HCO_3/2.5-mM tartaric acid at pH 8.2 delivered at 1 mL/min isocratic flow. This evaluation was carried out initially using matrix-free calibration standards containing the species of interest and refined using an oyster tissue extract that contained arsenocholine (AsC), two arsenosugars (As-sug. B and As-sug. D), $TMAs^+$ and several unknown species in addition [5]. The injection volume for samples and standards was 50 µL.

In order to enhance the ionization of the As-species [6, 7], methanol was added to the mobile phase at concentrations ranging from 0.5% to 5% v/v. At concentrations above 1%, the chromatographic separation degraded significantly to the degree that base-line resolution between AsB and As(III) was no longer achieved. However, the addition of 1% MeOH to the mobile phase resulted in a significant improvement in the sensitivity (3–4-fold increase in peak height) for all analytes. A chromatogram for a 5-ng/g mixed calibration standard with the final chromatography conditions is shown in Figure 1.

Variations in Signal Response for Different As-Species

The chromatogram shows that the four species analyzed here have very different response factors with this method, even when made up to contain the same concentration of As in solution. This is further illustrated by the calibration curves and their respective slopes, as shown in Figure 2. Such differences in the analyte signal intensity were reported previously in the literature [7] and appear to be due to a combination of the ICP-MS hardware used and the plasma conditions, which are in turn affected by the mobile phase composition. This points to possible differences in the nebulization, transport and/or ionization of different species by such methods. In order to determine whether this effect could be attributed to the coupling of the ICP-MS with a liquid chromatograph, aqueous standards of AsB and As(III) were made up to equivalent concentrations as As and analyzed by direct aspiration without chromatography. This indicated that the signal response of AsB was ~10%–15% higher compared to the inorganic As standard and, therefore, the difference in signal response does not appear to be related to the coupling with a liquid chromatograph.

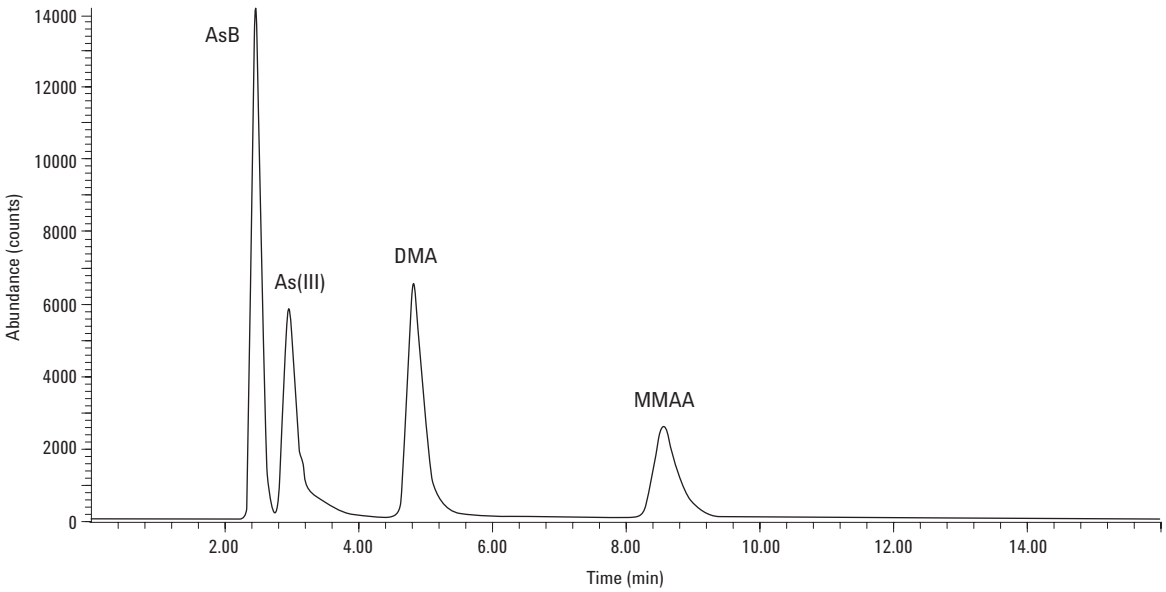

Figure 1. Chromatography A: 2.2-mM NH_4HCO_3 2.5-mM tartaric acid, 1% MeOH, pH 8.2, Hamilton PRP X-100 column. Concentration of standard ~ 5 ng/g as As.

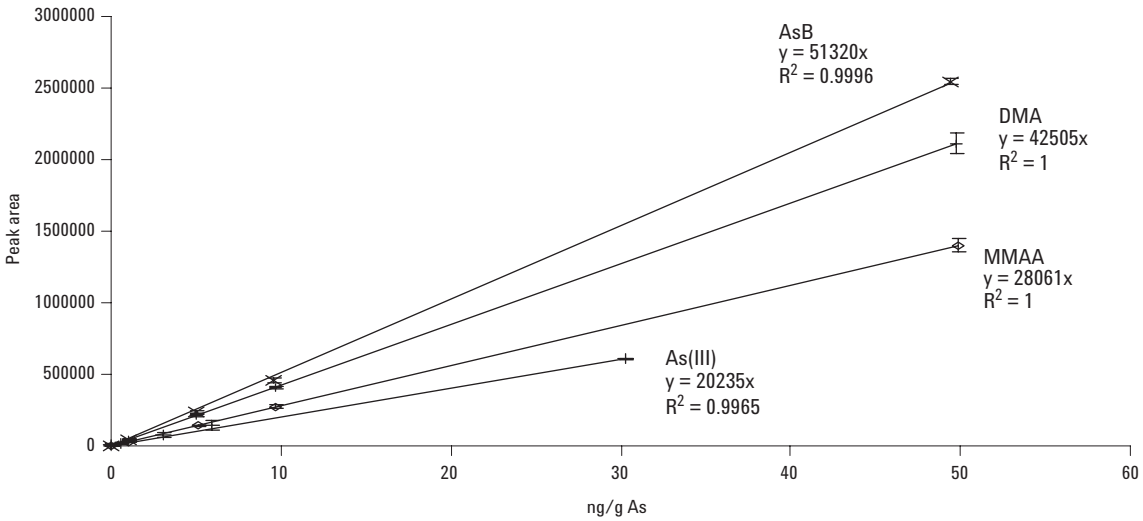

Figure 2. Calibration curves for AsB, DMA, MMAA and As(III) over a range of 0–50 ng/g as As.

In order to increase the signal intensity for species such as As(III) and MMAA by the approach described here, additional MeOH was added via a T-piece post-column so as not to impact on the chromatographic resolution. Although the relative volume of MeOH could be increased by 50%–70% in this way without deteriorating plasma stability, the relative signal responses of the four species were not influenced significantly. Because the relative signal response was stable on a day-to-day basis, no further attempts were made to equalize the signal responses.

The instrumental detection limit for AsB by this method was 0.04 ng/g as As. The linearity obtained, as indicated by the correlation coefficient of the calibration line, was 0.999–1.000 over a calibration range of 0–700 ng/g as As.

Plasma Disturbance Due to Elution of MeOH

During the analysis of fish samples, which had been extracted under the ASE conditions highlighted in Table 1, a disturbance of the plasma was observed between ~2.3 to 4.3 min after injection. This affected all of the isotopes monitored and the effect on ^{75}As and ^{103}Rh is highlighted in Figure 3. As can be seen from the chromatogram, the effect on these two isotopes is nonlinear. The ^{103}Rh signal decreases significantly during this time, whereas the ‘shoulder’ on the tailing side of the AsB peak indicates an increase in the ^{75}As signal.

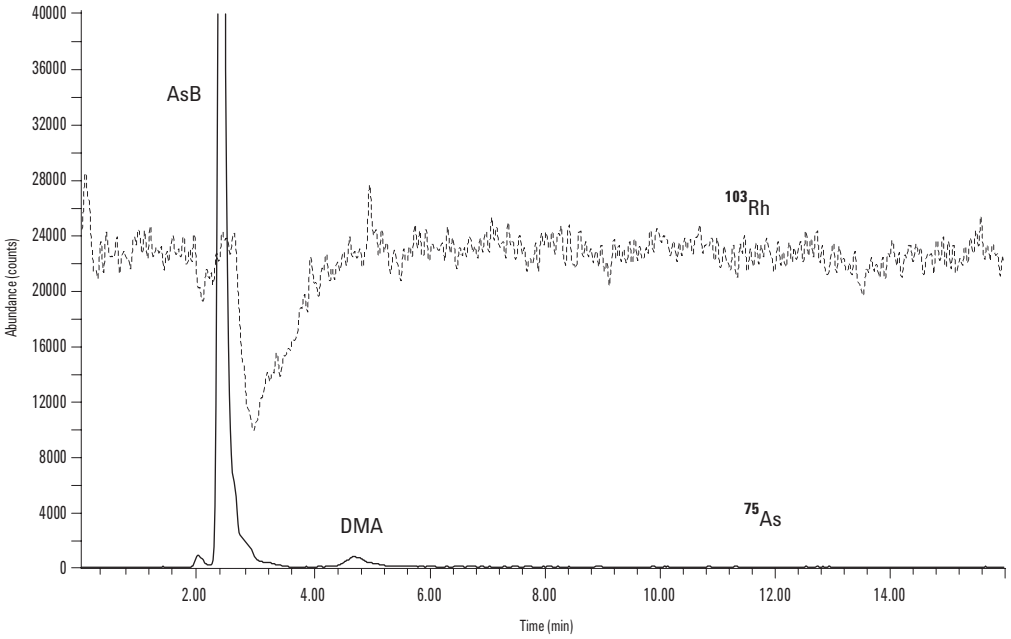

Figure 3. Signals for ^{103}Rh and ^{75}As in an undiluted fish extract. Notice the increase in the ^{75}As signal at the tailing side of the major peak (AsB) coinciding with the decrease in the ^{103}Rh signal.

The observed fluctuation in the signal intensities for the different isotopes coincides with the elution of the organic methanol fraction of the fish extracts from the analytical column. This effect could be reduced slightly by lowering the temperature of the spray-chamber from 5 °C to 0 °C, but the effect was not completely eliminated. During the injection of undiluted sample extracts, the volume of methanol that passes through the column and into the ICP-MS is ~10%. It has already been discussed that the addition of MeOH enhances the ^{75}As signal by increasing the ionization efficiency of this analyte; this effect is observed on a small scale here. Although there is no detectable As(III) in this fish material, the accurate quantitation of this compound (compared to aqueous calibration standards) could obviously lead to an overestimation if the signal of this analyte is enhanced due to the simultaneous elution of MeOH from the column. In this case, a standard addition calibration would represent a more accurate approach for quantitation. However, the spiking of each sample extract at different levels, which is necessary for this type of calibration, would make such an approach less suitable for a high sample-throughput application. In addition, the accurate integration of AsB is influenced by the signal increase on the tailing side of the peak.

In order to eliminate the effect of these signal variations on the accurate quantitation of the As-species in the methanolic extracts, the methanol fraction could either be reduced by evaporation or dilution with water. Dilution was chosen as the preferred option over evaporation in order to avoid possible analyte losses and because of time-efficiency. Whereas evaporation would either involve passing an inert gas over the solution or using rotary evaporation equipment, gravimetric dilutions were easily and quickly achieved by pipetting an aliquot of the extract into a sealed HPLC autosampler vial, weighing, and then adding the appropriate amount of water. In order to observe the effect of different dilution factors on the observed plasma disturbance, a fish extract was diluted 10-, 5-, and 2-fold in water and also injected undiluted. The effects of the different dilutions on the ^{103}Rh signal are shown by the chromatograms in Figure 4.

As demonstrated in Figure 4, a 10-fold dilution is sufficient to eliminate the plasma disturbance sufficiently; therefore, all extracts were diluted 1:10 in water prior to injection.

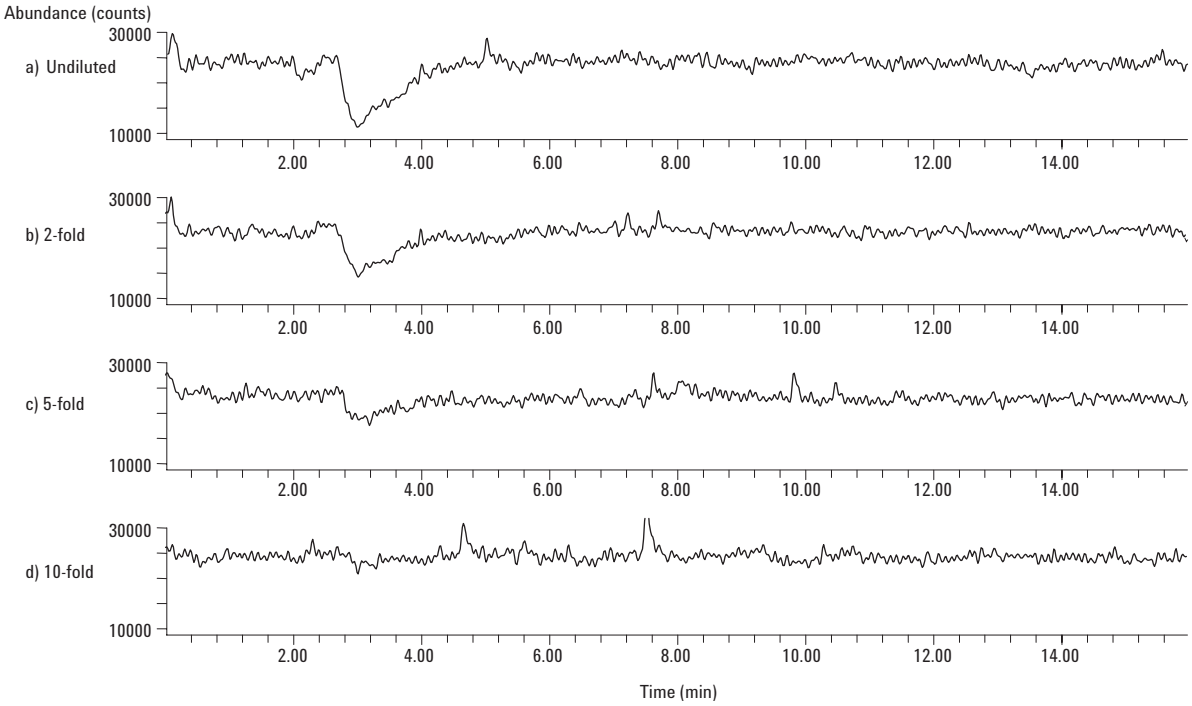

Figure 4. Signal of the internal standard ^{103}Rh, for fish sample extracts a) undiluted and diluted b) 2-fold, c) 5-fold and d) 10-fold.

Comparison of External Calibration and Standard Addition for the Quantitation of AsB in Fish Tissues

Due to the fact that arsenic is mono-isotopic, isotope dilution analysis cannot be used for the high-accuracy quantitation of this compound by LC-ICP-MS. In such circumstances, calibration by standard additions is often used in order to achieve matrix matching of standards and samples. It is also a useful technique in chromatographic applications where the possibility of retention time (RT) shifts of analytes due to matrix components exists. This can result in misidentification, and thus erroneous results. However, standard addition calibration can be very time- consuming because several aliquots of the sample require spiking with different levels of a calibration standard, and at least three levels of standard addition are needed for accurate quantitation of the same sample. External calibration by non-matrix matched standards can be used for applications where the difference in the matrix between samples and standards does not influence the accuracy of the result to a significant extent.

Standard addition calibration and non-matrix matched external calibration were compared for AsB in two certified reference materials (DORM-2, Dogfish muscle, NRC Canada and BCR 627, Tuna Fish, BCR EU) in order to assess whether the calibration technique used significantly influenced the accuracy or precision of the analytical result. The results showed that there was no significant difference in the mean results determined by the different calibration techniques with this method. The mean results for repeat analysis of both materials showed that the difference in the DORM-2 material was less than 1.4% and less than 4.5% for the BCR 627 material. When taking into account the standard deviations (SD) associated with the mean result obtained by each calibration technique, there was no statistically significant difference between the AsB results obtained by either approach in either of the fish tissue certified reference materials (CRMs).

Results of CRM Analysis

In order to test the accuracy of the developed ASE extraction and HPLC-ICP-MS method, a variety of certified and candidate reference materials of marine origin were extracted and analyzed. The samples included the certified fish reference materials DORM-2 and BCR 627, as well as an oyster tissue material (BCR 710)*, which is pending certification.

* The "MULSPOT" project has been financed by the SM&T Program (EU) (Contract SMT4-CT98-2232) and coordinated by ENEA (IT). The Project is at the certification stage and the material is not yet available on the market.

Table 3. Data Obtained for AsB in Two CRMs and a Candidate Reference Material

Expressed as mg/kg As unless otherwise stated	Measured value	Certified value
DORM-2 (Dogfish muscle)	16.3 ±0.9 (±1 SD)	16.4 ±1.1 (±95% C.I.)
BCR 627 (Tuna fish)	3.69 ±0.21 (±1 SD)	3.90 ±0.22 (±95% C.I.)
BCR 710 (Oyster tissue)† (Concentration as species)	31.8 ±1.1 (±1 SD)	32.7 ±5.1 (±1 SD)

† The data shown for this material is based on the consensus mean of the final certification round after the removal of statistical outliers.

Subsamples of the different materials (n = 4–6) were extracted, diluted in water, and analyzed as described above. The data for AsB determined in these samples is shown in Table 3. A chromatogram of the tuna fish material BCR 627 is shown in Figure 5.

The chromatogram indicates that the major species in this sample is AsB with two minor species, which were also extracted and detected. One peak was identified as DMA, and the peak labelled P1 is most likely to be AsC from RT matching. The data in Table 3 shows that the combined ASE/HPLC-ICP-MS methodology is capable of delivering accurate and reproducible results for AsB in these matrices. In addition, the extraction of other minor species, such as DMA and AsC, was achieved in the fish tissues; up to six species were extracted and separated in the oyster material, although none of these (apart from DMA) were quantified during this study. This DMA data for BCR 710 (730 ±30 ng/g DMA) showed a good agreement with the consensus mean value of the certification round (820 ±200 ng/g DMA).

Evaluation of Method Performance During a CRM Feasibility Study

The method performance was assessed in comparison to a number of European expert laboratories during the “SEAS” feasibility study organized by the The University of Plymouth Enterprise Limited and sponsored by the European Union (BCR, EU)‡. A fish material was prepared for this intercomparison by the University of Plymouth and distributed to participating laboratories. Participants were asked to determine AsB in a fish material from two different bottles using a methodology of their choice and making their determinations, at least, in duplicate on separate days.

The developed As-speciation method was used to extract and analyze the fish samples provided. A total of 12 subsamples from the two bottles were

‡The “SEAS” feasibility study was co-ordinated by The University of Plymouth Enterprise Limited (Plymouth, UK) under the EC contract: G6RD CT2001 00473 "SEAS" with the title: 'Feasibility Studies for Speciated CRMs For Arsenic in Chicken, Rice, Fish and Soil and Selenium in Yeast and Cereal'.

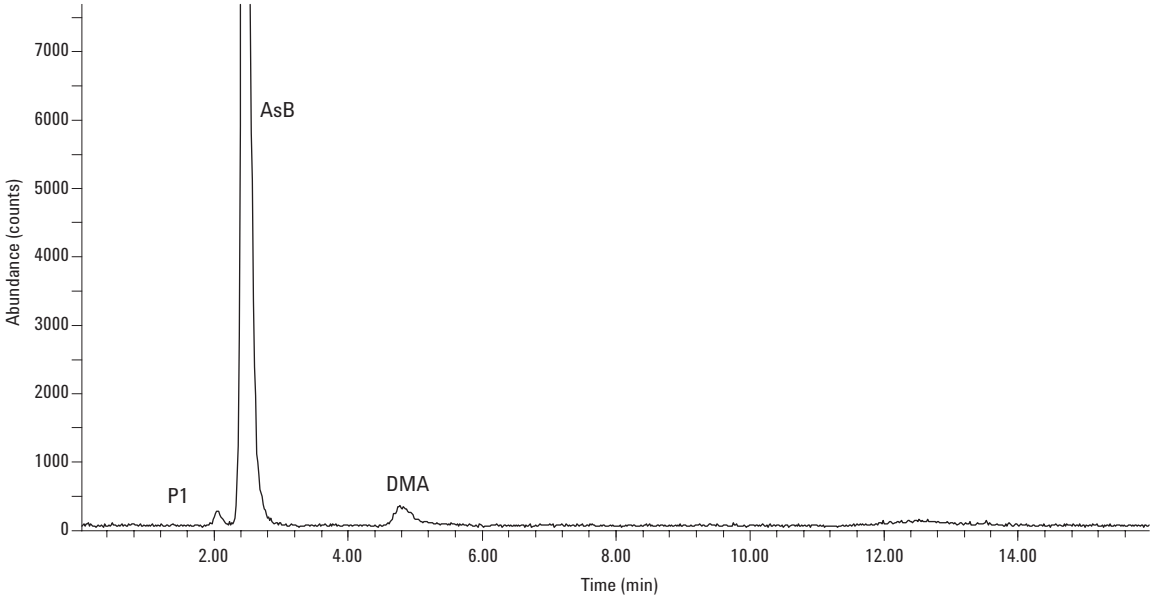

Figure 5. Chromatogram of a tuna fish extract (BCR 627) enlarged to show the detection of minor species in this material.

extracted and analyzed on 3 different days. The data were combined to provide the value labelled "LGC" in Figure 6 below. The error bars indicate the SD of the mean of individual results. The mean of all result (excluding a statistical outlier) together with 1 SD above and below the mean is indicated by the solid and dashed horizontal lines, respectively. The data provided by the combined ASE extraction and developed LC-ICP-MS methodology (94.92 ±3.95 mg/kg AsB) is in very good agreement with the mean result of all labs (95.72 ±7.79 mg/kg AsB, n = 11). The precision achieved was also satisfactory at 4.2% (RSD) for 12 subsamples from different bottles analyzed on 3 separate days. The performance of the method in this international intercomparison is highlighted by the good agreement with data provided by several European expert laboratories with longstanding expertise in As-speciation analysis. It should also be noted that the intercomparison was carried out with a blind sample of unknown concentration, rather than based on the analysis of a CRM with known certified values.

Conclusions

A robust and practical method has been developed based on accelerated solvent extraction and HPLC-ICP-MS analysis for the fast and accurate determination of AsB in fish samples. The benefits of the methods include automated extraction of up to 24 samples, minimal sample preparation steps (dilution only) after extraction, and rapid and automated analysis by HPLC-ICP-MS. The separation of four to six species of toxicological interest is achieved within 10 min using an isocratic elution. This increases the sample throughput by negating the column equilibration period needed with most gradient elution profiles.

The method was validated using commercially available CRMs and during a European intercomparison study with a fish sample of unknown concentration. The performance of the method was very satisfactory in terms of both accuracy and precision compared to several other expert laboratories.

This method can be used to rapidly determine the nontoxic proportion (AsB) in fish samples with high total As content and could therefore be used to determine whether a particular sample poses a toxicological risk in the food chain.

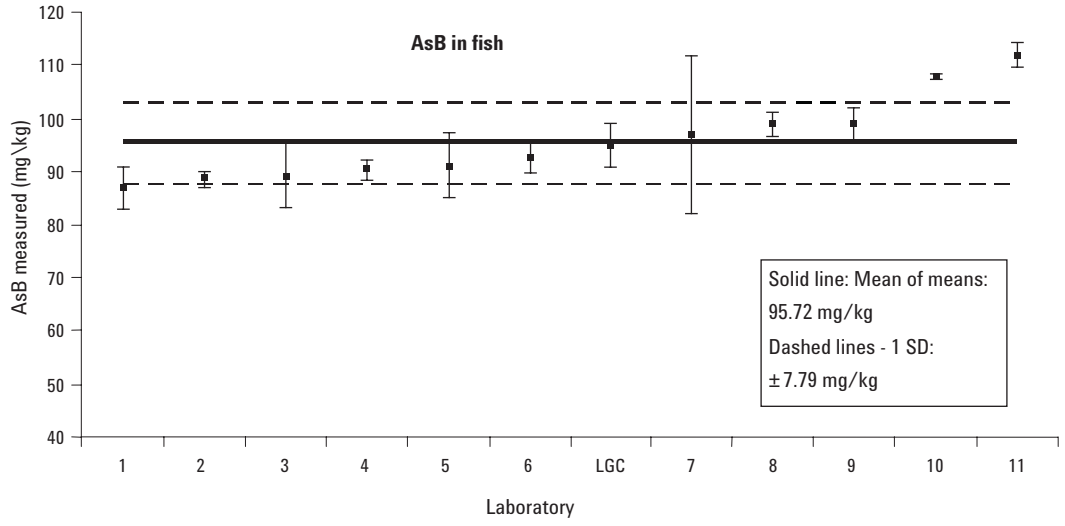

Figure 6. Comparison of data submitted by 12 participants for the determination of AsB in fish during the "SEAS" feasibility study. The error bars associated with the individual data points represent 1 SD of analysis of separate subsamples.

Acknowledgements

The work described in this application note was supported under contract with the Department of Trade and Industry (UK) as part of the National Measurement System Valid Analytical Measurement (VAM) program.

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Printed in the USA
October 14, 2003
5988-9893EN

Agilent Technologies

Speciation of Organic Compounds, Using a Newly Developed, Experimental GC-ICP-MS Interface

Application Note

ICP-MS

Environmental

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Introduction

Tin has been an important metal for thousands of years, having been used in the formulation of alloys such as bronze, in mirrors and in the production of glass. More recently, organotin compounds have been used as industrial materials such as stabilizers in polymers. The trialkylated forms are efficient biocides and fungicides and their properties have been used in many applications. For example, triphenyltin (TPhT) has been used as a pesticide and tributyltin (TBT) was used extensively both as a wood preservative and as the active component in marine anti-fouling paints, applied to the hulls of sailing vessels. While organotin compounds degrade rapidly under photolytic conditions, some trialkyltin compounds are persistent once introduced in the environment (e.g. TBT). Despite of the fact that TBT has been banned from use on small boats for over a decade, it is still commonly used on the hulls of large ships, to prevent the growth of marine organisms. In 1989, TBT was banned in all states of the USA on vessels of 25 meters or less in length. Despite a general reduction in the use of organotin compounds, they can accumulate in sediments over many years and can be ingested and absorbed by marine organisms, leading to accumulation in the marine food chain and ultimately presenting a potential threat both to the environment and later to human consumption.

Recent studies provide strong evidence that many organotin compounds can act as endocrine disruptors, even at very low concentrations. Endocrine disruptors interfere with the action of many hormones, and can be very damaging to the development of animal embryos. As a consequence, there is an increasing demand for a new analytical method for these compounds, which is fast, sensitive and offers high chromatographic resolution.

Capillary Gas Chromatography (GC) offers fast and high-resolution speciation, and is well suited for organotin compounds. Measurement limits using currently available GC detectors (FPD, MS and AED) are good, but the need for determination of organotins at ever-lower levels of concentration has fuelled the investigation of alternative detection systems. Further the presence of sulfur compounds in many of the samples requires a highly selective and sensitive detector.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) offers ultra trace detection limits and high selectivity for most elements. The principles of ICP-MS are summarized in Figure 1. Samples are introduced into a high temperature argon plasma, where they are decomposed, atomized and ionized. The resultant ions are transported, through a sampling interface, into a mass spectrometer for measurement. The high temperature in the ICP source means that all forms of an element are decomposed into individual atoms, so ICP-MS results represent total element levels. However, in combination with an on-line separation technique, such as Liquid Chromatography (LC), Ion Chromatography (IC) or Capillary Electrophoresis (CE), ICP-MS is increasingly being used as a sensitive and highly specific detector in a wide variety of speciation applications.

Combining the separation capabilities of a GC with the selectivity and sensitivity of ICP-MS could offer benefits in the measurement of ultra-trace levels of organically bound metals. In this paper, we describe some initial investigations into the coupling of GC to ICP-MS, for the analysis of organotin compounds.

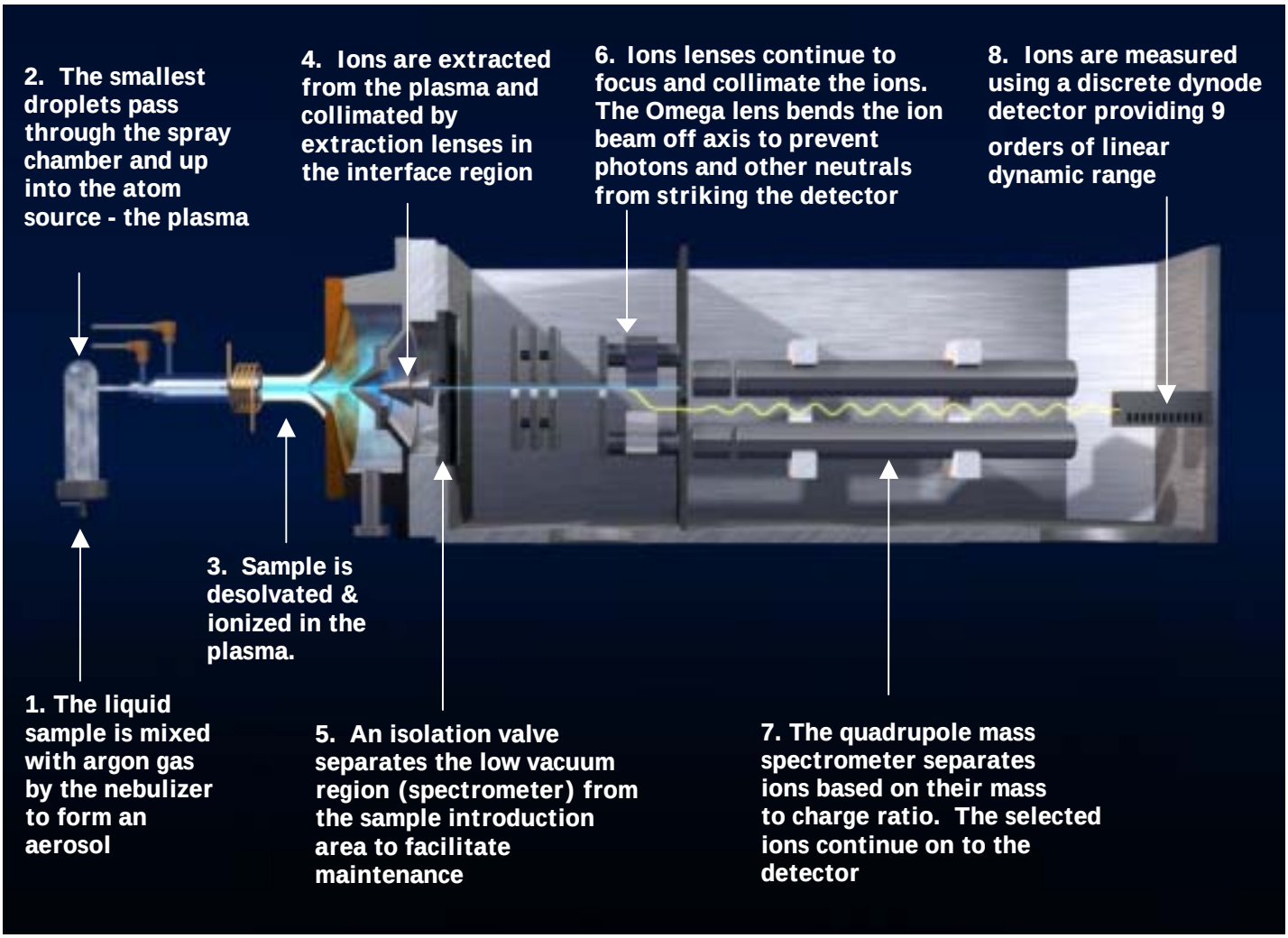

Figure 1. Schematic of an ICP-MS

The interface is not yet commercially available, but it shows some promise. We have used the GC-ICP-MS device to separate and quantify organotin compounds – monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), monophenyltin (MPhT), diphenyltin (DPhT), and triphenyltin (TPhT) - in marine environmental samples of oyster tissue and sediment, collected from the same locality.

Instrumentation

The GC used in these studies was a model 5890, and the ICP-MS was a 4500, both from Agilent Technologies.

The general principal of combining GC with ICP-MS is simple. The end of the capillary GC column is fastened to the base of the ICP torch, so that separated species are carried directly into the plasma by a heated Ar flow.

Using a heated transfer line to connect the GC to the ICP-MS prevents material condensing within the interface and so enables the analysis of high boiling point compounds. Figure 2 is a schematic that describes the interface used during these experiments. Xenon was added to the argon make-up gas as a means of optimizing the ICP-MS operating conditions. The Xe:Ar gas mixture was preheated by passing it through a stainless steel coil mounted within the GC oven.

Initial evaluations of the interface were undertaken at the laboratory of O.F.X. Donard at the University of Pau in France. Figure 3 is a chromatogram of a GC-ICP-MS separation of a 1µL injection of a standard, which contained a mixture of organotin species. Each peak in Figure 3 represents the equivalent of 5 pg tin.

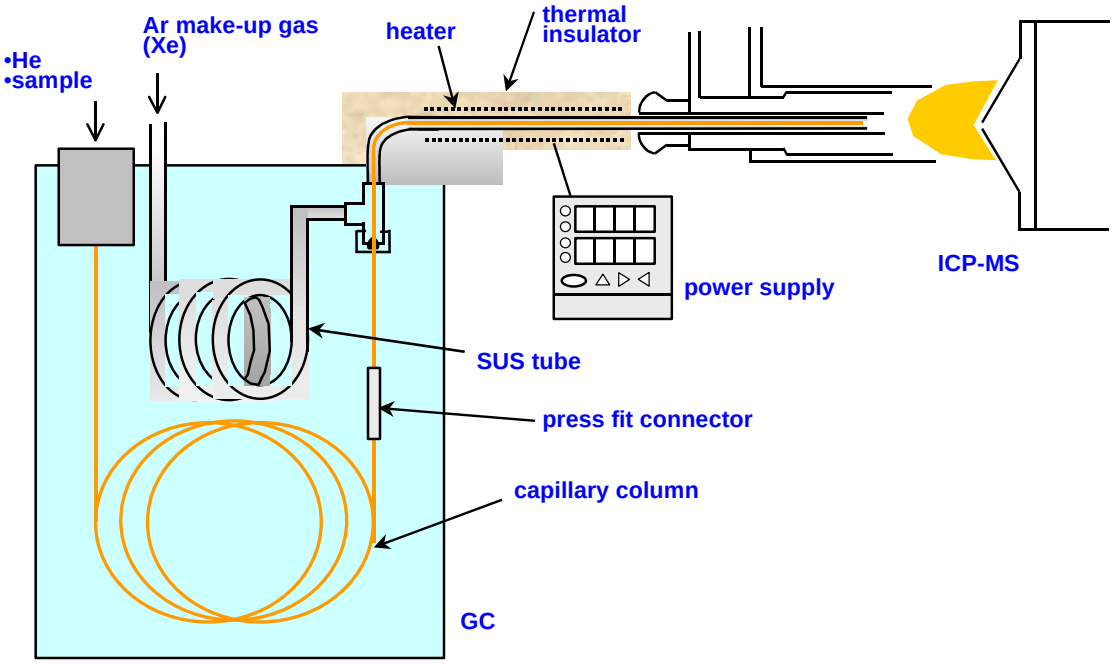

Figure 2. Schematic of the GC-ICP-MS and Interface

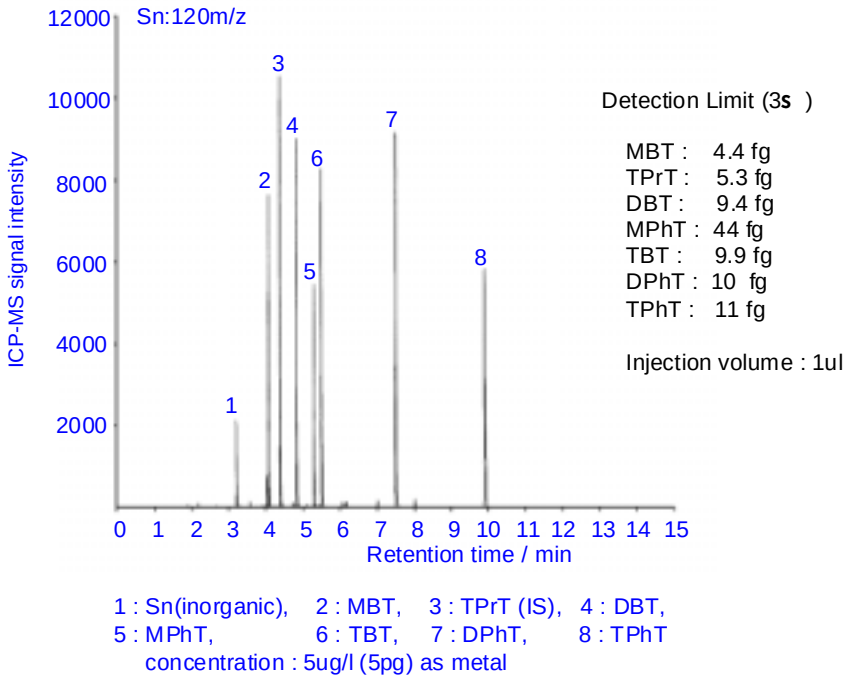

Figure 3. GC-ICP-MS Chromatogram of a 1uL Injection of a 5ppb Organotin Mixed Standard

Table 1 GC-ICP-MS Acquisition Conditions

GC	
Injection mode	Splitless
Injection volume	1uL
Inlet temp	290° C
Column	Non-polar capillary – HP-1 (30m,0.32mm, 0.25um)
Carrier gas	He – 1.0mL/min
Oven program	70°C (1 min):ramp to 190°C (30°C/min): ramp to 270°C (15°C/min)
Interface temperature	250° C
ICP-MS	
RF power	1300W
Sampling depth	8mm
Carrier gas flow	0.8L/min
Oxygen gas flow	0.02L/min (added to auxiliary gas)

Ethylation using sodium tetraethylborate ($NaBEt_4$) was chosen as the derivatization method for this work. The reaction between the organotin compound and the $NaBEt_4$ is in aqueous conditions, which makes it much more suitable for environmental and biological samples than a normal Grignard reagent.

As the chromatogram illustrates, the peak shapes obtained are excellent, suggesting little or no broadening caused by the interface.

Initial results

Preliminary studies on organotin content of oysters and sediments were done in collaboration with the University of Pau . We have taken here samples from the Bay of Arcachon since it is one of the most productive areas for oyster farming. Despite of the fact that organotin concentrations have declined in the water and the sediment of the bay, organotin compounds can be founds in oysters, due to bioaccumulation. In general there is an increasing concern about the occurrence of organotin in shellfish worldwide, particularly as many species are used for human consumption. Samples of oysters and sediment were collected and analysed using the GC with ICP-MS detection.

Some care had to be exercised in the preparation of the oyster tissue, to prevent any potential decomposition of the analyte. The sample preparation method is summarized in Figure 4. Tripropyl tin (TPrT) was added to each sample as an internal standard.

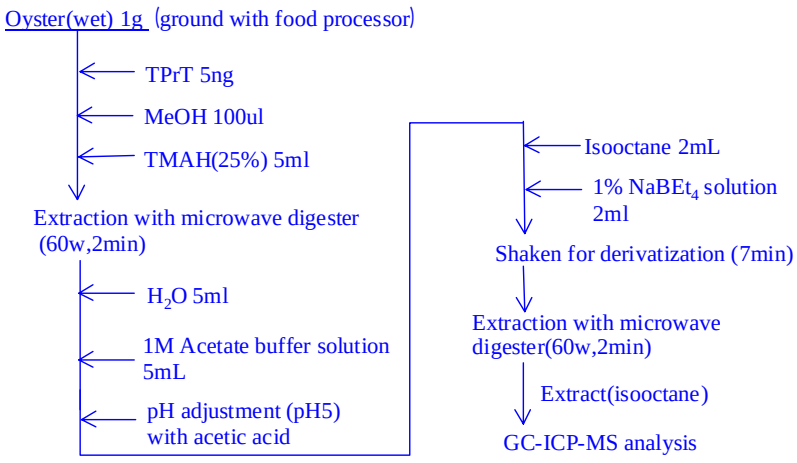

Figure 4. Sample Preparation Steps for the Oyster Tissue

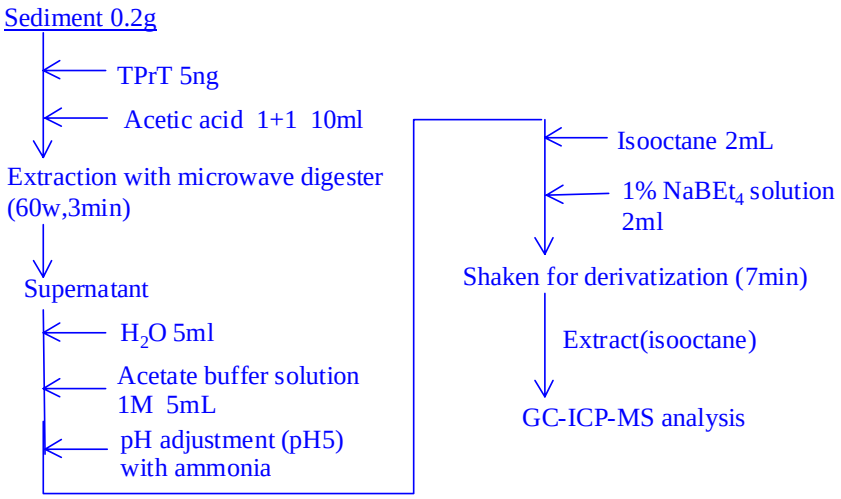

Figure 5. Sample Preparation Steps for the Sediment

A similar procedure was used for the preparation of sediment samples (Figure 5), except that acetic acid was used instead of Tetramethyl ammonium hydroxide (TMAH).

Figure 6 is a typical GC-ICP-MS chromatogram from one of the oyster extracts, illustrating excellent separation and peak shape. As the data show, there are substantial and measurable amounts of a variety of organotin compounds in the sample.

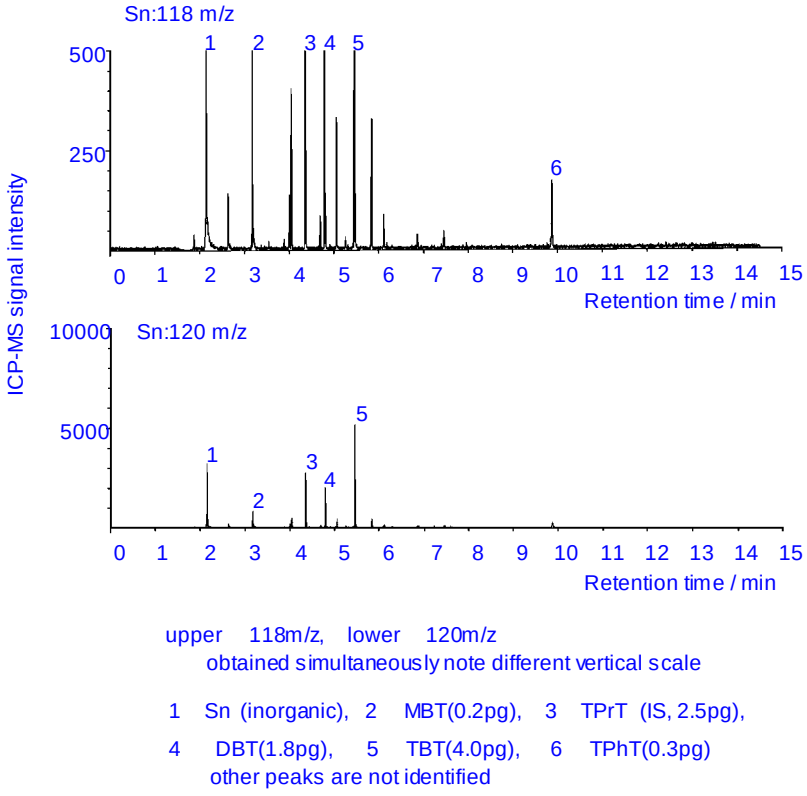

Figure 6. GC-ICP-MS Chromatogram from the Analysis of One of the Oyster Tissue Samples

Table 2. Results Summary from the Analysis of Oyster Samples across Arachon Bay, Plus Analysis of a Sediment Sample

Area	water(%)	concentration / ng/g (Dry)					
		MBT	DBT	TBT	MPhT	DPhT	TPhT
Area1	85	8.3	13	28	0.3	*N.D.*	*N.D.*
Area2	81	0.8	9.5	21	*N.D.*	*N.D.*	1.6
Area3	85	3.7	15	46	1.3	*N.D.*	*N.D.*
Area4	81	1.1	12	42	*N.D.*	*N.D.*	*N.D.*
Area5	86	0.7	11	51	*N.D.*	*N.D.*	2.1
Area6	83	1.8	13	39	*N.D.*	*N.D.*	*N.D.*
Area7	81	0.5	5.3	16	*N.D.*	*N.D.*	1.6
Area8	82	1.7	18	54	*N.D.*	*N.D.*	*N.D.*
Area9	83	2.4	20	141	*N.D.*	*N.D.*	*N.D.*
sediment	36	6.0	5	7	3.0	*N.D.*	2.0

Table 2 summarizes data from oysters sampled in and around Arachon Bay, as well as a sediment sample. Tributyltin (TBT) is the single largest component in each case, although there are several other species present at significant levels. Monobutyltin (MBT) and Dibutyltin (DBT) are breakdown products of TBT. Although the use of TBT in marine antifouling paints has been discontinued in France, the organotin compound still exists in the sediment where the oysters develop. Of particular interest is Area 9, which is a part of the Bay where oyster production has been poor. This also coincides with the largest level of TBT.

The exceptional resolution of the chromatographic separation allows an anticipation of the formation of metabolite products from organotin compounds (e.g. methylation of butyltin compounds) opening the way to new understanding of environmental and biometabolic pathways for these contaminants after further identification.

Summary

These preliminary results suggest that GC-ICP-MS offers a highly sensitive and selective method for the determination of organometallic compounds in environmental matrices The exceptional chromatographic separation capability of the CGC, coupled to the sensitivity, selectivity and multielemental capability of the ICP-MS detector, certainly makes this combination a very promising tool for environmental studies. The interface used in these studies is not yet commercially available and will require some further refinement and characterization. The robustness of the heating system will require some refinement to ensure long-term reliability. Future applications work is under way evaluating the potential of this interface for the simultaneous determination of other organometals such as tin, lead and mercury compounds.

Printed in Japan (07/03)
5980-0336E

Separation and Analysis of Toxic Arsenic Species, Using LC-ICP-MS

Application Note

ICP-MS

Environmental

Tetsushi Sakai

Introduction

Arsenic is a common element in the natural environment and in biological materials. It is used for industrial purposes such as in agricultural chemicals, semiconductor materials, industrial gases and so on. Arsenic exists as arsenopyrite in nature. Water from some volcanic hot springs can contain large amounts of arsenic.

Arsenic in environmental water is generally assumed to exist primarily as its anionic forms, such as As(III) or As(V).

Arsenic takes various chemical forms and is known to be "bio-active", which means that it is easily converted from one form to another by biological processes. Of the various forms of As, some are essentially harmless to human life (such as arsenobetaine and arsenocholine), while others, notably the inorganic forms, are not only specified as toxic, but have also been shown to be carcinogenic. Issues relating to As toxicity are of interest all over the world and several million people are affected by arsenic pollution, which has been highlighted in West Bengal in India, Bangladesh and Inner Mongolia.

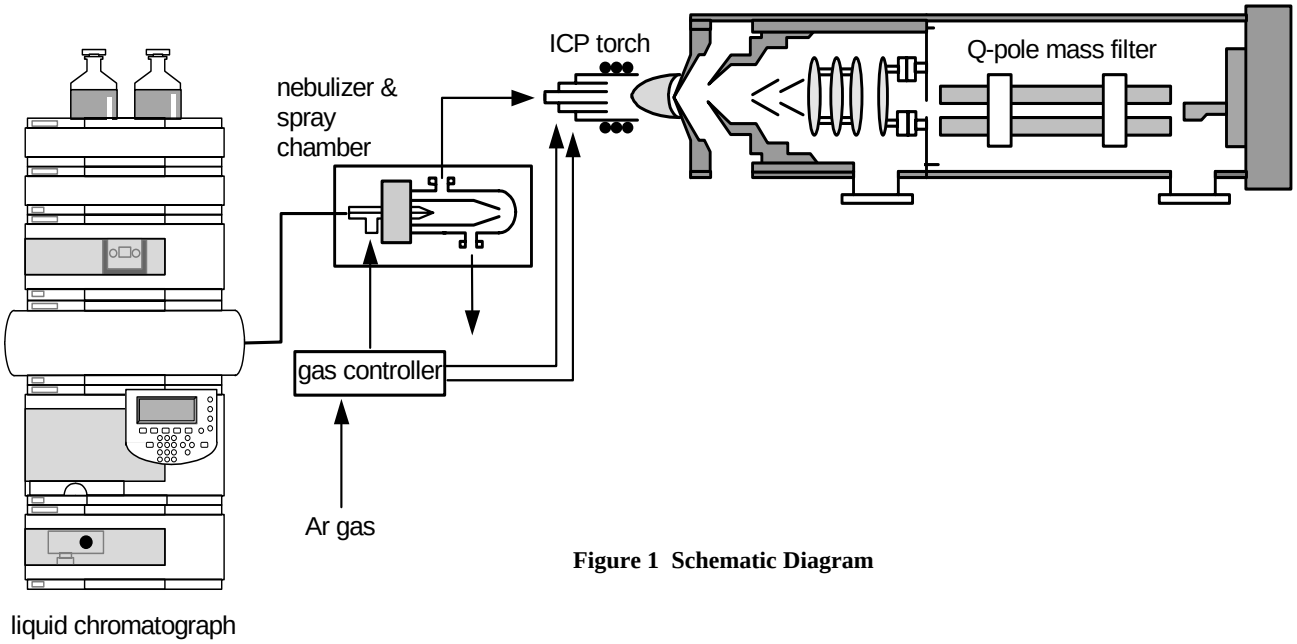

Figure 1 Schematic Diagram

Nowadays, arsenic analysis is required under various laws. According to the World Health Organisation (WHO) drinking water guidelines, Japan's drinking and environmental water quality standards strictly require that the concentration of arsenic should be less than 10 ug/L(ppb) . Ensuring a low total As level will automatically mean that the toxic forms of the element are also low, but separate identification and quantification of the individual forms of As would be of much greater use in assessing the potential toxicity.

Since the toxicity and the metabolism of As alters depending upon its form, evaluation of each chemical form is essential, to correctly measure the potential impact of the arsenic content of environmental, nutritional and other inputs to the human body.

In this application note, a system combining liquid chromatography (LC) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to separate and analyze several arsenic species. This LC-ICP-MS system allows highly sensitive and selective analysis of arsenic species contained in drinking water, river water and many other sample types.

Mechanism and Configuration of LC-ICP-MS System

Generally, there are no cationic organo-arsenic compounds present in either environmental water or drinking water, therefore the target analytes selected for this study were As(III) (which is both toxic and carcinogenic), As(V), dimethylarsinic acid (DMAA) (which functions as a carcinogen promoter) and methylarsonic acid (MMAA).

As only total arsenic concentration is specified in many existing regulations, AAS, ICP-OES or ICP-MS (either alone or in combination with hydride generation) can be used to analyze arsenic in many cases. However, with improved understanding of the potential for toxic effects at very low concentrations, it is becoming increasingly important to acquire information on each different chemical form, to fully understand the potential toxicity.

Separation technique such as LC is generally effective analytical method to separate the individual chemical forms of an element. However, there are some problems in anion exchange chromatography when it is applied to the arsenic speciation analysis in environmental water.

It is not easy to perform rapid, simultaneous analysis of different arsenic compounds because the ionicity varies with each form. For example, As(III) has weak ionicity, and it is difficult to separate from the cationic arsenic compounds, also some species such as As(V) can react with other metallic species forming a precipitate. Due to its reactivity, some arsenic containing samples can react with metallic elements on the column, and the correct results cannot be obtained. Moreover, analysis at ug/L(ppb) level is almost impossible with LC's inadequate sensitivity.

On the other hand, ICP-MS has very high sensitivity and selectivity, making it ideally suited to the analysis of trace elements, albeit with the limitation that it is an elemental analyser and so provides no information on the different forms of an element. Virtually the only potential difficulties in the determination of As by ICP-MS relate to its high first ionisation potential (which reduces the proportion of ions formed and therefore the sensitivity) and the potential overlap on As from $^{40}Ar^{35}Cl$ at mass 75. Both of these potential problems can be reduced or virtually eliminated, through optimisation of the plasma and sample introduction parameters. The potential for suppression effects and interface clogging due to the presence of highly ionic eluent and buffer solutions is also reduced, as the Agilent 7500 is designed to operate under sample introduction conditions which ensure complete matrix decomposition.

The combination of LC and ICP-MS makes use of the best features of each technique, to give efficient separation of the various forms of an element, followed by sensitive and selective detection. The commercial availability of arsenic speciation kits, which contain all the required columns and the LC connection kit, makes this application a routine possibility in high-throughput and commercial laboratories.

One reported limitation of coupled chromatographic techniques relates to the speed of the ICP-MS detector, which is typically operated in "dual-mode", meaning signals are recorded simultaneously in high sensitivity pulse-count and low gain analog modes. In conventional ICP-MS detectors, the response time of the Analog mode is much slower than the pulse-count mode, so measurement speed is compromised when using dual mode. The Agilent 7500, by contrast, features a true simultaneous dual mode detector with a new, high-speed log amplifier, which allows the system to acquire data at the same high speed, whether analyzing in pulse counting mode, analog mode or both. This new detector also covers a linear range of 9 orders of magnitude, making the Agilent 7500 the ultimate tool for time resolved measurements, such as those required for chromatographic analysis.

Method developed with the LC-ICP-MS column and eluent configuration and operating conditions allowed separation and analysis of four arsenic species, As(III), DMAA, MMAA and As(V), in only 10 minutes. On-column loss of As was prevented by the addition of a complexing agent (EDTA) to the eluent. The high sensitivity of the ICP-MS allowed these As species to be determined easily at the ug/L(ppb) levels required under current legislation.

Analysis Example

The operating conditions used for this experiment are shown in Table 1. The data were processed using the ICP-MS Chromatographic Software, which integrates the LC and ICP-MS modules to allow completely automatic acquisition and data calculation from chromatographic measurements, in conjunction with the standard ICP-MS ChemStation.

Figure 2a shows the chromatogram from the measurement of a standard solution which contained 20 ug/L(ppb) of each arsenic species, illustrating the complete separation of the four As species, As(III), DMAA, MMAA, As(V) in only 10 minutes. Figure 2b shows a comparable chromatogram from the analysis of a drinking water sample.

Due to the fact that ozonation or other oxidative methods are frequently used during the treatment of drinking water supplies, the various forms of As present in the source water may be converted to As(V) following treatment, so only this form is found in the final water. Table 2 shows the results of reproducibility tests (n=6) of these four species under the same column and analytical conditions. All species showed excellent limits of detection (3 sigma) in the region of 0.1 ug/L(ppb) and the reproducibility (RSD%) at 10 ug/L(ppb) was less than 2%.

Figure 3 shows 5-point calibration curves within the concentration range from 1 and 100 ug/L(ppb) for each of the four As species studied, As(III), DMAA, MMAA and As(V). The results demonstrate exceptional linearity with correlation coefficients (R2) better than 0.9997 for all four species.

Table 1 Operating Conditions

LC

LC	Agilent 1100 Series
Column	Anion exchange columns (G3154A/101, G3154A/102))
Mobile phase	2.0 mM PBS/0.2 mM EDTA solution
Flow rate	1.0 mL/min
Column temperature	Ambient
Injection volume	0.05 mL
Run time	10 min (600 sec)
Number of injection	1

ICP-MS

ICP-MS	Agilent 7500
RF power	1.4 kW
Plasma gas	15 L/min
Aux. gas	1.0 L/min
Carrier gas	1.1 L/min
Sampling depth	7.5 mm
Acquired mass	75
Points/mass	1
Dwell time	0.5 sec/mass

Conclusion

Recently, anionic arsenic compounds in environmental water have received widespread attention due to their potential toxicity to humans. They can be analyzed quickly and precisely at the low concentrations required under current legislation, using an optimized coupled technique consisting of the Agilent 1100 LC, coupled to the Agilent 7500 ICP-MS system. The compatibility and automation of this coupled system means that LC-ICP-MS can be considered a routine, high sample throughput method for monitoring levels of potentially toxic arsenic species in environmental and nutritional samples.

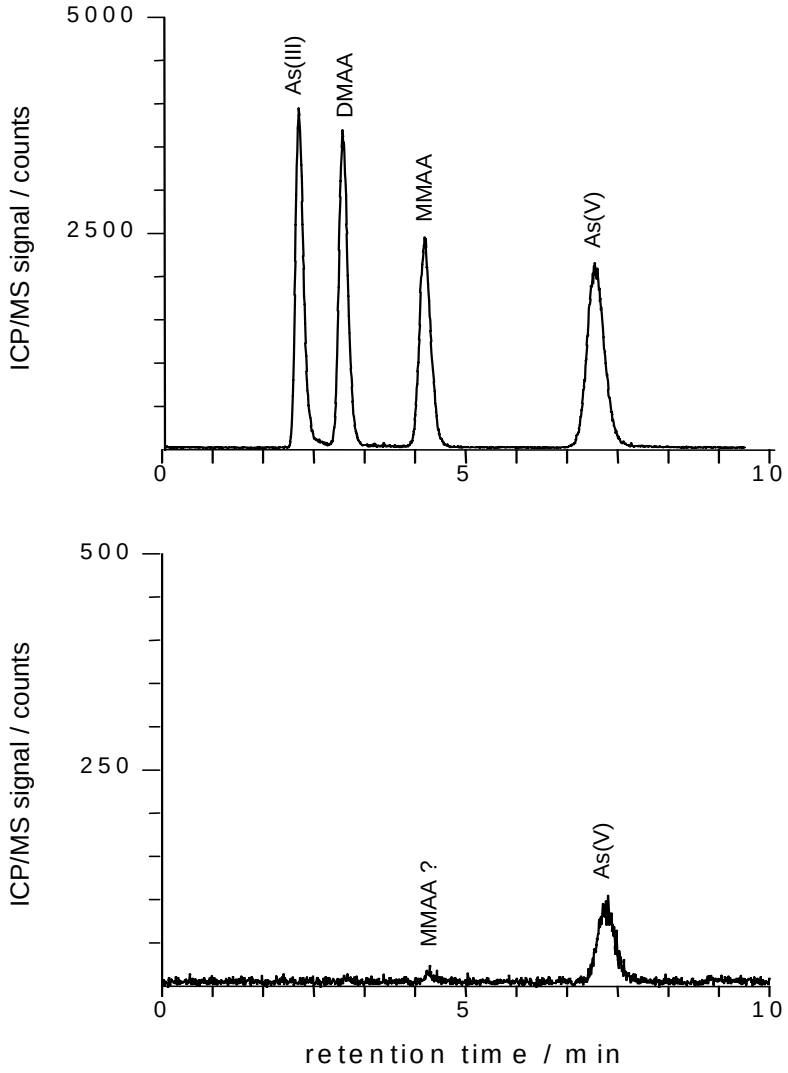

Figure 2 Chromatograms of As Species
a) 10 ug/L(ppb) As species mixed standard solution
b) drinking water

Table 2 Repeatability and Detection Limits

	Repeatability (n=6)	DL (ug/L)
As(III)	2.0%	0.1
DMAA	1.5%	0.1
MMAA	1.3%	0.1
As(V)	1.6%	0.2

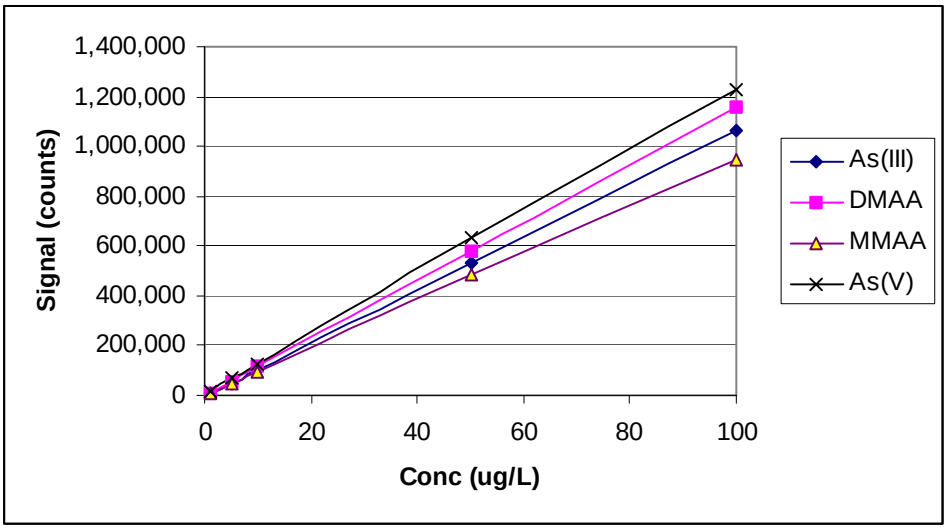

Figure 3 Calibration Curves for As Species

Printed in the USA. 17 June, 2003
5980-0262E

Meeting Worldwide Regulatory Requirements for the Analysis of Trace Metals in Drinking Water Using the Agilent 7500c ICP-MS

Application

Environmental

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Abstract

The Agilent 7500c ICP-MS can be used to meet the regulatory requirements for trace metals in drinking water around the world. Elements previously relegated to other techniques, such as GFAA or ICP-OES due to very high or low concentrations or the presence of interferences, can now be measured in a single analysis.

Introduction

Virtually all developed countries have adopted programs and regulations to monitor and maintain the quality of public water systems. In the US, water quality is regulated by the United States Environmental Protection Agency (USEPA) as mandated by the *Safe Drinking Water Act of 1974*. In the European Union, drinking water is regulated by the *Council Directive 98/83/EC of 3, November, 1998 on the Quality of Water Intended for Human Consumption*. In Japan, quality of drinking water is regulated by the *Japan Water Supply Act*, dating from 1957, and most recently updated in 2001. Most of the rest of the world's developed countries have adopted drinking water quality standards based on World Health Organization (WHO) Standards, *Guidelines for Drinking Water Quality, 1996, 1998*, or on the USEPA standards. While these guidelines, as they pertain to trace metals, vary somewhat in their lists of regulated metals and concentrations, they are fundamentally similar. They all require accurate, precise measurement of multiple toxic metals in drinking waters at the lowest practical limits of quantification. This application note will demonstrate that the sensitivity, accuracy, and precision requirements for the analysis of trace metals in drinking water worldwide can be met by a single, robust technique using the Agilent 7500c ICP-MS system with Octopole Reaction System (ORS) technology.

US Regulations

In the US, the quality of public drinking water is safeguarded by the provisions of the Safe Drinking Water Act of 1974.

The Safe Drinking Water Act (SDWA) was originally passed by Congress to protect public health by regulating the nation's public drinking water supply. The law, amended in 1986 and 1996, requires many actions to protect drinking water and its sources in rivers, lakes, reservoirs, springs, and ground water wells (SDWA does not regulate private wells, which serve fewer than 25 individuals). SDWA authorizes the USEPA to set primary national health-based standards for drinking water to protect against both naturally-occurring and man-made contaminants that may be found in drinking water. These primary national drinking water standards include maximum contaminant level goals (MCLGs), levels below which there is no known or expected health risk. From these MCLG values, EPA determines maximum contaminant levels (MCLs), which are

enforceable levels that may not be exceeded. The MCLs are set as closely as possible to the MCLGs and are based on best available current technology and economic feasibility. These limits are reviewed and updated periodically as new information becomes available and technology improves.

Japanese Regulations

Drinking water quality in Japan is regulated by the Japan Water Supply Act, which was first promulgated in 1957 with the Quality Standard for Drinking Water set the following year. This standard currently regulates the drinking water quality of more than 97% of the population. The Quality Standard sets maximum allowable concentrations (MAC) for 17 metals. It also requires that quantification limits be set at 1/10 of the MAC to assure accurate measurements at trace levels. Because of this, in 2001 the Drinking Water Test Method was revised and expanded to include the use of ICP-MS for 14 of the 17 metals. The approval of the use of ICP-MS has eliminated the need for costly and time consuming preconcentration, which was required to meet the required detection limits using ICP-OES.

European Union Regulations

Currently, water quality in the European Union (EU) is regulated by Council Directive 80/778/EEC. This directive applies to all waters intended for human consumption, except natural mineral waters or waters which are medicinal products. As of December 2003, Directive 80/778/EEC will be repealed and replaced by Council Directive 98/83/EC *Directive on the Quality of Water Intended for Human Consumption,* which came into force on December 25, 1998. The standards are based largely on recommendations by the WHO[1]. Member states of the European Community, while they must comply fully, are permitted to implement regulation and enforcement locally. As a result, no single regulation exists for the analysis of trace metals in water throughout Europe.

[1]World Health Organization Guidelines and International Standards for Drinking-Water Quality, 1998

Table 1. Drinking Water Standards for Trace Metal Content from WHO Recommendations, EU Regulations, Japan Drinking Water Regulations and USEPA.

Analyte	WHO Standard (mg/L)	EC Directive 98/83/EC (mg/L)	Japan Drinking Water Standard (mg/L)	USEPA Primary MCL (mg/L)	Agilent 7500c MDLs*** (mg/L)
Aluminum (Al)		0.2	0.2	0.02-0.2*	0.000054
Antimony (Sb)	0.005**	.005	0.002	0.006	0.000035
Arsenic (As)	0.01**	.01	0.01	0.01	0.000052
Barium (Ba)	0.7			2	0.000027
Beryllium (Be)				0.004	0.000028
Boron (B)	0.5**	1	1.0		
Cadmium (Cd)	0.003	0.005	0.01	0.005	0.000025
Chromium (Cr)	0.05**	0.05		0.1	0.000019
Copper (Cu)	2**	2	1.0	1.3	0.000023
Iron (Fe)		0.2	0.3	0.3*	0.00125
Lead (Pb)	0.01	.01	0.05	0.015	0.000017
Manganese (Mn)	0.5**	.05	0.05	0.05*	0.000020
Mercury (Hg)	0.001	0.001	0.0005	0.002	0.000005
Molybdenum(Mo)	0.07				0.000030
Nickel (Ni)	0.02**	0.02	0.01		0.000024
Selenium (Se)	0.01	0.01	0.01	0.05	0.000047
Silver (Ag)				0.01*	0.000027
Sodium (Na)		200	200		0.0276
Thallium (Tl)				0.002	0.000021
Uranium (U)	0.002**		0.002	0.030	0.000015
Zinc (Zn)				5.0*	0.000101

*Secondary Standard, **Provisional Guideline Value, ***MDLs Calculated as Three Sigma of 10 Replicates of Low Standard, as Described in this Work. MDLs Reported in mg/L to Match Regulatory Requirements.

Table 1 includes the trace metals that are regulated by various worldwide regulatory and advisory agencies. ICP-MS is the only analytical technique capable of meeting all the required detection limits for all the regulated trace metals. Therefore, while not mandated as the only acceptable technique for most regulations, ICP-MS is becoming the instrument of choice for trace metals analysis in water worldwide.

While the details of QA/QC criteria and reporting requirements vary significantly from jurisdiction to jurisdiction, Table 1 shows that the actual detection limit requirements are very similar. In addition, the fundamental goals of the QA/QC requirements in all jurisdictions are the same. This is to insure that the reported values for all samples meet commonly accepted guidelines for accuracy and precision. Typically, these guidelines are met through the analysis of periodic QC samples inserted into the sample queue. Such QC samples should include: a check on the accuracy of the initial instrument calibration; a control sample of known concentration similar to that of the analytes in a similar matrix; a sample designed to test the ability of the system to eliminate interferences as false positives; a sample designed to detect sample carryover or memory problems; and periodic calibration check samples to check for instrument drift. If samples are to be analyzed outside the calibration range of the analytical method, then a linear range check sample must also be analyzed. It is outside the scope of this application note to detail the specific QA/QC requirements for each regulation where they exist at all. Instead, a general QA/QC protocol will be outlined which will demonstrate the ability of the Agilent 7500c to meet generally accepted guidelines while easily meeting the required reporting limits for drinking water monitoring worldwide. Simple modifications to this procedure can be implemented to insure strict compliance with detailed local requirements.

Advantages to the Use of the ORS for Drinking Water Analysis

Generally, drinking water is not considered a particularly difficult matrix for analysis by ICP-MS. There are, however, a few significant challenges. These challenges are due to the very low desired reporting limits for several elements (Table 1), as well as the possibly high concentrations for others, such as Ca and Na. This combination of very low and very high analyte concentrations presents a challenge that no other analytical technique can overcome. In order to measure all elements simultaneously, the ICP-MS must be able to accurately measure mercury at 0.05 ppb or less and Na or Ca as high as 1000s of ppm. In addition, the ICP-MS must be able to eliminate common interferences on Fe, As, Se, Cu, V, and other elements which originate in the plasma and interface region. If unmanaged, these interferences make trace level analysis of the above elements difficult or impossible in many water samples.

The ORS serves two purposes. First, it uses collision/reaction cell technology to virtually eliminate polyatomic interferences on most elements. This allows the analyst to select the most abundant isotope of each analyte for analysis and avoid the use of mathematical correction factors. The result is sub-ppb detection limits for virtually all elements of interest. Second, it allows the analyst to use passive collisions in the ORS to reduce the ion current for high concentration, low-mass elements such as Na and Ca. In this way, the dynamic range for these elements is shifted to allow accurate, linear measurement at levels previously impossible by ICP-MS. It is this ability to simultaneously improve the sensitivity for ultra-trace analytes and extend the dynamic range upward for matrix analytes that is unique to the ORS system.

Instrument Conditions

Table 2 shows the instrument conditions used for typical water analysis. Listed are the preferred isotope, the tune mode (normal, hydrogen reaction, or helium collision), integration time, calibration range, and approximate detection limit based on normal commercial laboratory conditions. RF power is typically set high, 1400–1500 W, to maximize decomposition of the matrix. Other tune conditions such as ion optics, quadrupole, and detector parameters are set according to standard instrument tune guidelines. No special tuning is required.

Table 2. Elements of Interest with Appropriate Isotopes, ORS Acquisition Mode, Integration Time, Calibration Range and Measured MDLs for Each Isotope

Analyte	Isotope	ORS mode	Integration time (s)	Calibration range (ppb)	~MDL (ppb)
Aluminum (Al)	27	Normal	0.1	0.5–100	0.054
Antimony (Sb)	121	Normal	0.1	0.5–100	0.035
Arsenic (As)	75	Helium	0.5	0.5–100	0.052
Barium (Ba)	137	Normal	0.1	0.5–100	0.027
Beryllium (Be)	9	Normal	0.1	0.5–100	0.028
Boron (B)	10	Normal	0.1	0.5–100	
Cadmium (Cd)	111	Normal	0.1	0.5–100	0.025
Calcium (Ca)	40	Hydrogen	0.1	50–200,000	2.02
Chromium (Cr)	52	Helium	0.5	0.5–100	0.019
Copper (Cu)	63	Helium	0.5	0.5–100	0.023
Iron (Fe)	56	Hydrogen	0.1	50–200,000	1.25
Lead (Pb)	Sum of isotopes 206, 207, 208	Normal	0.1	0.5–100	0.017
Manganese (Mn)	55	Normal	0.1	0.5–100	0.020
Mercury (Hg)	202	Normal	1.0	0.05–1.0	0.005
Molybdenum(Mo)	95	Normal	0.1	0.5–100	0.030
Nickel (Ni)	60	Helium	0.5	0.5–100	0.024
Potassium (K)	39	Helium	0.5	50–200,000	3.02
Selenium (Se)	78	Hydrogen	0.5	0.5–100	0.047
Silver (Ag)	107	Normal	0.1	0.5–100	0.027
Sodium (Na)	23	Hydrogen	0.1	50–200,000	27.6
Thallium (Tl)	205	Normal	0.1	0.5–100	0.021
Uranium (U)	238	Normal	0.1	0.5–100	0.015
Vanadium (V)	51	Helium	0.5	0.5–100	0.034
Zinc (Zn)	66	Normal	0.1	0.5–100	0.101
Useful Internal Standards					
6Li	6	Normal	0.1	50	
Sc	45	All	0.1	50	
Ge	70,72,74	All	0.1	50	
Y	89	Normal	0.1	50	
In	115	Normal	0.1	50	
Tb	159	Normal	0.1	50	
Pt	195	Normal	0.1	50	
Bi	209	Normal	0.1	50	

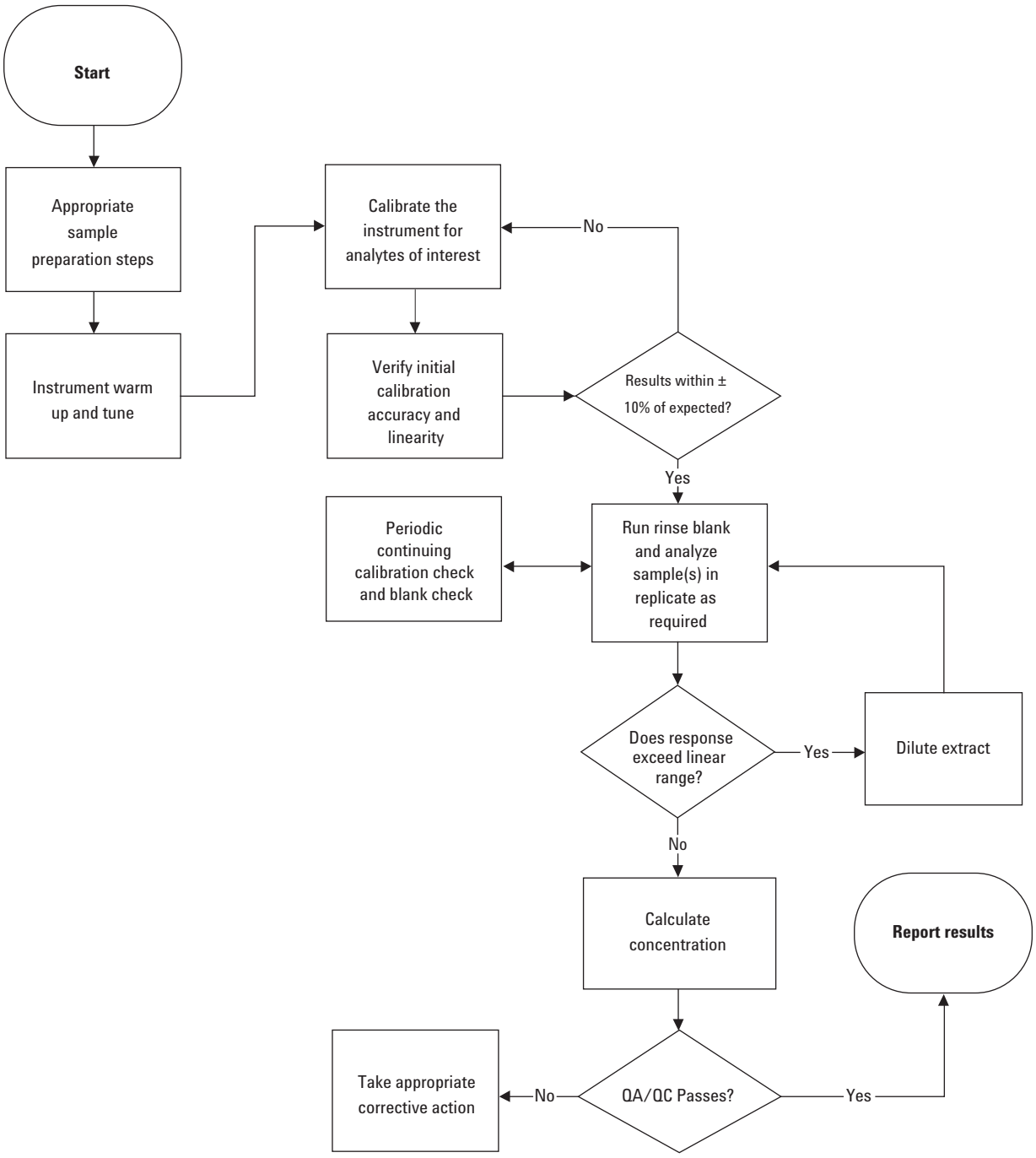

Figure 1. Summary of general water analysis protocol.

Figure 1 depicts the general flow of sample analysis and QA/QC that would be performed to meet the daily requirements of most drinking water regulations. Specific QA/QC details vary from jurisdiction to jurisdiction and are not outlined here. In addition to the daily requirements, less frequent, periodic QA/QC documentation must be performed to ensure ongoing accuracy and precision. Such periodic requirements include: verification of method detection limits, dynamic range, management of interferences (both isobaric and memory effects), as well as general instrument condition and performance. Specific examples of these requirements are found in USEPA Method 200.8 and the UK Drinking Water Inspectorate publication, "NS-30."

Interference Correction

Because the ORS is capable of efficiently removing polyatomic interferences and most isobaric

elemental interferences in unknown, complex matrices, the use of mathematical interference correction is all but eliminated. The elements which typically require interference correction in water, Ca, V, Fe, As, Se, Mo, and Cd can all be analyzed without the need for mathematical correction. This simplifies the analysis and improves confidence in the results. In this work, only Li-6, In-115, and Pb are corrected (see Table 3). The Li-6 correction is used to correct the abundance of the Li-6 internal standard in the presence of high concentrations of Li-7 in some samples. The In-115 correction is used to correct an internal standard, In, in the presence of high concentrations of tin. Neither of these cases is common and normally these can be ignored. The Pb correction is used to normalize the lead response in the case of varying lead isotope ratios and is not an interference correction.

Table 3. Typical Mathematical Corrections Used for Water Matrices with the Agilent 7500c ORS System

Mass	Equation
6	(6)*1 - (7)*0.082
115	(115)*1 - (118)*0.014
208	(208)*1 + (206)*1 + (207)*1

Experiment

The following data and results were all obtained from a single sequence of 44 analyses of standards, blanks, QC samples, unknown groundwater samples, and seawater samples. All calibrations are based on a single set of standards prepared in 1% nitric acid/0.5% hydrochloric acid. No attempt at matrix matching beyond simple acidification was made. The instrument and conditions were like those of a typical commercial environmental laboratory. “Clean room” conditions or ultra-high purity reagents were not employed. The Agilent 7500c ICP-MS with ORS and Integrated Sample Introduction System (ISIS), configured for autodilution, was used.

Quality Control

Quality control in this experiment consisted of four components:

- Verification of tune performance for each ORS mode
- Initial Calibration linearity check
- Verification of accuracy of initial calibration using NIST 1640 standard reference water
- Periodic verification of calibration accuracy through measurement of continuing calibration verification (CCV) samples

Autodilution

The Agilent 7500c was configured with an ISIS for rapid sample uptake and autodilution. ISIS uses flowing stream autodilution rather than discrete sample dilution. This greatly enhances the throughput and minimizes the possibility of contamination compared with other types of autodiluters. In the ISIS autodiluter, the sample stream is mixed with a flowing stream of diluent in an entirely closed system. Dilution factor is controlled by high precision peristaltic pumps that are automatically and periodically monitored for accuracy throughout the run. Autodilution is invoked automatically by the intelligent sequencing software whenever the system encounters a user-definable out-of-range condition, such as an analyte outside the calibration range or an internal standard outside predefined bounds. Autodilution was invoked in a number of the samples in this work. An excellent check on both the linearity of the instrument and the accuracy of the autodilution can be obtained by comparing the results for diluted and undiluted samples. If the results match well, both the instrument linearity and autodilution accuracy are in control. Tables 5 and 7 show excellent examples of this.

Results

QC results are depicted in Tables 4 (CCV results) and 5 (NIST 1640 results). Examples of calibration linearity are depicted in Figures 2, 3, and 4, which are representative. Calibration “R” values of .9998 or greater are considered linear.

Table 4. Recovery of Periodic Calibration Check Standard in a Sequence of Water Samples Including Drinking Waters, Ground Waters, and Seawaters. Calibration Checks were Run After 30 and 43 Real Samples in this Experiment

	CCV Actual value	CCV 50/5000/0.5	% Recovery	CCV 50/5000/0.5	% Recovery
Total DF:		**1**		**1**	
File:		**031_CCV.D#**		**044_CCV.D#**	
Be/9 [#1]	50	50.62	101.2	50.01	100.0
Na/23 [#2]	5000	4933.00	98.7	4838.00	96.8
Mg/24 [#1]	5000	4700.00	94.0	4802.00	96.0
Al/27 [#1]	50	47.09	94.2	46.84	93.7
K/39 [#3]	5000	5260.00	105.2	5076.00	101.5
Ca/40 [#2]	5000	5053.00	101.1	5063.00	101.3
V/51 [#3]	50	51.52	103.0	50.84	101.7
Cr/52 [#3]	50	51.43	102.9	50.78	101.6
Mn/55 [#1]	50	49.92	99.8	50.89	101.8
Fe/56 [#2]	5000	5067.00	101.3	5068.00	101.4
Co/59 [#1]	50	49.88	99.8	50.16	100.3
Ni/60 [#3]	50	51.99	104.0	51.36	102.7
Cu/63 [#3]	50	52.64	105.3	51.74	103.5
Zn/66 [#1]	50	49.27	98.5	49.44	98.9
As/75 [#3]	50	51.63	103.3	51.58	103.2
Se/78 [#2]	50	50.90	101.8	50.61	101.2
Se/80 [#2]	50	51.45	102.9	51.10	102.2
Mo/95 [#1]	50	49.44	98.9	48.11	96.2
Ag/107 [#1]	50	48.73	97.5	47.02	94.0
Cd/111 [#1]	50	49.34	98.7	48.40	96.8
Sb/121 [#1]	50	47.71	95.4	47.03	94.1
Ba/137 [#1]	50	50.35	100.7	49.19	98.4
Hg/202 [#1]	0.5	0.49	98.3	0.47	94.8
Tl/205 [#1]	50	49.68	99.4	50.46	100.9
Pb/208 [#1]	50	49.41	98.8	49.25	98.5
Th/232 [#1]	50	48.54	97.1	49.09	98.2
U/238 [#1]	50	49.46	98.9	49.84	99.7

Values in brackets after element mass are tune mode, 1= normal, 2 = hydrogen, 3 = helium.

Table 5. Analysis of Certified Reference Water NIST 1640 as a Calibration Check. Sample was Measured Neat and Autodiluted 1/20 (actual measured DF = 21.72), since Na Value Exceeded Upper Calibration Limit. Note that Even in the Undiluted Sample, the Recovery for Na is 101.2%

	Certified value (ppb)	NIST 1640	% Recovery undiluted	NIST 1640	% Recovery diluted
Total DF:		**1**		**21.72**	
Be/9 [#1]	34.94	35.750	102.3	34.860	99.77
Na/23 [#2]	29350	29690.000	101.2	29140.000	99.28
Mg/24 [#1]	5819	5893.000	101.3	6154.000	105.76
Al/27 [#1]	52	49.180	94.6	69.290	133.25
K/39 [#3]	994	947.900	95.4	858.800	86.40
Ca/40 [#2]	7045	7328.000	104.0	7488.000	106.29
V/51 [#3]	12.99	13.030	100.3	12.930	99.54
Cr/52 [#3]	38.6	37.470	97.1	38.540	99.84
Mn/55 [#1]	121.5	119.500	98.4	120.100	98.85
Fe/56 [#2]	34.3	35.840	104.5	31.820	92.77
Co/59 [#1]	20.28	19.400	95.7	20.010	98.67
Ni/60 [#3]	27.4	26.920	98.2	28.000	102.19
Cu/63 [#3]	85.2	86.450	101.5	92.350	108.39
Cu/65 [#3]	85.2	86.350	101.3	91.340	107.21
Zn/66 [#1]	53.2	55.380	104.1	55.560	104.44
As/75 [#3]	26.67	26.910	100.9	28.080	105.29
Se/78 [#2]	21.96	21.990	100.1	20.930	95.31
Mo/95 [#1]	46.75	45.310	96.9	43.280	92.58
Ag/107 [#1]	7.62	7.210	94.6	7.497	98.39
Cd/111 [#1]	22.79	22.560	99.0	22.420	98.38
Sb/121 [#1]	13.79	13.090	94.9	12.590	91.30
Ba/137 [#1]	148	143.900	97.2	142.100	96.01
Hg/202 [#1]		0.017		0.019	
Tl/205 [#1]		0.009		-0.042	
Pb/208 [#1]	27.89	26.690	95.7	26.370	94.55
Th/232 [#1]		0.011		-0.429	
U/238 [#1]		0.725		0.698	

Values in brackets after element mass are tune mode, 1= normal, 2 = hydrogen, 3 = helium.

Table 6. Replicate Analyses of Low Standard After Sequence of 33 High Level Samples, Standards, and Blanks for MDL Calculations. Three Sigma MDL are Calculated in ppb

	MDL rep 01	MDL rep 02	MDL rep 03	MDL rep 04	MDL rep 05	MDL rep 06	MDL rep 07	MDL rep 08	MDL rep 09	MDL rep 10	3 Σ MDL
Be/9 [#1]	0.50	0.50	0.49	0.49	0.50	0.47	0.49	0.49	0.50	0.49	**0.028**
Na/23 [#2]	53.45	47.78	43.96	39.85	40.52	36.48	34.69	30.58	30.17	22.08	**27.617**
Mg/24 [#1]	49.82	49.13	49.75	48.94	48.83	48.92	49.32	48.84	48.24	48.41	**1.530**
Al/27 [#1]	0.30	0.26	0.25	0.25	0.25	0.23	0.24	0.24	0.25	0.26	**0.054**
K/39 [#3]	56.28	55.34	55.09	53.35	55.02	55.15	53.73	53.25	54.17	53.70	**3.023**
Ca/40 [#2]	52.33	51.76	51.55	51.81	52.32	51.86	51.28	51.33	53.42	51.15	**2.023**
V/51 [#3]	0.51	0.53	0.53	0.53	0.54	0.51	0.53	0.50	0.52	0.52	**0.034**
Cr/52 [#3]	0.52	0.52	0.51	0.51	0.50	0.51	0.52	0.51	0.51	0.51	**0.019**
Mn/55 [#1]	0.49	0.49	0.49	0.47	0.48	0.48	0.49	0.48	0.48	0.47	**0.020**
Fe/56 [#2]	53.84	53.69	53.43	53.46	53.97	53.18	53.10	52.91	53.17	52.65	**1.251**
Co/59 [#1]	0.48	0.48	0.49	0.48	0.48	0.48	0.49	0.49	0.48	0.47	**0.016**
Ni/60 [#3]	0.50	0.49	0.49	0.48	0.50	0.48	0.48	0.49	0.48	0.49	**0.024**
Cu/63 [#3]	0.48	0.48	0.46	0.47	0.46	0.48	0.48	0.47	0.46	0.47	**0.023**
Zn/66 [#1]	0.50	0.45	0.43	0.43	0.43	0.42	0.42	0.46	0.44	0.42	**0.074**
As/75 [#3]	0.50	0.53	0.53	0.49	0.54	0.54	0.54	0.53	0.52	0.52	**0.052**
Se/78 [#2]	0.52	0.51	0.53	0.52	0.49	0.52	0.52	0.51	0.48	0.51	**0.047**
Se/80 [#2]	0.58	0.62	0.56	0.56	0.55	0.57	0.58	0.55	0.54	0.57	**0.066**
Mo /95 [#1]	0.47	0.46	0.46	0.46	0.45	0.46	0.48	0.44	0.47	0.45	**0.030**
Ag/107 [#1]	0.45	0.47	0.46	0.44	0.46	0.46	0.46	0.45	0.44	0.45	**0.027**
Cd/111 [#1]	0.45	0.43	0.44	0.44	0.44	0.44	0.44	0.44	0.45	0.43	**0.025**
Sb/121 [#1]	0.46	0.45	0.44	0.45	0.43	0.44	0.46	0.45	0.43	0.45	**0.035**
Ba/137 [#1]	0.49	0.47	0.47	0.49	0.48	0.47	0.47	0.47	0.46	0.48	**0.027**
Hg/202 [#1]	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	**0.005**
Tl/205 [#1]	0.40	0.42	0.42	0.43	0.42	0.42	0.42	0.42	0.41	0.41	**0.021**
Pb/208 [#1]	0.46	0.46	0.46	0.45	0.46	0.45	0.45	0.45	0.45	0.45	**0.017**
Th/232 [#1]	0.29	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	**0.050**
U/238 [#1]	0.43	0.44	0.44	0.43	0.43	0.43	0.44	0.44	0.43	0.43	**0.015**

Values in brackets after element mass are tune mode, 1= normal, 2 = hydrogen, 3 = helium.

Detection Limits

The method detection limits reported in Table 6 were generated at the end of a sequence of 33 real world samples, standards, and blanks. Column one lists the isotope and ORS acquisition mode, #1 = Normal Mode, #2 = Hydrogen Mode, #3 = Helium Mode. Actual method detection limits will vary depending on instrument and laboratory conditions. These detection limits should be achievable with normal levels of laboratory cleanliness, using trace metal grade acids and ASTM type 1 water. The instrument used for this work was equipped with the ISIS, which typically improves DLs somewhat by increasing sample introduction precision and minimizing carryover.

Dynamic Range

One of the advantages of using the ORS is its ability to reduce interferences on certain trace level analytes and simultaneously attenuate the signal on high concentration or matrix elements. In this work, calibrations were generated from a low of 50 ppt for Hg to a high of 200 ppm for the mineral elements, Na, K, Ca, Mg, and Fe. Sample calibration curves follow. Additionally, while Na was calibrated only as high as 200 ppm, which is the highest regulated concentration in any of the elements in the worldwide drinking water regulations (see Table 1), it yields linear response at much higher concentrations.

Table 7. A Series on Analyses on Three High Dissolved Solids Ground Water Samples. Each Sample was Analyzed Undiluted and Automatically Autodiluted. Elements which were Undetected were Removed for Simplicity.

	Water 1	Water 1	Water 2	Water 2	Water 3	Water 3
Total DF:	**1**	**21.72**	**1**	**21.72**	**1**	**21.72**
File:	**014SMPL.D**	**015SMPL.D**	**016SMPL.D**	**017SMPL.D**	**018SMPL.D**	**019SMPL.D**
Na/23 [#2]	489100.000	492500.000	330500.000	324100.000	563700.000	554000.000
Na/23 [#3]	480300.000	505800.000	337200.000	342800.000	563000.000	571800.000
Mg 24 [#1]	559.000	599.900	511.700	534.800	3099.000	3407.000
K/39 [#3]	1564.000	1365.000	794.000	721.400	2513.000	2333.000
Ca/40 [#2]	8708.000	8760.000	2337.000	2255.000	13350.000	13400.000
Mo/95 [#1]	0.776	0.773	1.482	1.535	49.070	49.180
Ba/137 [#1]	17.070	16.990	29.250	28.800	5.263	5.154
U/238 [#1]	0.043	0.037	0.036	0.034	0.115	0.103

Values in brackets after element mass are tune mode, 1= normal, 2 = hydrogen, 3 = helium.

Table 7 shows the results of the analysis of three brackish ground water samples. Each sample was analyzed directly and then autodiluted. Both sets of results show both the dynamic range of the Agilent 7500c and the accuracy of the autodilution. The autodilution factor of 21.72 is the result of the system automatically calibrating the dilution factor at the beginning of the sequence and periodically, as needed. Note that for the uranium result, where the undiluted concentration is only 30–40 ppt, the autodiluted result agrees very well. This translates to accurate measurement of uranium in the diluted samples of ~35/21.7 = 1.6 ppt.

Table 8. Results of Analysis of a 1/10 "Synthetic Seawater" Blank (High Purity 0.3% NaCl) Plus a Spike at 5 ppb for Trace Elements and 500 ppb for Matrix Elements.

	1/10 Synth Sea H_20	Spike 1/10 Synth Sea H_20 + 5 ppb	% Recovery 5/500 ppb spike
File:	**020SMPL.D#**	**021SMPL.D#**	
Be/9 [#1]	0.000	4.591	91.8
Na/23 [#1]	over range	over range	N/A
Na/23 [#2]	1233000.000	1215000.000	N/A
Na/23 [#3]	1193000.000	1193000.000	N/A
Mg/24 [#1]	2.382	477.000	94.9
I/27 [#1]	-0.409	4.250	93.2
K/39 [#1]	13.730	491.500	95.6
K/39 [#2]	8.195	548.600	108.1
K/39 [#3]	16.510	597.400	116.2
Ca/40 [#2]	6.740	532.600	105.2
V/51 [#3]	0.031	5.426	107.9
Cr/52 [#3]	0.045	5.287	104.8
Mn/55 [#1]	-0.003	4.497	90.0
Fe/56 [#2]	-0.258	508.600	101.8
Co/59 [#1]	0.122	4.569	89.0
Ni/60 [#1]	0.024	4.318	85.9
Ni/60 [#3]	-0.040	4.801	96.8
Cu/63 [#3]	-0.117	4.691	96.2
Cu/65 [#3]	-0.117	4.564	93.6
Zn/66 [#1]	0.025	4.520	89.9
Zn/67 [#1]	0.007	4.714	94.1
As/75 [#3]	0.011	5.027	100.3
Se/78 [#2]	0.006	4.366	87.2
Se/80 [#2]	0.143	4.620	89.5
Mo/95 [#1]	0.043	5.040	99.9
Ag/107 [#1]	-0.010	4.254	85.3
Cd/111 [#1]	0.033	4.545	90.2
Sb/121 [#1]	0.034	4.598	91.3
Ba/137 [#1]	0.010	4.789	95.6
Hg/202 [#1]	0.017	0.020	N/A
Tl/205 [#1]	-0.003	4.883	97.7
Pb/208 [#1]	0.175	5.066	97.8
U/238 [#1]	0.000	4.968	99.4

Values in brackets after element mass are tune mode, 1= normal, 2 = hydrogen, 3 = helium.

Table 8 shows the results of the analysis of a 0.3% 3000 ppm NaCl or 1180.5 ppm Na, both unspiked and spiked with trace elements and other matrix elements. Recoveries are reported in column 4. Note that in this case, for demonstration purposes, Na was acquired in all three ORS modes (normal, hydrogen, and helium). As expected, in the normal mode, the sodium signal was over range and the detector was protected from excessive signal. However, sodium was measurable in both hydrogen and helium modes at 1233 and 1193 ppm respectively, yielding recoveries of 104% and 101% respectively without further dilution or any other manipulation of instrument conditions. Under identical conditions, in the same run at the same time, Arsenic in the spike was also measured using He collision mode at 5.03 ppb to give 100.3% recovery.

1180.5 ppm sodium

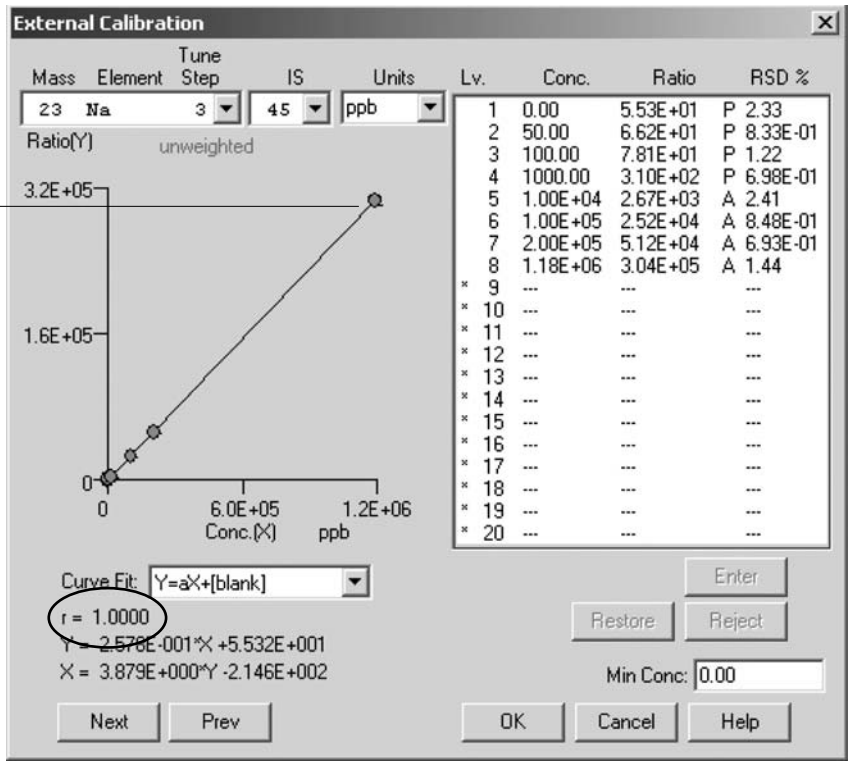

Figure 2. Calibration curve for Na in Helium collision mode showing linearity from 50 ppb to 1180 ppm (0.3% NaCl).

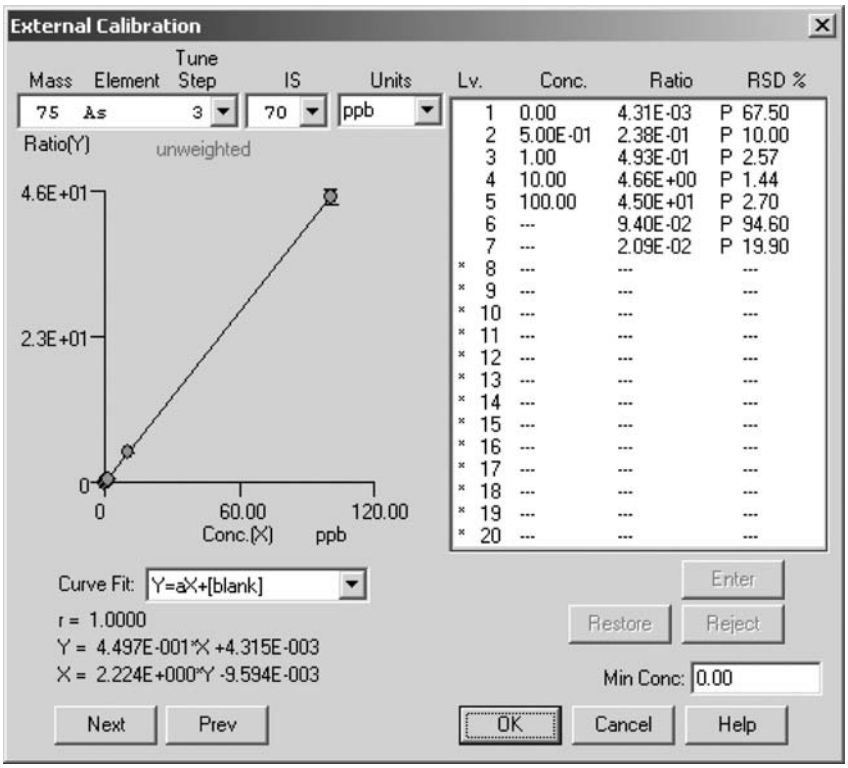

Figure 3. Arsenic calibration acquired in helium collision mode (same as Na in Figure 2) from 0.5 to 100 ppb.

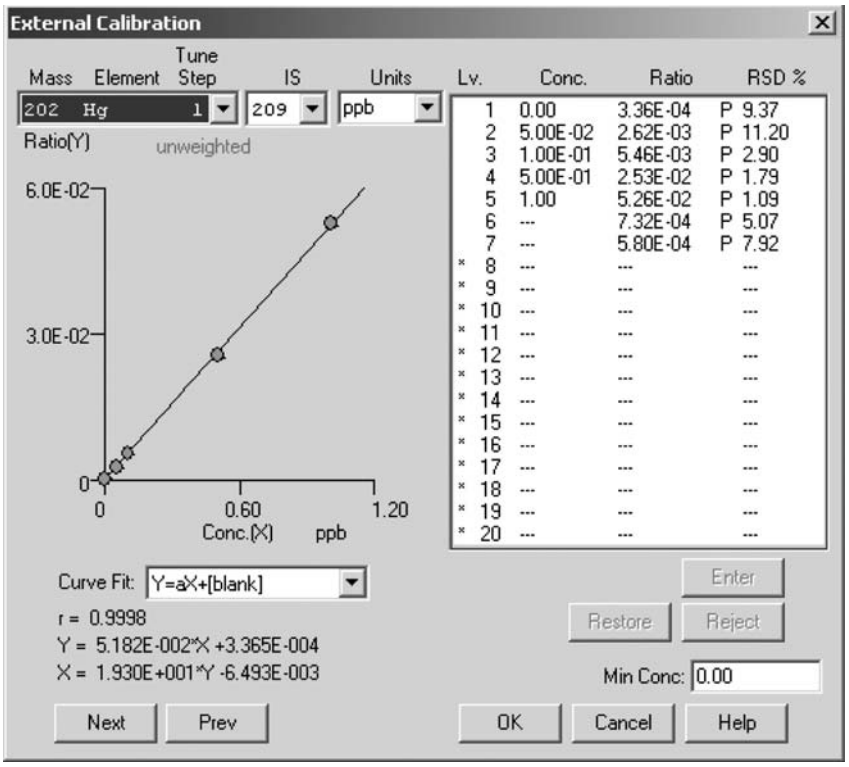

Figure 4. Mercury calibration acquired in normal (no gas) mode from 0.05–1 ppb.

The calibration curves in Figures 2–4 were all acquired from the same mixes of standard elements in dilute nitric/hydrochloric acid. That means that the low standard contained 50 ppt of mercury, 500 ppt of the other trace elements and 50 ppb of the mineral elements (Na, K, Ca, Mg, and Fe), and so on through the levels. In the sodium curve, the actual calibration was performed up to 200 ppm (level 7 in Figure 2); the 1180.5 ppm level was the 1/10 "synthetic seawater" NaCl solution.

Conclusions

While the specific details for drinking water monitoring vary from country to country around the world, the overall requirements, both from a reporting limit and quality control standpoint, are very similar. Currently, of the many available techniques for monitoring trace metals in water, only ICP-MS has the sensitivity and elemental coverage to meet all worldwide requirements. In addition, the use of collision/reaction cell technology in the form of the Agilent 7500c ORS allows the user both to easily meet the strictest ultra-trace reporting limits and to measure mineral or matrix elements at 1000s of ppm simultaneously, without fear of false positives from polyatomic interferences or out-of-range elements.

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Printed in the USA
February 19, 2003
5988-8902EN

Agilent Technologies

A Comparison of GC-ICP-MS and HPLC-ICP-MS for the Analysis of Organotin Compounds

Application

Environmental

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Abstract

An inductively coupled plasma mass spectrometer (ICP-MS) was used as a detector for gas chromatography (GC) and high performance liquid chromatography (HPLC) analysis of organotin compounds. ICP-MS is a highly sensitive detector with detection limits in the pg–ng range, as well as enabling calibration by isotope dilution mass spectrometry (IDMS). Calibrating using isotopically labeled organotin species reduces measurement uncertainties and leads to greater precision compared to external calibration methods. This application note details the relative merits of the two techniques for the analysis of organotin compounds.

Introduction

The toxic effects of organotin compounds in the environment have been well documented [1] and have led to extensive research into analytical methodologies for their determination in a variety of matrices. The widespread use of organotin compounds has resulted in their detection in most marine and fresh-water sediments as well as in open-ocean waters [2]. In recent years, the focus of research in organotin analysis has begun to include matrices with human health implications, such as seafood [3], manufactured products (PVC pipes used for drinking water distribution [4]), and human blood samples [5].

Organotin analysis has traditionally been performed by chromatographic separation (gas chromatography (GC) or high performance liquid chromatography (HPLC)) coupled to a variety of detectors. GC separations enable the analysis of many different groups of organotin compounds (for example, butyl-, phenyl-, octyl-, and propyl) in a single analysis after derivatization [6]. However, derivatization is time-consuming and yields may vary between species and in terms of efficiency depending on matrix components. GC-ICP-MS has the potential to facilitate simultaneous multi-elemental speciation analysis, because species of Se [7], Pb [8], Hg [9], and Sn [10] have volatile forms and could be analyzed in a single analysis. Organotin separations by HPLC offer the advantage that derivatization is not required, which eliminates a potential source of uncertainty in the final result and can reduce analysis time significantly. However, the range of compounds that can be analyzed in a single run are limited compared to GC. The use of ICP-MS as a detector enables calibration by isotope dilution mass spectrometry as well as providing very low limits of detection (pg–ng range). In conjunction with isotopically labeled organotin species, this approach offers many advantages from an analytical point of view including reduced measurement uncertainties and greater precision compared to external calibration methods.

Experimental

Reagents and Standards

Acetonitrile (UpS™ ultra-purity solvent grade) was obtained from Romil (Cambridge, UK). Glacial

acetic acid (TraceSelect) and anhydrous sodium acetate (Microselect ≥ 99.5% NT) were obtained from Fluka (Gillingham, Dorset, UK). Triethylamine, methanol and hexane were used as HPLC grade. Deionized water was obtained from a water purification unit at >18MΩ (Elga, Marlow, UK). Sodium tetra-ethylborate ($NaBEt_4$) was obtained from Aldrich (Gillingham, Dorset, UK).

Tributyltinchloride (TBTCl), Dibutyltinchloride ($DBTCl_2$), Triphenyltinchloride (TPhTCl) and Diphenyltinchloride ($DPhTCl_2$) were obtained from Aldrich and purified according to the procedure described by Sutton et al [11]. The ^{117}Sn isotopically enriched TBTCl was synthesized according to the procedure described in the same paper. Monobutyltinchloride ($MBTCl_3$) and Tetrabutyltinchloride (TeBTCl) were obtained from Aldrich, and Dioctyltin (DOT), Tripropyltin (TPrT), and Tetrapropyltin (TePrT) were obtained from Alfa Aesar (Johnson Matthey, Karlsruhe, Germany).

Instrumentation

Accelerated solvent extraction was carried out using a Dionex ASE 200 system. An Agilent 7500i ICP-MS was used for time-resolved analysis of ^{120}Sn, ^{118}Sn, and ^{117}Sn. The ShieldTorch system was used, and a second roughing pump was added in-line to increase sensitivity.

An Agilent Technologies (Palo Alto, California, USA) 1100 HPLC system was used for HPLC separations. All stainless steel parts of the HPLC system that come into contact with the sample were replaced by polyether ether ketone (PEEK) components. A 100-cm length piece of PEEK tubing was used to connect the analytical column to the 100-µL min^{-1} PFA MicroFlow nebulizer of the ICP-MS. Optimization of the ICP-MS conditions was achieved prior to HPLC analysis by adjusting the torch position and tuning for reduced oxide and doubly charged ion formation with a standard tuning solution containing 10 ng g^{-1} of ^{7}Li, ^{89}Y, ^{140}Ce, and ^{205}Tl in 2% HNO_3. After this preliminary optimization, the HPLC system was coupled to the nebulizer and a final optimization was carried out using ^{103}Rh added to the HPLC mobile phase. To reduce the solvent loading on the plasma, the double-pass spray-chamber was Peltier-cooled to -5 °C. Oxygen (0.1 L min^{-1}) was mixed into the make-up gas and added post-nebulization to convert organic carbon to CO_2 in the plasma and avoid a carbon build-up on the cones. The final optimization was important because the nebulizer gas and make-up gas flows had to be adjusted to ensure plasma stability with the organic mobile phase conditions. HPLC separations were performed using a C-18 ACE column (3-µm particle size, 2.1 mm × 15 cm) with a mobile phase of 65: 23: 12: 0.05 % v/v/v/v acetonitrile/ water/ acetic acid/TEA. The flow rate was 0.2 mL min^{-1}, and 20 µL of sample blends and mass-bias blends were injected. See Table 1.

GC separations were performed on an Agilent 6890 GC. The Agilent G3158A GC interface [12] was used to couple the GC to the ICP-MS. The GC method was used as described by Rajendran et al [6]. The analytical column was connected to a length of deactivated fused silica, which was inserted along the ICP transfer line and injector. After installation of the interface, the torch position and the ion lenses were tuned using a 100-ppm xenon in oxygen mixture, which was added to the ICP-MS carrier gas at 5% volume via a T-piece. The isotope monitored for this adjustment was ^{131}Xe.

Table 1. ICP-MS Parameters Used

	HPLC-ICP-MS	GC-ICP-MS
Interface cones	**Platinum**	**Platinum**
Plasma gas flow	14.5–14.9 L min^{-1}	14.5–14.9 L min^{-1}
Carrier gas flow	0.65–0.75 L min^{-1}	0.80–0.85 L min^{-1}
Make-up gas flow	0.15–0.25 L min^{-1}	Not used
RF power	1350–1550 W	1100–1200 W
Sampling depth	4–7 mm	6.5–7.5 mm
Integration time per mass	300 ms	100 ms
Isotopes monitored	^{120}Sn ^{117}Sn ^{103}Rh	^{120}Sn ^{118}Sn ^{117}Sn
Other parameters	ICP torch injector diameter: 1.5 mm Peltier cooled spray chamber at -5 °C 5% O_2 added post-nebulization ShieldTorch fitted	5% N_2 or O_2 added to enhance sensitivity ShieldTorch fitted

Extraction of Organotin Compounds

The ASE extraction cells were fitted with PTFE liners and filter papers and filled with dispersing agent. The sediment and the isotopically enriched spike were added and left to equilibrate overnight. Each cell was extracted using five 5-minute cycles at 100 °C and 1500 psi after a 2-minute preheat and 5-minute heat cycle. 0.5 M sodium acetate/ 1.0 M acetic acid in methanol was used as the extraction solvent [13]. A calibrated solution (mass-bias blend) was prepared by adding the appropriate amounts of both ^{120}Sn TBTCl and ^{117}Sn TBTCl into an ASE cell filled and extracting under the same conditions as the samples. Digestion blanks were prepared by extracting ASE cells filled with hydromatrix and PTFE liners. After the extraction, each cell was flushed for 100 seconds with 60% of the volume and purged with N_2. Prior to analysis, the extracts were diluted two- to five-fold in ultrapure water for HPLC-ICP-MS analysis. For GC-ICP-MS analysis, 5 mL of sample-, blank-, and mass-bias blend solutions were derivatized with 1 mL of 5% $NaBEt_4$ and shaken for 10 minutes with 2 mL of hexane. An aliquot of the hexane fraction was then injected for analysis.

Isotope Dilution Mass Spectrometry (IDMS) Methodology

The method used for IDMS consisted of analyzing a blend of the sample together with a mass-bias calibration blend. Each sample blend was injected four times and bracketed by injections of the mass-bias calibration blend. The mass-bias calibration blend was prepared to match the concentration and isotope amount ratio in the sample by mixing the same amount of spike added to the sample with a primary standard of the analyte of interest [14], [15]. The estimation of the standard uncertainties for the measured isotope amount ratios was different to the one described in [14] as they were calculated as peak area ratios and not spectral measurement intensities. The chromatographic peaks were integrated manually using the RTE integrator of the Agilent ICP-MS chromatographic software. The mass fraction obtained from the measurement of each sample blend injection was then calculated according to:

$$w'_X = w_Z \bullet \frac{m_Y}{m_X} \bullet \frac{m_{Zc}}{m_{Yc}} \bullet \frac{R_Y - R'_B \bullet \frac{R_{Bc}}{R'_{Bc}}}{R'_B \bullet \frac{R_{Bc}}{R'_{Bc}} - R_Z} \bullet \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}}$$

R'_B	Measured isotope amount ratio of sample blend (X+Y)
R'_{Bc}	Measured isotope amount ratio of calibration blend (Bc=Z+Y)
R_{Bc}	Gravimetric value of the isotope amount ratio of calibration blend (Bc=Z+Y)
R_Z	Isotope amount ratio of Primary standard Z (IUPAC value)
R_Y	Isotope amount ratio of spike Y (value from certificate)
w'_X	Mass fraction of Sn in sample X obtained from the measurement of one aliquot
w_Z	Mass fraction of Sn in primary standard Z
m_Y	Mass of spike Y added to the sample X to prepare the blend B (=X+Y)
m_X	Mass of sample X added to the spike Y to prepare the blend B (=X+Y)
m_{Zc}	Mass of primary standard solution Z added to the spike Y to make calibration blend Bc (=Y+ Z)
m_{Yc}	Mass of spike Y added to the spike Y primary standard solution Z to make calibration blend Bc (=Y+ Z)

The representative isotopic composition of Sn taken from IUPAC was used to calculate the isotope amount ratios of the primary standard. For the spike TBTCl, the isotopic composition was obtained from the certificate supplied with the ^{117}Sn enriched material from AEA Technology plc (UK). For the measured isotope amount ratio of the calibration blend (R'_{Bc}), the average of the two ratios measured before and after each sample blend isotope amount ratio (R'_B) were taken. A mass fraction was calculated for each sample blend injection and the average of the bracketing mass-bias calibration blend injections. The average of the four mass fractions was then reported as the mass fraction obtained for the blend analyzed. The final mass fraction was recalculated back to the original sample and corrected for moisture content.

Results and Discussion

General Comparison

Analysis of mixed organotin standard solutions showed that the GC method could separate a greater number (10–12) of compounds in a single run compared to HPLC-ICP-MS (5–6). The injection-to-injection time was ~40% shorter for HPLC-ICP-MS, due to the temperature profile used for GC separations. Because of the cost of the derivatizing agent, the reagent cost per sample is approximately double for GC sample preparation.

Sensitivity Enhancement of GC-ICP-MS by Using Additional Gases

Figure 1 and Table 2 illustrate the effect of adding different additional gases on the signal response for a range of organotin compounds. Adding 5% O_2 results in an increase in the measured peak area ranging from 9-fold (DBT and MPhT) to 12-fold (MBT). The addition of N_2 results in a further increase compared to analysis without addition of an optional gas. Response factors range from 105 (DBT and TPhT) to 136 for MBT and 150 for TeBT. This translates to a reduction of the method detection limit (3s) for TBT from 0.4 ng mL^{-1} (no gas) to 0.03 ng mL^{-1} (with 5% O_2 added) to 0.006 ng mL^{-1} (with 5% N_2 added). The table below summarizes detection limits based on analysis of a calibration standard for MBT, DBT, and TBT.

Detection limits (ng mL^{-1} as Sn) by GC-ICP-MS

	No gas added	5% N_2 added
MBT	0.7	0.01
DBT	0.5	0.008
TBT	0.4	0.006

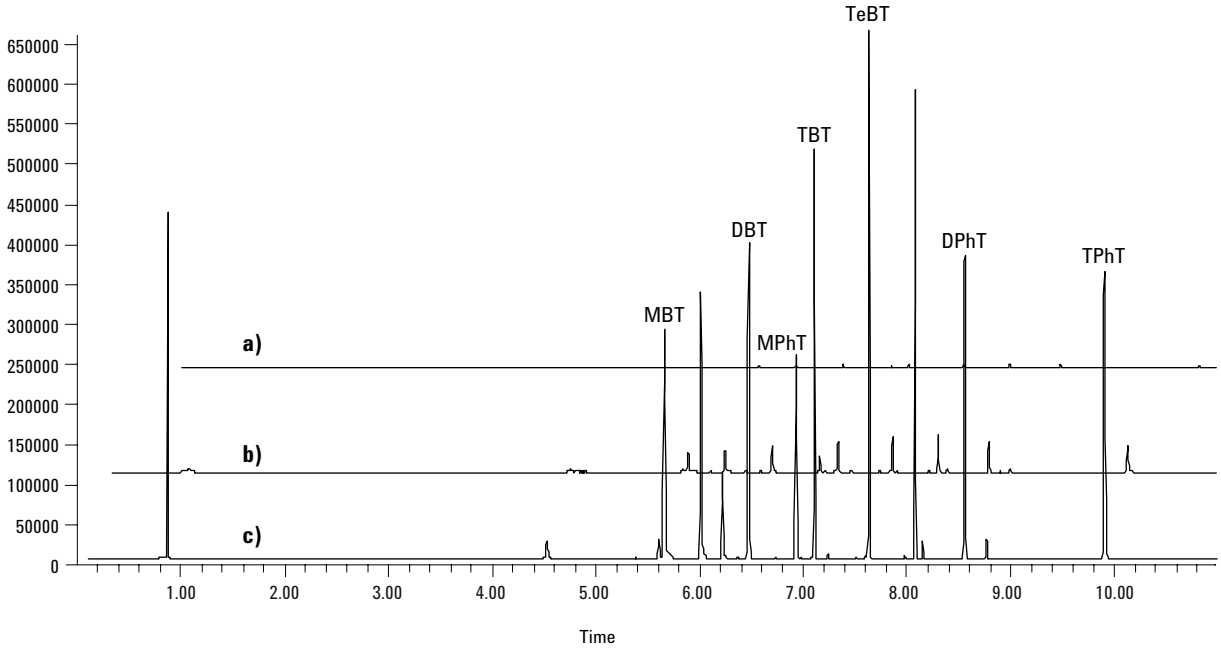

Figure 1. Sensitivity increase on a 20 ng mL^{-1} mixed standard by using a) no additional gas, b) 5% O_2, and c) 5% N_2.

Table 2. Effect of Different Additional Gases on Sensitivity of Organotin Compounds by GC-ICP-MS

Compound	Retention time (min)	a) No gas added (peak area)	b) 5% O_2 added (peak area)	Response factor compared to a)	c) 5% N_2 added (peak area)	Response factor compared to a)	Response factor compared to b)
MBT	5.57	2274	27029	12	309702	136	12
DBT	6.38	3247	29238	9	340436	105	12
MPhT	6.84	2026	18173	9	215182	106	12
TBT	7.02	3490	33132	10	399868	115	12
TeBT	7.54	3717	34225	9	558916	150	16
DPhT	8.46	3181	29665	9	338057	106	11
TPhT	9.81	4287	41119	10	450803	105	11

Comparison of HPLC-ICP-MS and GC-ICP-MS for Analysis of TBT in Sediment

Table 3 shows the comparative data obtained by analysis of the same sediment extracts by both methodologies. There is no statistically significant difference between the two data sets. This confirms that the chromatographic separation and the different sample pretreatment (dilution/derivatization) used has no influence on the analytical result obtained. The chromatography for both methods appears in Figure 2 and Figure 3. The isotope amount ratio measurement precision, measured for 15 injections over a 6–8 hour period, is good for both methods (1.6% for HPLC-ICP-MS and 1.7% for GC-ICP-MS). The uncertainty estimates provided by HPLC-ICP-MS tend to be larger than for GC separations. This is a result of broader peaks (50–60s by HPLC, compared to 4–6s by GC) and greater baseline noise.

Detection limits for sediment analysis are estimated by peak height measurements (3s) as 3 pg TBT as Sn for HPLC-ICP-MS and 0.03 pg TBT as Sn for GC-ICP-MS with 5% O_2 addition. This demonstrates the superior sensitivity of GC-ICP-MS even without sample preconcentration.

The accuracy of the analytical procedure was evaluated by measuring extractions of the certified reference sediment PACS-2 (NRC, Canada). The mean mass fraction obtained by the HPLC-ICP-MS analysis of four extracts was 864 ±35 ng g^{-1} TBT as Sn compared to a certified value of 980 ±130 ng g^{-1} TBT as Sn.

Table 3. TBT Data for Sediment Extracts

Sample	HPLC-ICP-MS (ng/g as Sn) n = 4	Standard uncertainty k = 1 (ng/g as Sn)	GC-ICP-MS (ng/g as Sn) n = 4	Standard uncertainty k = 1 (ng/g as Sn)
1	827	19	853	12
2	805	38	846	13
3	845	9	838	8
Mean	**826**	**22**	**846**	**11**
Expanded uncertainty (k = 2)	**±87**		**±39**	

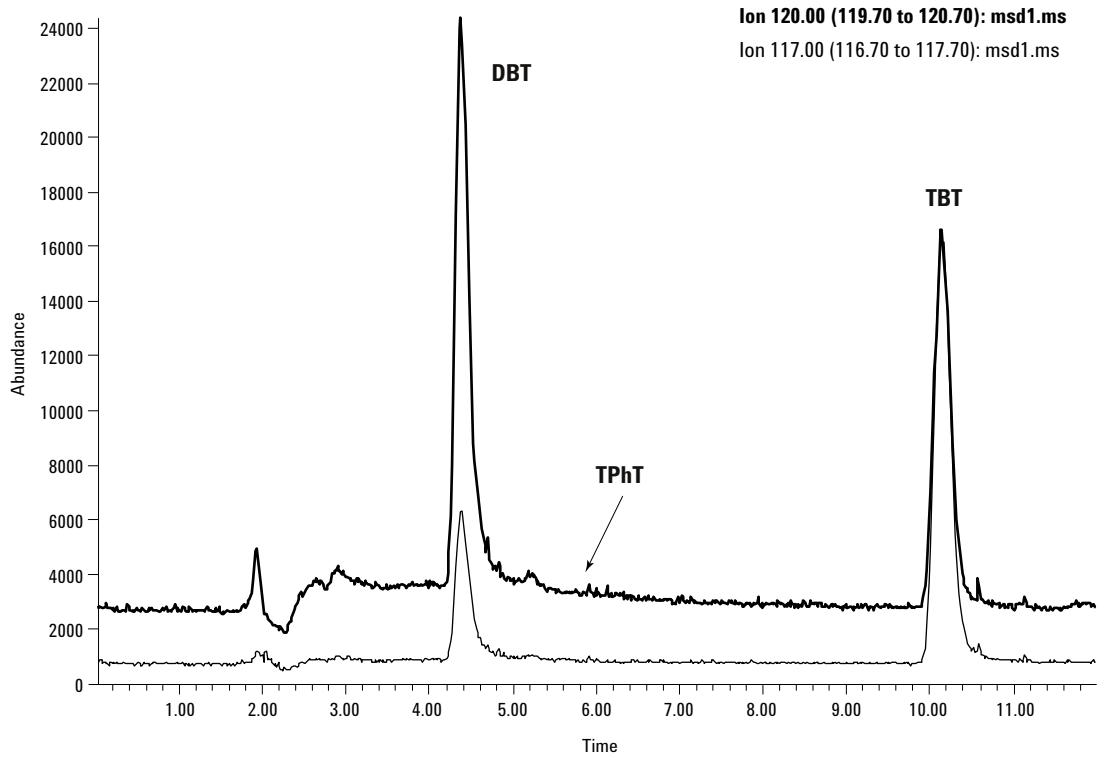

Figure 2. HPLC-ICP-MS chromatogram.

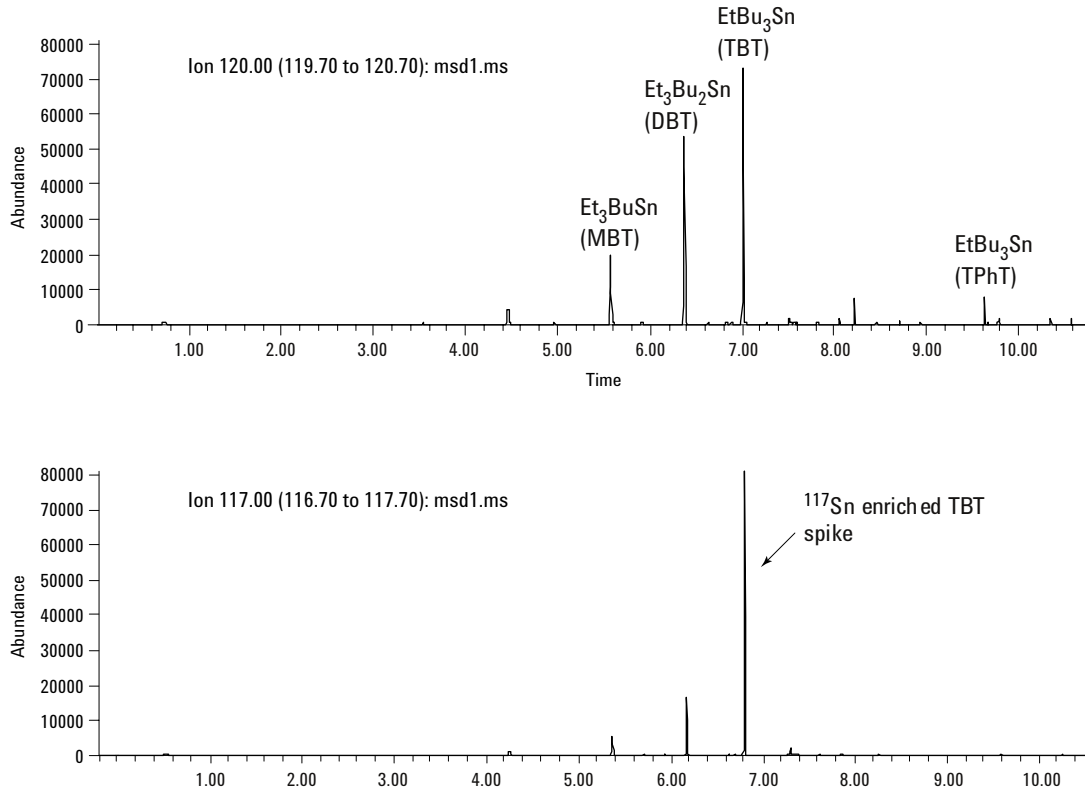

Figure 3. GC-ICP-MS chromatogram.

Conclusions

Both HPLC-ICP-MS and GC-ICP-MS offer advantages for organotin speciation analysis. While there is no statistical difference in the results obtained, HPLC-ICP-MS can be used for cheaper and faster determinations of large sample batches, while the superior sensitivity and the greater number of analytes separated make GC-ICP-MS an ideal tool for monitoring studies at the ultratrace level.

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Acknowledgments

The work described in this application note was supported under contract with the Department of Trade and Industry (UK) as part of the National Measurement System Valid Analytical Measurement (VAM) program.

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Printed in the USA
August 7, 2002
5988-6697EN

Agilent Technologies

Determination of Trace Levels of Phosphorus in Environmental Samples with the 7500c ICP-MS System

Application

Environmental

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Abstract

The determination of the element phosphorus at the single ppb level in ambient water samples is described. P is a difficult element to determine by inductively coupled plasma mass spectrometry due to its high first ionization potential and the presence of interfering polyatomic species directly at mass 31 and indirectly at mass 32 from $^{16}O_2$ and ^{32}S. The experimental work was carried out using Agilent's collision/reaction cell inductively coupled plasma mass spectrometer, the 7500c. The unique hardware features and design of the 7500c, which incorporates an Octopole Reaction System for effective interference removal, combine to fully resolve the phosphorus peak from the neighboring peak at mass 32. Removing the potential overlap so effectively allowed the accurate quantitation of phosphorus at low levels, even in the presence of ~700 ppm H_2SO_4.

Introduction

The determination of low concentrations of phosphorus presents several problems for inductively coupled plasma mass spectrometry (ICP-MS). As well as being relatively poorly ionized in the argon ICP, the phosphorus peak at mass 31 can suffer from overlaps by both isobaric and adjacent interfering species.

Analysis of phosphorus in environmental samples and in particular ambient waters is important because the element promotes the growth of aquatic algae. Even when present at trace levels, it can promote excessive and undesirable growth of aquatic algae and subsequent oxygen reduction. At high enough levels, resultant anoxic conditions can cause odor problems and kill aquatic life. Typical anthropogenic sources of phosphorus include fertilizers and detergents.

The range of sample matrices typically encountered in environmental analytical laboratories is large and unpredictable. Ideally, phosphorus should be determined at biologically significant levels without the need for complicated sample preparation. It would be advantageous if phosphorus could be determined on the same instrument and at the same time as the other trace elements of interest, thereby reducing requirements for additional sample preparation, analysis, and data processing.

Phosphorus Analysis – the Importance of Good Abundance Sensitivity

Phosphorus has a high first ionization potential (1st IP) of 10.487 electron volts (eV). This means that there is relatively poor conversion of phosphorus (P) atoms to P+ ions in the inductively coupled plasma (ICP). A reasonable estimate for the central channel temperature of a well-optimized ICP-MS is around 7000K, which gives about 6% conversion of P atoms to P+ ions, a relatively low response factor for ICP-MS. In comparison, aluminum, which has a 1st IP of 5.986 eV, is 99% ionized at this temperature, a factor of 16× greater than for P.

A second major obstacle to accurate quantitative analysis of trace levels of P is the possibility of interfering polyatomic species. In addition to the isobaric overlap from $^{15}N^{16}O$ and $^{14}N^{16}O^{1}H$ at mass 31, P is only one mass unit lower than the major background peak observed at mass 32. In addition to a high background interference from $^{16}O_2$ (derived from the water matrix of aqueous samples), mass 32 is also the major isotope of sulfur, which is commonly found in environmental samples. Sulfur may be present due to the use of sulfuric acid during sample preservation and preparation as well as being a natural component in many environmental sample matrices.

The capability of an ICP-MS to resolve a trace peak at an adjacent mass to a major peak is referred to as abundance sensitivity. The 7500 Series (and also the preceding 4500 Series) has always possessed excellent abundance sensitivity due to three important factors:

- A very good operating vacuum of ~3×10^{-6} mbar
- High frequency quadrupole RF (3 MHz – the highest available in ICP-MS)
- The use of true hyperbolic cross section quadrupole rods unique in ICP-MS

Round cross-section rods, while significantly cheaper to manufacture, can only approximate the theoretically-correct hyperbolic field. The theoretically-correct field generated by true hyperbolic cross-section rods offers significantly better abundance sensitivity and transmission, allowing trace peaks to be easily resolved from major peaks without sacrificing sensitivity. This leads to better data integrity, particularly with unknown sample matrices, and also eliminates the requirement to adjust the resolution on a per mass basis to compensate for poor inherent peak separation. The profiled rods of the quadrupole used in the 7500 series ICP-MS are pictured in Figure 1.

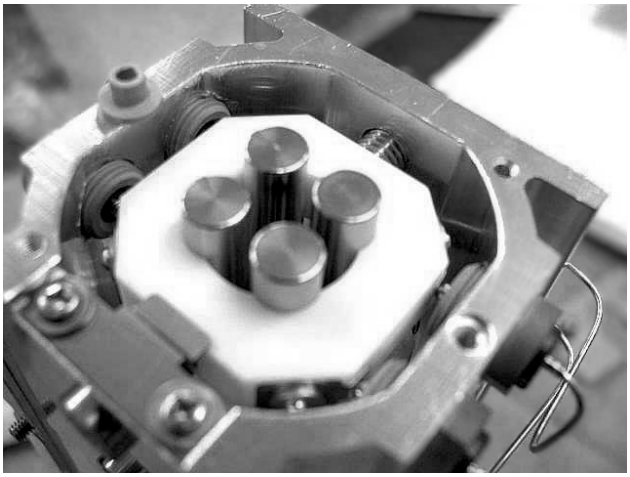

Figure 1. Photograph of hyperbolic profile quadrupole rods from the Agilent 7500.

Phosphorus in High Sulfur Matrix Samples – Benefits of the 7500c

Even with the excellent abundance sensitivity of the 7500 Series, the complete separation of ^{31}P from S and O_2 at mass 32 is difficult when high levels of sulfate are present. However, with the introduction of the Octopole Reaction System (ORS), a collision/reaction cell, even this application is possible. The 7500c is the latest addition to the 7500 Series and brings the unique combination of interference removal through collision/reaction technology together with the robustness and ease of use that are trademarks of the 7500 Series mainframe. Figure 2 is a schematic of the 7500c that highlights the major components of the instrument, including the unique off-axis lens system (mounted immediately behind the skimmer) and ORS.

However, for this application, the advantage of the 7500c lies not in the resolving power of the ORS itself, but in the Agilent ShieldTorch interface, fitted as standard on the instrument and a key contributor to the effectiveness of the collision/reaction cell. The ShieldTorch interface reduces and narrows the energy distribution of ions entering the mass spectrometer and virtually eliminates low mass peak tailing. In this example, the 7500c was operated in a non pressurized or "normal" mode.

Phosphorus in a sulfate-rich matrix can be resolved from the very intense S (and O_2) peak at mass 32 at the low and sub-ppb level on the 7500c. The background equivalent of phosphorus is less than 10 ppb, due largely to trace contamination. See Table 1 for the operating conditions used for the analysis of trace levels of P.

Table 1. Agilent 7500c Operating Conditions

Plasma RF power	1500 W
Sample depth	11 mm
Carrier gas flow	1.2 L/min
Extract voltage	5.5 V (soft extraction mode)
Mass peak width (10% peak height)	0.4 amu
Spray chamber temperature	1 °C
Reaction mode	No gas
Octopole bias	-2 V
Quadrupole bias	1 V

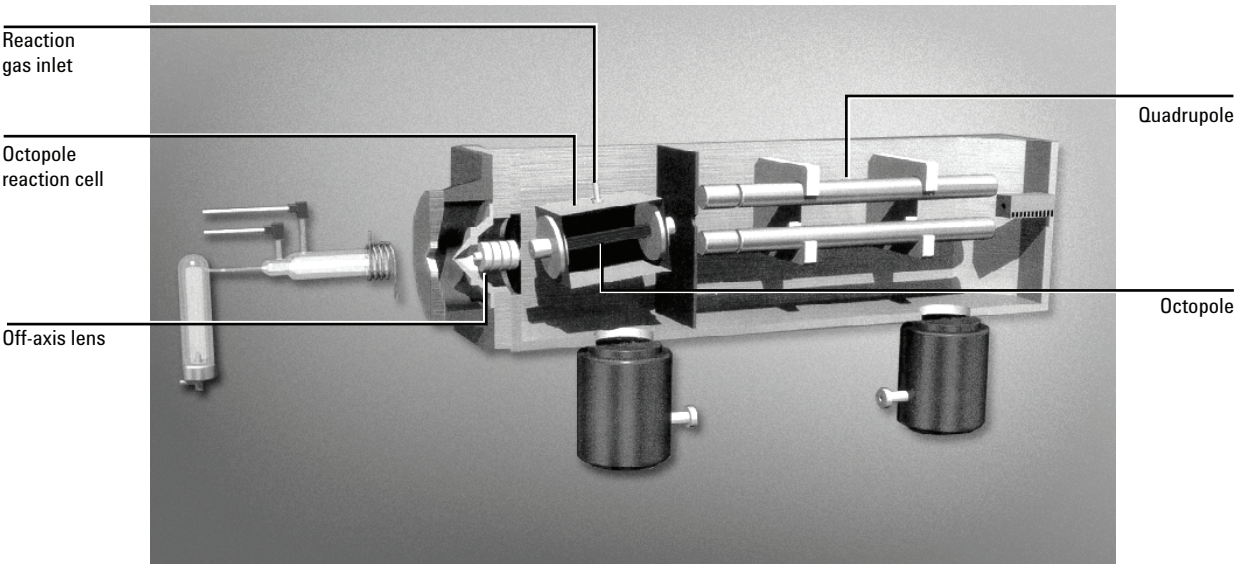

Figure 2. Schematic diagram of the Agilent 7500c Octopole Reaction System.

Results and Discussion

The practical effect of all these factors is the quantification of P at single ppb levels, even in a matrix containing ~700 ppm H_2SO_4. Figure 3 is a calibration graph of P standards from 500 ppt to 20 ppb and highlights the effectiveness of the instrument. Table 2 summarizes the results obtained in a blind analysis of ambient waters using the Agilent 7500c, in non-pressurized mode, and compares them with data obtained using a traditional wet chemical method. The quantitative analysis of P in the ambient water samples demonstrates that both the 7500c and the operating conditions are robust and tolerant of the changing matrix composition found in naturally occurring samples.

Table 2 Comparison of Results for Phosphorus in Waste Water

Ambient water sample	Agilent 7500c values (ppb)	*Given values (ppb)
QC	3.9	4
L16892-1	0.40	1
L16892-2	50.1	52
L16898-14	14.3	17
L16998-14	11.3	12
L16912-14	11	12
L16957-14	9.4	11

*Values obtained by traditional wet chemical method

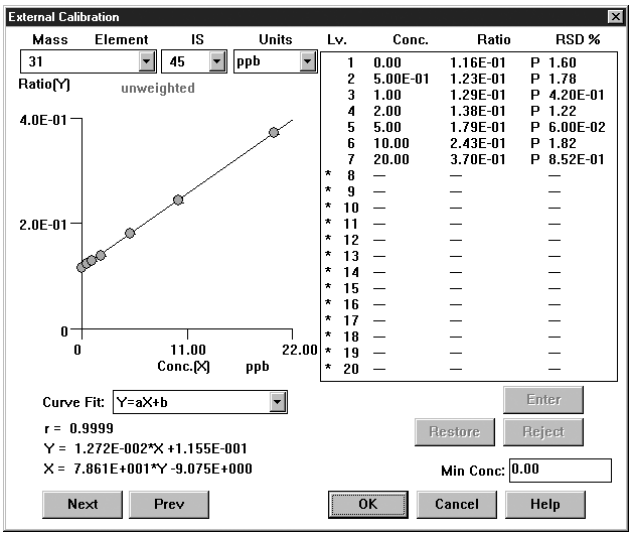

Figure 3. Calibration for phosphorus in a matrix containing 700 ppm sulfuric acid.

Conclusions

Trace phosphorus analysis was previously an application that was not addressed reliably by ICP-MS, particularly in the presence of a large concentration of sulfate. This analysis is now made accessible due to developments in instrumentation uniquely featured in the Agilent 7500c. The accurate quantification of P at low and even sub-ppb levels in natural and waste waters is possible, even where sulfate was added as a preservative.

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Printed in the USA
November 19, 2001
5988-4286EN

Measuring Elemental Ratios in Corals by LA-ICP-MS

Application

Environmental

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Abstract

A rapid analytical technique of laser ablation inductively coupled plasma mass spectrometry was developed to measure trace element constituents in corals and hard sponges. This technique is illustrated by measuring B, Mg, Ca, Mn, Zn, Sr, Ba and U in a Porites coral collected from the Great Barrier Reef, Australia. The accuracy of laser ablation inductively coupled plasma mass spectrometry for the measurement of Sr/Ca and Ba/Ca in corals by comparison with solution isotope dilution inductively coupled plasma mass spectrometry is discussed. We demonstrate the significance of the Sr/Ca ratio by correlation with in situ sea surface temperature data to show that Sr/Ca in corals provides a means to retrospectively measure sea surface temperature prior to available instrumental records. Coral Ba/Ca ratios as an indicator of riverine sediment input during times of runoff into the Great Barrier Reef are also examined.

Introduction

General circulation models (GCM's) describe the time-evolving circulation and thermodynamics of the atmosphere and oceans, the two main components of the Earth's climate system. Understanding the processes that control the Earth's climate and making accurate predictions of future changes in climate requires computer models of the climate system that are more realistic then those currently available. One of the principal impediments to the development of better models is the lack of accurate reconstructions of paleoclimate records (e.g. sea surface temperature).

Reliable high-resolution paleoclimate records are needed to understand the patterns and mechanisms of natural climate variability. Tropical air-sea interactions that affect the global distribution of water vapor (the most potent greenhouse gas) are largely dependent on sea surface temperature (SST) and sea surface salinity. Therefore, understanding the sensitivity of tropical SST and salinity in response to major global climate change is of particular importance. Until a good understanding of natural climate variability is obtained, the impacts on climate attributable to anthropogenic causes (increases of CO_2, etc.) cannot be fully understood.

An organism capable of providing this information is reef-building coral (Figure 1). Corals provide a continuous "time series record" of the marine environment through chemical records preserved in their aragonite skeletal lattice. Elemental ratios

(Sr/Ca) in corals have been shown to provide geochemical proxies for reconstructing the SST during the corals growth. Obtaining long time series by traditional methods (thermal ionization mass spectrometry, solution ICP-MS) is very time consuming and expensive. The development of rapid multi-element analysis by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) improves the quantity and quality of information that can be extracted from corals, an essential prerequisite for their practical application as environmental proxies.

Figure 1. A diver drilling a Porites coral to obtain a core for geochemical analysis.

LA-ICP-MS is gaining wide use as an analytical tool for the analysis of trace elements in a diverse range of sample materials. Semi-quantitative and quantitative elemental concentrations using LA-ICP-MS are being reported using chemically and matrix-matched standards as well as the NIST Standard Reference Material glass suite (NIST 610, 612, 614). Laser ablation has the technical advantage of analyzing elements in situ without time-consuming sample dissolution, thereby providing a rapid and relatively non-destructive technique. In this paper, we describe the LA-ICP-MS method used to measure the Sr/Ca ratio in corals as a monitor of sea surface temperature variations and the Ba/Ca ratio as an indicator of terrestrial runoff from the Great Barrier Reef.

Experimental

Coral Collection

The massive Porites coral can live for hundreds of years. To measure elemental ratios in corals, we take a core from the center of the coral colony using an underwater drilling system (Figure 1). The 5-cm diameter core is sliced into 7-mm thick slabs and X-rayed to show the growth bands (Figure 2). The coral is then cut into 45 × 25 mm pieces to fit into the laser-sampling chamber.

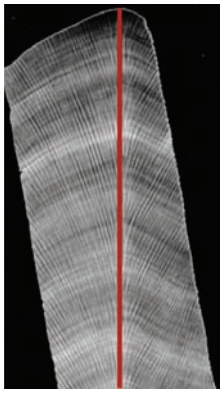

Figure 2. X-Ray positive of a Porites coral core slab. Light and dark bands correspond to different coral skeletal densities and growth. The red line indicates the track the laser follows along a main growth axis.

ICP-MS

Our LA-ICP-MS system uses an ArF excimer laser (193 nm wavelength) coupled to an Agilent 7500s. The operating parameters for the ICP-MS are listed in Table 1. The laser system consists of an in-house constructed sample chamber that holds a 45 × 25 mm coral piece for ablation. The laser illuminates a rectangular aperture which is imaged onto the flat surface of a coral slice and with each laser pulse ablates a rectangle 0.1 microns deep, 50 µm wide parallel to the growth axis, and 500 µm wide perpendicular to the growth axis (Figure 3). This rectangular laser beam is a crucial component of LA-ICP-MS of corals to ensure representative analysis across the interlocking branch-like structural elements of the corals. The long focal length (150 mm) of our excimer laser optical system and this large sampling window minimizes the depth related fractionation observed for material removed from the bottom of holes. The time resolution depends on the growth rate of the coral and the slit width, and we usually obtain sub-weekly resolution in corals (>100 samples year $^{-1}$). The fast mass switching (1 msec mass to mass, or 2 msec for large jumps) and high sensitivity of the 7500s allows us to measure many elements together without compromising this spatial resolution. A significant time interval between masses would result in noisy element ratios because a combination of the

coral surface topography and the sample cell and carrier gas transport system gives signal intensity variations with about a 1-second time constant. The laser has a pulse length of 25 nsec and the power density on the sample surface is 0.32 GW/cm^2.

For coral analysis, the laser is pulsed at 5 Hz. The material is ablated in helium and entrained in argon for analysis by ICP-MS. A side view of the laser sample cell is shown in Figure 4. The isotopes ^{10}B, ^{25}Mg, ^{46}Ca, ^{55}Mn, ^{66}Zn, ^{84}Sr, ^{138}Ba and ^{238}U are measured with ^{46}Ca (0.004% abundance) as an internal standard to compensate for variations in ablation yield due to coral surface porosity. The data is standardized to a pressed powder disc

Table 1. Agilent 7500s Operating Parameters

	Laser Mode
ICP-MS	Agilent 7500s
Forward power	1250 W
Reflected power	< 1 W
Gas flow rate:	
Cool gas	14 L min^{-1}
Carrier gas Ar	1.14 L min^{-1}
Optional gas He (into cell)	0.3 L min^{-1}
Cone composition	Nickel
Detector mode	Dual simultaneous
Acquisition mode	Time resolved
Isotope dwell time	25 to 60 ms
Points per peak	3
Time slice	~ 1 s

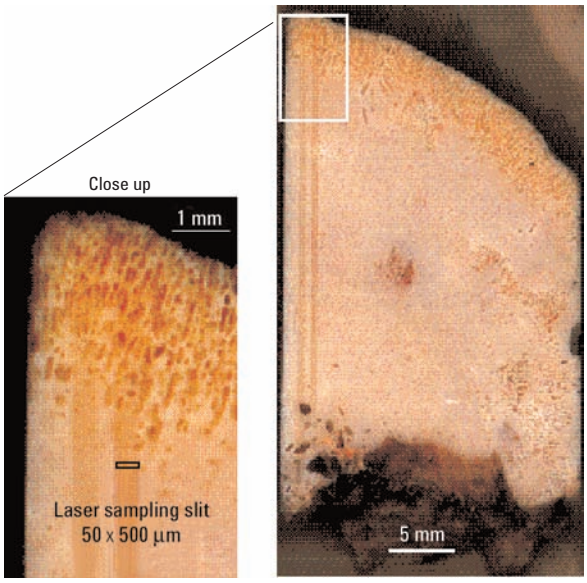

Figure 3. Laser sampling on a coralline sponge showing parallel tracks (right side) and close up of boxed section (left side) of the laser sampling slit. Laser tracks are not visible on corals due to surface porosity.

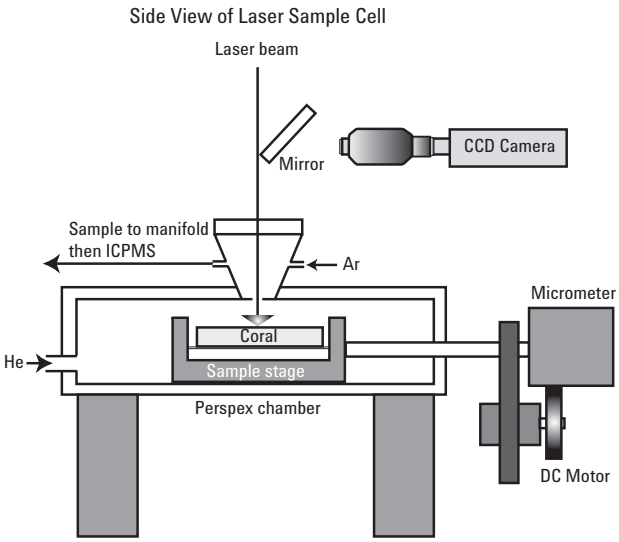

Figure 4. Side view of the laser sample cell. Coral is ablated in a sealed Perspex chamber under a helium atmosphere. The sample stream is entrained in argon and enters a smoothing manifold before entrance to the Agilent 7500s ICP-MS. The sample is viewed by CCD video camera. A DC motor with a high-ratio gearbox scans the sample beneath the laser, enabling continuous sampling.

prepared from a cleaned and finely crushed Porites coral from the Great Barrier Reef. Some other elements we routinely measure are lead and rare earth elements. They are standardized using the NIST 614 glass. The dual simultaneous detector system of the Agilent 7500s enables us to measure the low concentration elements (manganese and zinc) simultaneously with the more abundant elements (Figure 5).

The analytical procedure is shown in Figure 5. Background and standards are collected for 60 seconds each before and after the coral analysis, a motor scans the coral sample and standards beneath the laser at a speed of 0.03 mm s^{-1}. The entire protocol takes around 40 minutes for each piece of coral.

Results and Discussion

Accuracy: Comparison with Isotope Dilution Solution ICP-MS

One of the methods used to check the accuracy of LA-ICP-MS was analysis of three other calcium carbonate samples. A coral from the Huon Peninsula, Papua New Guinea, an aragonite coralline sponge and a calcite coralline sponge were finely crushed and pressed to form pellets. The Sr/Ca and Ba/Ca

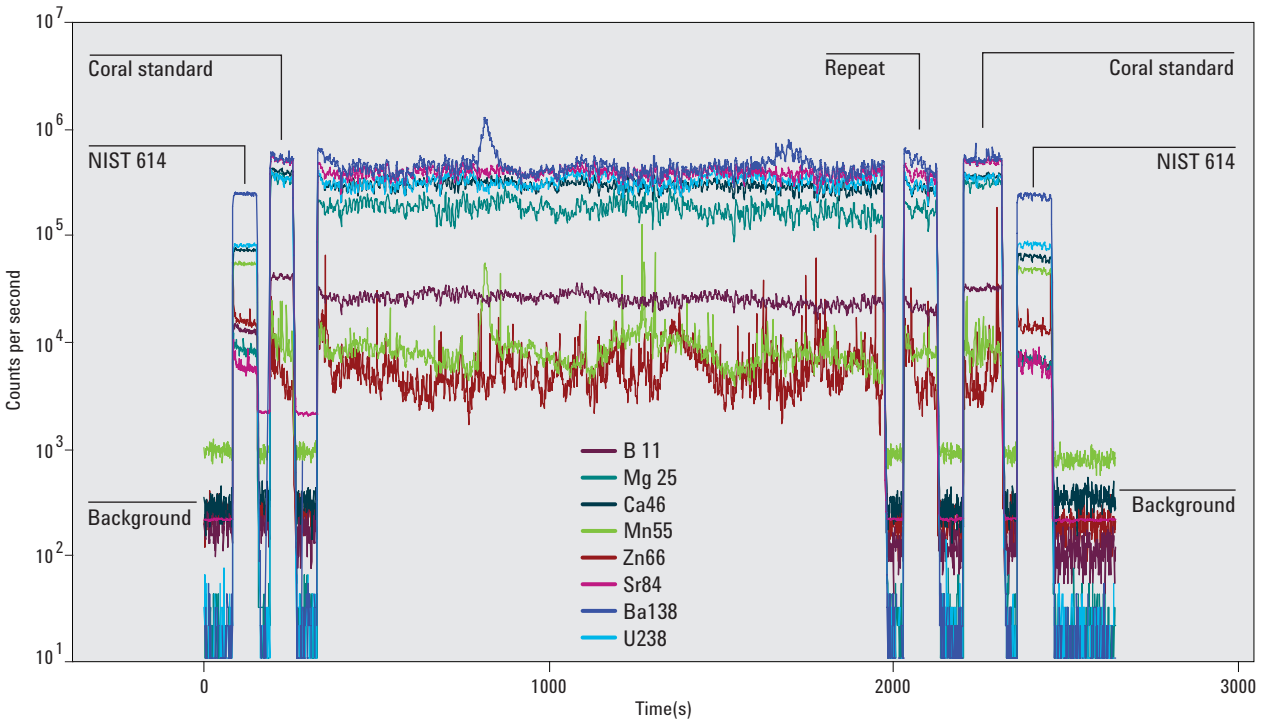

Figure 5. LA-ICP-MS protocol for coral analysis, in counts per second for each isotope.

concentration of the bulk powders was measured by isotope dilution solution ICP-MS. The pressed powder samples were also analyzed with LA-ICP-MS by scanning 2 to 3 mm long sections. The coral sample was measured 12 times over a 1-month period, the aragonite sponge sample 50 times over a 3-month period and the calcite sponge sample five times in 1 day. The LA-ICP-MS and solution measurements agree within the statistical error (Figure 6). Using a pressed powder standard constructed from a calibrated coral provides accurate fully quantitative LA-ICP-MS for $CaCO_3$ (corals and sponges) with differing concentrations.

Sea Surface Temperature Proxy: Sr/Ca

A Porites coral from the Great Barrier Reef was analysed with the LA-ICP-MS. The comparison between the coral Sr/Ca ratio and the known sea

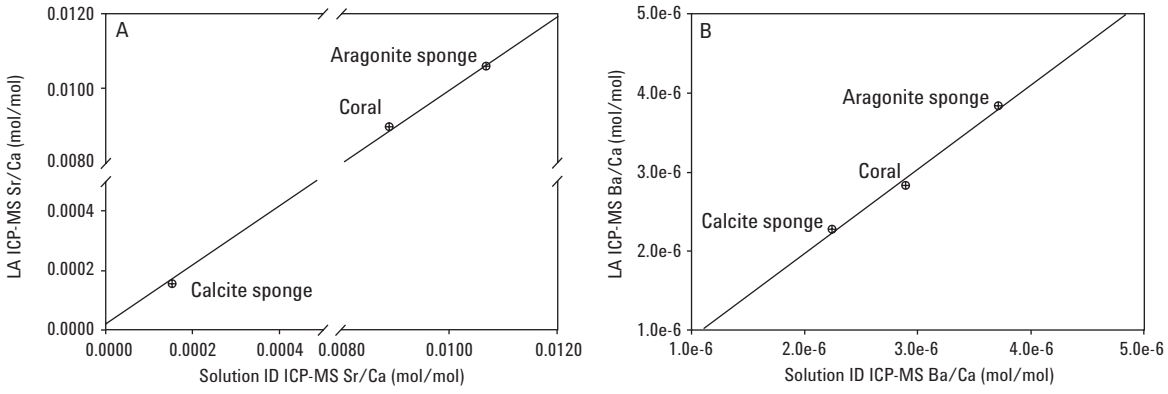

Figure 6. Accuracy of LA-ICP-MS method. Comparing solution ID ICP-MS (x axis) with LA ICP-MS (y axis) of three samples, a calcite sponge, a coral, and an aragonite sponge. Error bars and linear regression for the three samples are shown. A) Sr/Ca B) Ba/Ca.

surface temperature for about 90 mm of the core is shown in Figure 7. The correlation coefficient between the two data sets is r = - 0.93, or approximately 86% of the Sr/Ca variation is attributable to SST, indicating the value of coral Sr/Ca as a SST proxy. Sr/Ca has been measured by various labs and in many corals from different locations and it has been proven to be robust in terms of its ability to record SST. With such a calibration between Sr/Ca and SST, it is possible to reconstruct water temperatures before the advent of instrumental records from a particular reef.

Terrestrial Runoff: Ba/Ca

The Ba/Ca ratio in corals can be influenced by the upwelling of nutrient rich water, and in coastal corals by river runoff. This coral was collected from near shore on the Great Barrier Reef (GBR) near a major river system, the Burdekin. Sediment input to the GBR has elevated levels of barium, and heavy river flooding brings higher than normal barium into the surrounding seawater. The inshore corals of the Great Barrier Reef register floods by an increased Ba/Ca ratio. The influence of river runoff on GBR corals is also recorded as luminescent or fluorescent lines/bands in the coral skeletons. This coral records the floods of 1996, 1997 and 1998 (Figure 8). The relation between river discharge and coral Ba/Ca is not necessarily linear; with factors such as wind and ocean current conditions affecting the path of the flood plumes. Nevertheless the Ba/Ca runoff proxy in corals can be used as an indicator of sediment input into the GBR and for other areas around the globe.

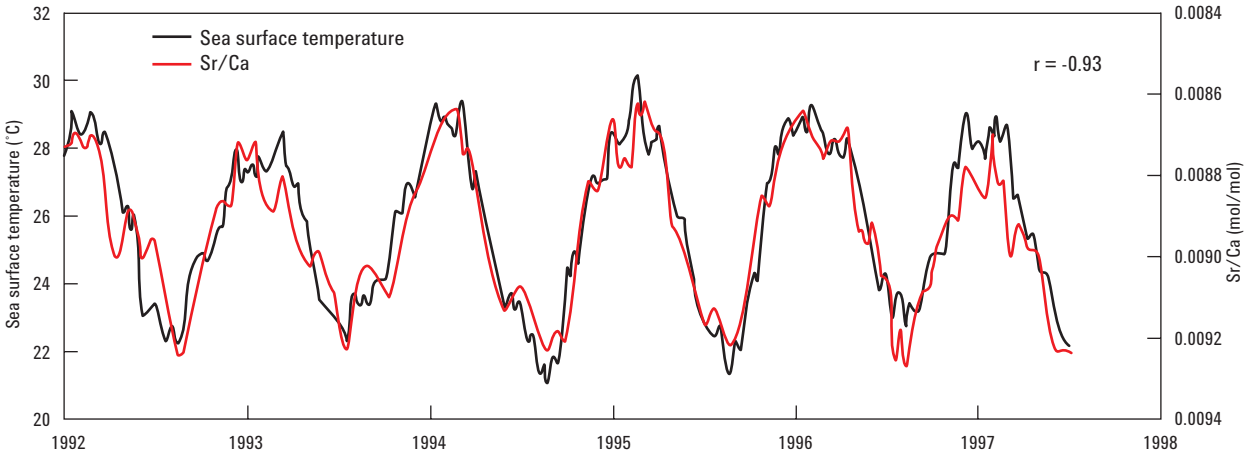

Figure 7. Measured coral Sr/Ca in red (right. axis) vs. in situ sea surface temperature record in black (left axis).

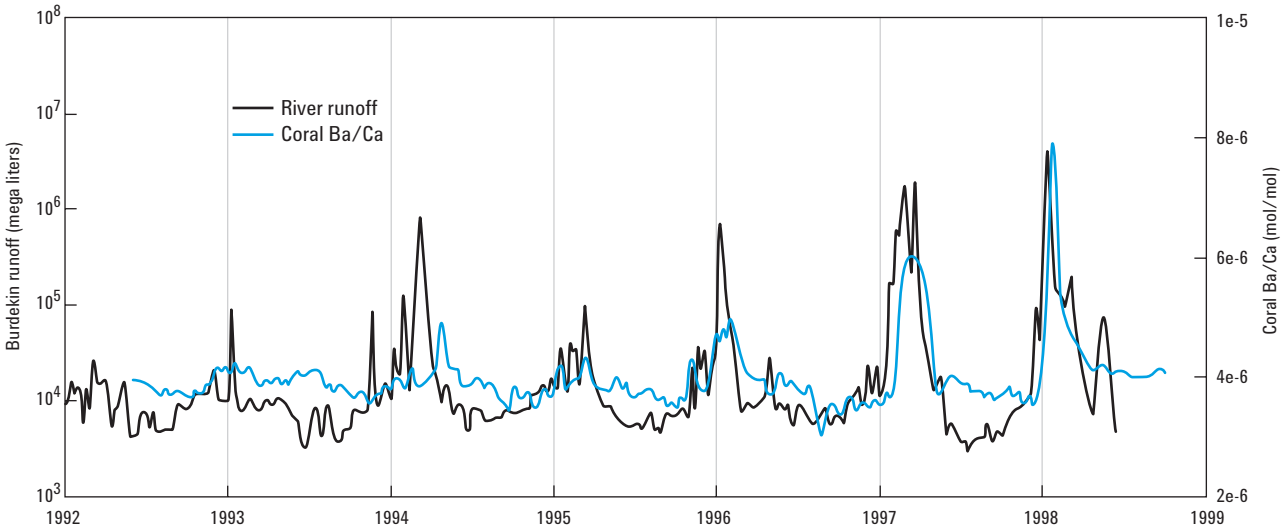

Figure 8. Comparison between terrestrial runoff (Burdekin River in mega liters, log scale) on the left axis and Coral Ba/Ca (mol/mol) on the right axis.

Conclusions

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) provides a rapid analytical technique for measuring B, Mg, Ca, Mn, Zn, Sr, Ba and U concentrations in corals. The power of this technique lies in its ability to measure these elements simultaneously with appropriate spatial resolution regardless of coral growth rate. Researchers have collected long coral cores spanning several centuries from various locations throughout the globe but producing long time series at monthly or higher resolution has previously been very time consuming and prohibitively expensive. The LA-ICP-MS discussed here makes it practical to produce long high-resolution climate reconstructions from corals, to enable a greater understanding of the role of the oceans in the dynamics of the Earth's climate.

Further Reading

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Printed in the USA
November 13, 2001
5988-3742EN

Agilent Technologies

Automated Real-Time Determination of Bromate in Drinking Water Using LC-ICP-MS and EPA Method 321.8

Application

ICP-MS

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Abstract

The suitability of coupling an HPLC to an ICP-MS for the fully automated, routine analysis of bromate in drinking water as per the proposed EPA Method 321.8 was investigated. The necessity to monitor the carcinogen bromate in ozonated drinking waters at single ppb levels has led the USEPA to investigate HPLC-ICP-MS as an alternative technique to the ion chromatography with conductivity detection method currently specified. During this investigation, a series of rigorous performance checks were used to assess the implementation of the proposed method including the determination of bromate in a series of EPA disinfection byproduct (DBP) standards.

Introduction

Ozonation is a common method used for the disinfection of drinking waters. In waters containing bromide (Br^-), such as those found in coastal regions subject to salt-water intrusion, a disinfection byproduct (DPB) of the ozonation process is the bromate ion (BrO_3^-). The bromate ion, produced by the oxidation of bromide, is very carcinogenic, with an estimated lifetime cancer risk of 1:10,000 for a concentration of 5 ppb.[1] The current method specified by the USEPA for the determination of bromate in drinking water uses ion chromatography (IC) with conductivity detection. One disadvantage of this method is the need for a tedious and time consuming sample pretreatment step.

The need for sample pretreatment arises from the potential for co-elution of chloride and bromide ions present in the sample, potentially resulting in false positive results. In order to prevent this from occurring, chloride present in the sample is precipitated out of solution using silver cartridges with subsequent pre-concentration of the bromate ions. This time consuming and lengthy clean-up procedure and pre-concentration step can result in pre-concentration of sulfate ions present in the water. Sulfate can subsequently displace the bromate ions on the resonating column resulting in false negatives.

For these reasons, ICP-MS has been investigated as an alternative, ion selective detector for this analysis. ICP-MS provides the resolution necessary to separate the bromate and chloride ion, thereby eliminating the need for a matrix elimination step. Furthermore, ICP-MS has been used successfully for the analysis of bromate in water samples containing concentrations of chloride in excess of 5000 ppb - much higher than the typical content of ozonated drinking water - without the need for sample pretreatment.[2]

This study will investigate the suitability of ion chromatography coupled to ICP mass spectrometry (ICP-MS) as an automated, real-time measurement approach, to determine low levels of bromate in ozonated drinking water samples, using the proposed EPA Method 321.8.[3]

Instrumentation

The Agilent Technologies 1100 Series HPLC system, coupled to a 7500 Series ICP mass spectrometer using the real-time Plasma Chromatographic software was used for this study. This system was specifically designed for the rigors of automated trace element speciation work, mainly in response to laboratory demands, particularly in the environmental, clinical and food application areas, that need to carry out routine elemental speciation. Its design takes advantage of Agilent's expertise in chromatography and its recognized leadership position in ICP-MS.

During the past few years, the potential of ICP-MS as a detector for elemental speciation studies has been realized.[4] When coupled to a chromatographic separation device, ICP-MS offers unmatched detection capability for laboratories interested in quantifying different species, forms, oxidation states or biomolecules associated with trace elements.[2, 5] Traditional approaches of coupling ICP-MS to chromatography devices are cumbersome, labor intensive and not readily automated. In fact, the majority of ICP-MS chromatography data handling software packages were designed specifically for liquid and gas chromatography (LC, GC) applications and required modification for use with ICP-MS. Some approaches even analyzed the chromatographic spectral peaks "post-run", meaning the data had to be imported into another software package after the analysis was completed, for quantitation purposes. It was clear that there was a real demand for a fully automated system, designed specifically for trace element speciation analysis. Agilent Technologies answered that demand with a fully integrated package for trace element speciation, comprising an 1100 Series HPLC system, coupled to a 7500 Series ICP mass spectrometer, using the Agilent ChemStation and real-time Plasma Chromatographic software.[6]

Methodology

ICP-MS Conditions

The ICP-MS instrumental conditions were optimized to give maximum signal at *m/z* 79, the most sensitive mass for Br. Because bromine is not completely ionized in argon ICP, sampling depth, nebulizer flow, RF power and ion lens voltages have to be optimized very carefully to guarantee the most efficient sampling of bromide ions. Operating conditions for the 7500 are shown in Table 1. These conditions gave an instrument response of 110,000 cps for a 100 ppb bromate standard, with a background of 1,800 cps (partially due to trace levels of bromide in the 18 MΩ deionized water).

Table 1: Optimized Operating Conditions for ^{79}Br Using the Agilent 7500 ICP-MS

Parameter	Optimized conditions
Nebulizer	Meinhard concentric - glass
Nebulizer flow rate	1.05 L/min
Spray chamber	Scott double pass - glass
Spray chamber temperature	2 °C
Sample flow rate	1 mL/min
RF power	1200 W
Sampling depth	Optimized for max signal at ^{79}Br
Ion lens voltages	Optimized for max signal at ^{79}Br

Chromatographic Conditions

See Table 2 for the chromatographic conditions for the separation. The column eluent was passed via a short length of PEEK tubing to a six-port Rheodyne injector equipped with a 100 µL (or 500 µL depending on the measurement) PEEK loop. A post column injection was performed at the beginning of each run (for internal standard purposes, specified in the proposed EPA Method) at the exact time the data acquisition began on the ICP-MS. See Figure 1 for a schematic of the HPLC instrumentation coupled to the ICP-MS.

Table 2: Chromatographic Conditions for the Bromate Study

Parameter	Specification
Eluent mobile phase	25 mM Ammonium nitrate, 5 mM Nitric acid (~pH 2.7) in 18 MΩ Deionized water
Injection volumes	100 µL, 500 µL loops
Post-column injector	Used for internal standardization
Pump flow rate	1 mL/min
Column	Dionex CarboPac PA-100 (94 × 250 mm) - with guard

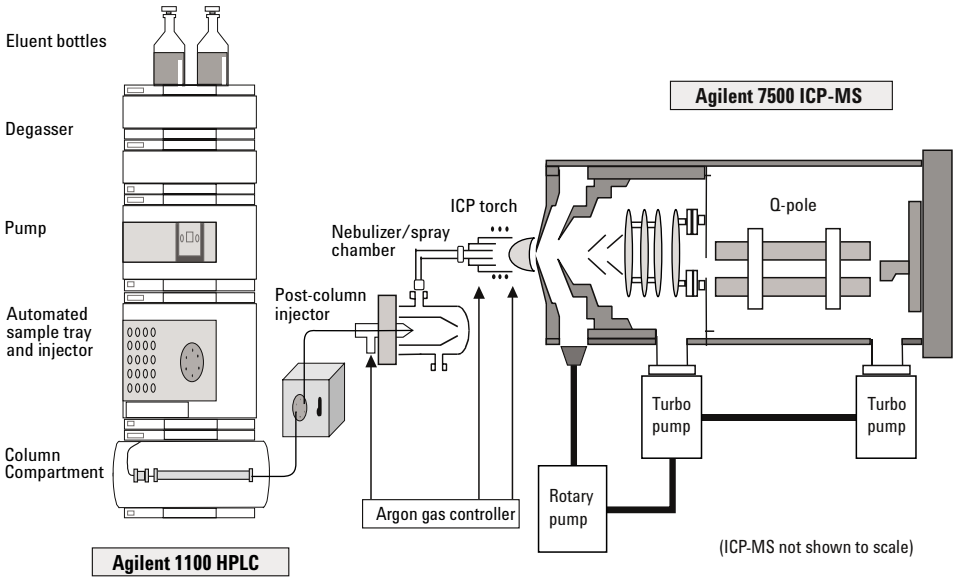

Figure 1: A schematic of the 1100 HPLC instrumentation coupled to the 7500 ICP-MS used for the bromate study.

Sample Preparation

The blank was 18 MΩ deionized water adjusted to pH 10 with NaOH. Standards were prepared daily from a USEPA 1 mg/mL bromate stock solution.

Demonstration of Instrument and Method Performance

As a way of maintaining data quality, the EPA uses performance checks to monitor the instrument and also ensure that the methodology is working correctly. Some of the more important performance checks for this proposed EPA method 321.8 include the measurement of:

- Abundance Sensitivity of ICP Mass Spectrometer
- Method Detection Limit
- Chromatographic Interferences
- Laboratory Fortified Blank
- Laboratory Fortified Matrix
- DBP Performance Sample

These measurements were used to assess the performance of the integrated system used for this study.

Abundance Sensitivity

A large argon dimer, $^{40}Ar^{40}Ar^{+}$ at mass 80 adjacent to the bromate ion $^{79}Br^{+}$ at mass 79, has the potential to bias results in the determination of bromate by ICP-MS. It is therefore critical that the abundance sensitivity, which is a measure of the instrument's ability to separate a trace peak from a major one,[7] is optimized to allow for maximum rejection of the ions at mass 80. The very high operating vacuum of the 7500, and the high frequency of its quadrupole, combined with optimization of the rod-bias voltages, ensures that it achieves clean separation of both peaks, even at a mass of 79.5 amu, where the tail of the $^{40}Ar^{40}Ar^{+}$ might interfere with the Br^{+} at mass 79. The excellent abundance sensitivity of the quadrupole's hyperbolic rods is demonstrated in Figure 2, which shows a spectral scan of 2% HNO_3. The effect of the large signal at mass 80 is shown to have minimal affect on the small bromine signal at mass 79.

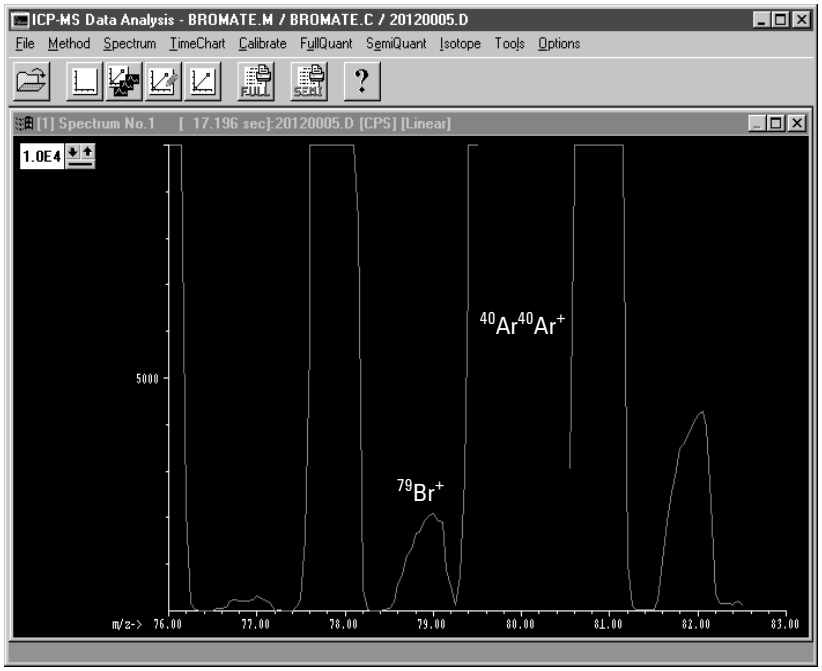

Figure 2: Mass spectrum showing clean separation of $^{79}Br^{+}$ from the argon dimer $^{40}Ar^{40}Ar^{+}$.

Method Detection Limit (MDL)

Two method detection limits were performed - one using a 500 µL loop, as specified in the method, and another using a 100 µL loop. A blank and three calibration standards (1, 5, and 25 ppb bromate) were used for both method detection limit tests.

Seven individually prepared bromate standards of 1 ppb (for the 100 µL loop) and 0.5 ppb (for the 500 µL loop) were then analyzed to determine the method detection limit (MDL). From this, an MDL was calculated for each loop by multiplying the standard deviation of the seven replicate results by 3.14, as indicated in the EPA method. Individual MDL replicate concentrations and statistics for both loops are shown in Table 3.

Table 3: Method Detection Limit Data for a 100 µL and 500 µL Loops

Replicate #	100µL Loop Concentration (ppb)	500 µL Loop Concentration (ppb)
MDL-1	1.1	0.46
MDL-2	0.98	0.39
MDL-3	0.77	0.35
MDL-4	0.77	0.46
MDL-5	0.97	0.45
MDL-6	0.83	0.48
MDL-7	0.90	0.41
Mean	0.90	0.42
SD	0.131	0.044
RSD (%)	14.6	10.5
MDL	0.41	0.14

Chromatographic Interferences

To show that other halogenated compounds do not elute at similar retention times as bromate, a haloacetic acid standard (HAA) standard solution, provided by the EPA, was analyzed. The stock solution was diluted 1:100 yielding final concentrations of six different halogenated compounds reported in Table 4.

Table 4: Concentrations of Six Haloacetic Acid Compounds that Could Potentially Interfere with the Determination of Bromate

Compound	Concentration (ppb)
Monochloroacetic acid	15
Dichloroacetic acid	15
Trichloroacetic acid	5
Monobromoacetic acid	10
Dibromoacetic acid	5
Bromochloroacetic acid	10

A chromatogram containing the haloacetic acid mixture and a 10 ppb bromate standard is shown in Figure 3. The retention time for bromate is 3.5 minutes. The bromine-containing HAA standards elute at 2.5 minutes, 5.9 minutes and 7.1 minutes indicating no chromatographic interference with bromate. Average bromate recovery (n = 2) for this standard spiked with 10 ppb bromate was 102%.

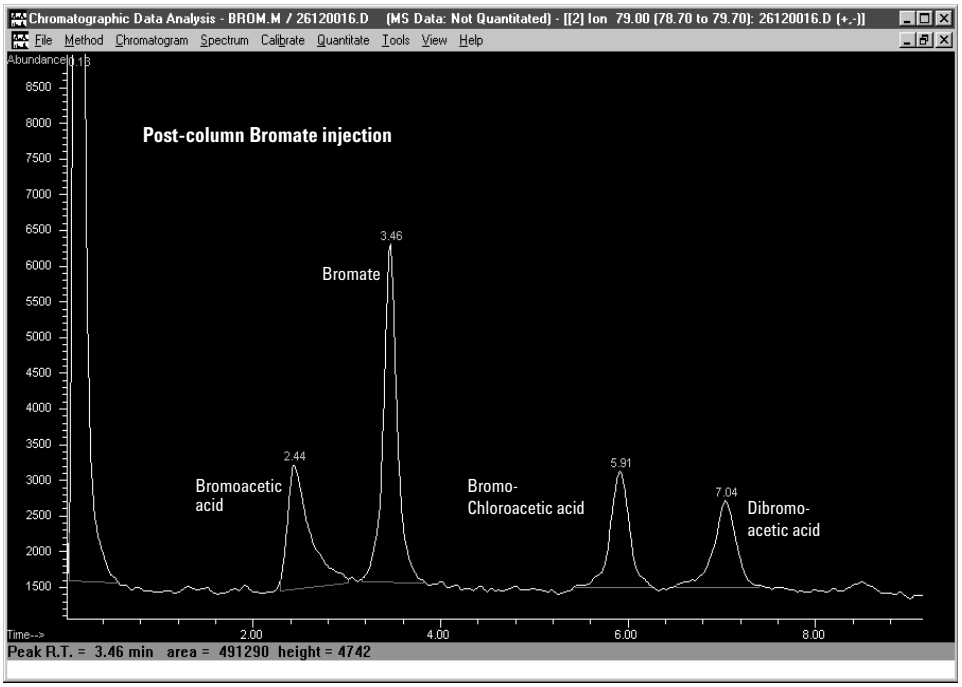

Figure 3: A chromatogram containing haloacetic acid mixture and a 10 ppb bromate standard.

Laboratory Fortified Blank

Ten replicates of a laboratory-fortified blank (LFB) were analyzed at a concentration of 5 ppb, which was approximately ten times the MDL. The LFB samples consisted of 18 MΩ deionized water adjusted to pH 10 with NaOH and spiked with 5 ppb bromate standard. The average for the replicates was 4.7 ppb (8.9% RSD) with a 93% recovery.

Laboratory Fortified Matrices

Four fresh samples supplied by the EPA, taken from ozonation utilities in the U.S., were analyzed using this methodology. Each sample was adjusted to pH 10 with NaOH, and analyzed twice, unfortified and fortified with 10 ppb bromate. The results for all four samples are shown in Table 5. The recovery results for these matrices are all within the EPA guidelines of 70-130% for this method.

Table 5: Bromate Results from Ozonation Utilities

Sample ID	Concentration of bromate in unfortified sample (ppb)	Concentration of bromate in fortified sample (ppb)	% Recovery
A	2.0	12	102%
B	2.7	12	89%
C	4.0	16	118%
D	8.9	18	100%

USEPA DBP Performance Evaluation Check

An EPA check ampule (USEPA ICR PE ampule for inorganic DBPs - Study 9), whose concentration was not known at the time of analysis, was also analyzed as a blind check sample. The ampule was prepared in duplicate by diluting 1:100 and analyzing immediately. Results are shown in Table 6. Once again, the recoveries are both within the recommended guidelines.

Table 6: Recovery of Inorganic DBPs in EPA Check Ampules

Sample	Concentration in original ampule (ppb)	% Recovery (917 ppb true value)
Ampule 1	1120	120
Ampule 2	1040	113

Conclusion

The ability to measure bromate in ozonated drinking waters at sub-ppb levels is essential to understanding its risk assessment as a carcinogen. Once USEPA Method 321.8 is validated for use, ICP-MS detection coupled to HPLC will become an approved method for achieving this. It has been shown that the instrumentation used in this study surpasses all the performance criteria specified in

the methodology, achieving a method detection limit of 0.14 ppb, with a 500 µL loop and 0.41 ppb with a much smaller injection volume (100 µL). Furthermore, this has been implemented in an automated fashion with real time data analysis using the Agilent 1100 LC and 7500 Series ICP-MS demonstrating that the technique is well suited for use as a routine analytical tool.

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Printed in the USA
June 22, 2001
5988-3161EN

Trace Level Determination of Fe in Drinking Water, and the Measurement of Fe Isotope Ratios Using the T-mode Interface

Application

ICP-MS Environmental

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Abstract

Both drinking water quality and the natural water used as the drinking water source require controls for health reasons. Routine monitoring is also performed on organic chemicals, bacteriological tests, and several inorganic components. These elements range from the major components (Na, K, Ca, etc) to the potentially toxic trace elements such as Cr, As, Se, Cd and Hg. While conventional ICP-MS can determine all of the required metals at the regulated levels in most countries, there is a trend to reduced levels of acceptable contamination, with the result that elements such as Fe cannot be measured accurately at the new Japanese and EU legislated levels.

The Agilent 7500 Series ICP-MS, fitted with the optional T-mode interface, has the ability to determine trace levels of contaminant metals in river water. The T-mode system employs a unique method of interference reduction that improves the BEC and LOD for all elements including Fe, As, and Se by effectively decomposing the ArN, ArO and CaN overlaps that normally compromise detection of these elements at levels below 10 ppb. Detection limits at the single ppb level can be achieved for Fe, and at sub ppb level for As and Se.

Introduction

The measurement and control of the levels of trace metals in drinking water is highly regulated and, in most countries, legislation exists regarding the quality of river water that is used for drinking water abstraction. As river water contains many elements with a variety of concentrations, it is necessary to determine not only trace elements, but also major and minor elements such as Na and Ca.

In the past, in order to measure all required metals in river water, both ICP-OES and GFAAS were necessary, often with element specific techniques for elements such as As, Se, Sb and Hg. ICP-MS has been used routinely for drinking water analysis for several years, but has insufficient detection capability for some elements such as Fe, forcing the need to use additional techniques. The regulated level for Fe in Japan, for example, requires a background equivalent concentration (BEC) of 30 ppb (1/10 of the Maximum Contaminant Level - MCL) and an LOD of <10 ppb.

As these levels cannot be achieved routinely by conventional ICP-MS instruments, Agilent Technologies have developed a unique method of interference reduction - T-mode - that improves the background equivalent concentration (BEC) and limit of detection (LOD) for Fe. The polyatomic interferences ArN, ArO and ArOH that form in the interface region of the ICP-MS compromise the measurement of Fe. The T-mode interface employs collision technology to significantly reduce these species, lowering detection limits to the single ppb level for Fe. A high temperature plasma is used which, along with the efficient matrix decomposition of the 7500 Series also

reduces the level of CaN (in high Ca matrices) which would otherwise interfere with Fe at *m/z* 54. Other polyatomics such as ArCl and Ar_2 are also reduced, lowering LODs to the sub-ppb level for As and Se. For these reasons, T-mode has found wide spread acceptance in Japan and Germany for the analysis of river and drinking waters.

T-mode Interference Reduction System

The key component of the T-mode system is a new, patented collision interface that promotes interion collisions inside the interface area. The design of the skimmer orifice allows it to act like a small collision cell, effectively reducing the argon based molecular species such as ArN, ArO and Ar_2, by collision with Ar ions as shown in Figure 1. No collision gas is required.

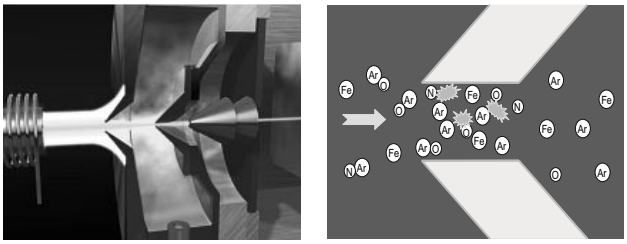

Figure 1. Interface and collision process inside the skimmer orifice.

As a result, the BECs of Fe and Se show significant improvement. Figure 2 shows the reduction of BECs of some important elements. Typical calibration curves of Fe and Se, as obtained under T-mode operation, appear in Figure 3. The BECs of ^{54}Fe, ^{56}Fe, and ^{82}Se improved to 12 ppb, 16 ppb, and 0.6 ppb respectively using the T-mode, compared to 100 ppb for Fe and 1 ppb for Se under conventional conditions.

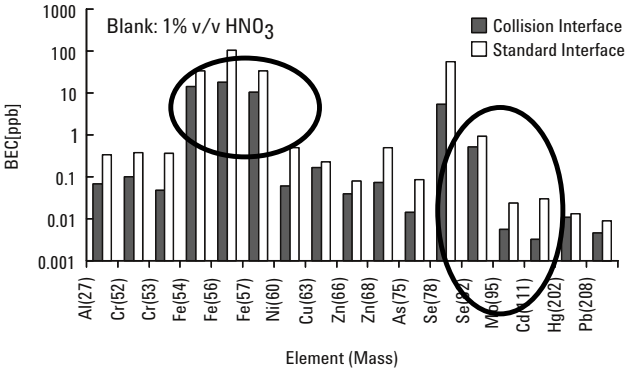

Figure 2. BEC reduction with the T-mode interface.

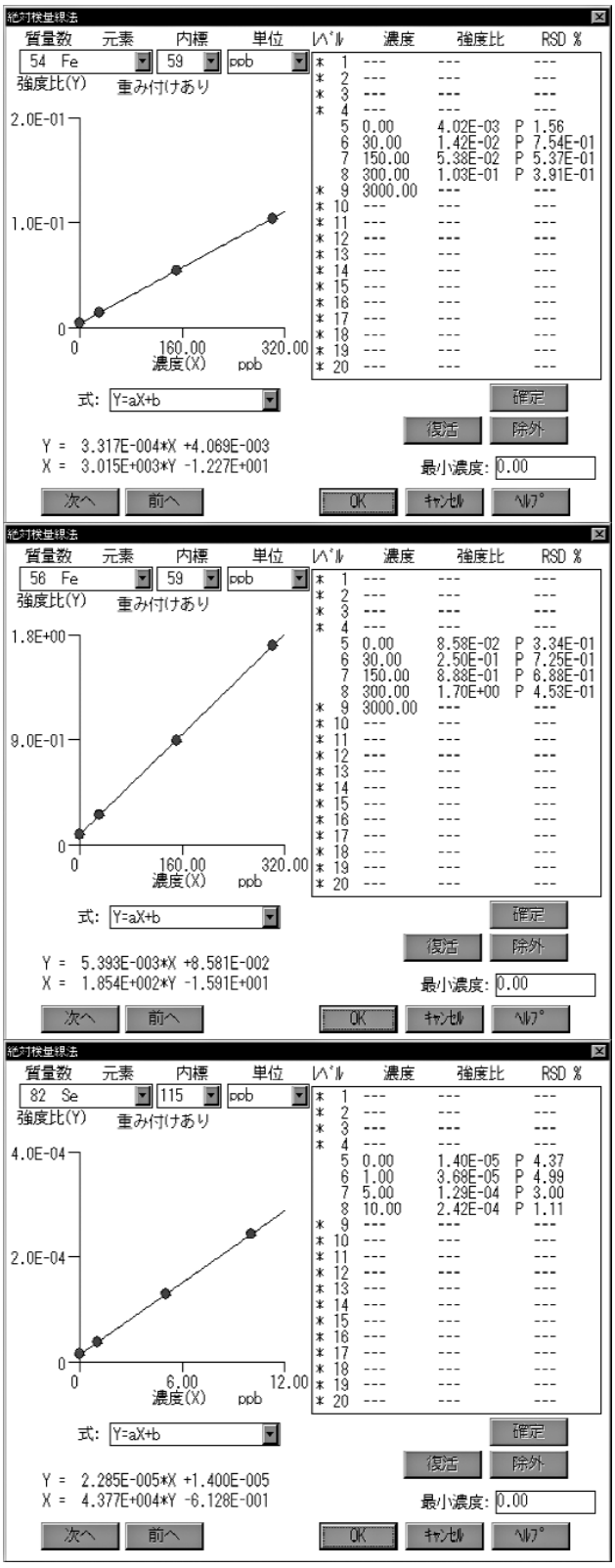

Figure 3. Calibration curves of ^{54}Fe, ^{56}Fe and ^{82}Se. BECs are 12 ppb, 16 ppb and 0.6 ppb respectively.

The use of a high temperature plasma (forward power 1550W), low sample uptake rate (0.5 mL/min) and a large diameter torch injector (2.5 mm) all contribute to the efficient breakdown of polyatomic species so that even in the presence of a high Ca matrix, Fe 54 can be determined accurately at the low ppb level despite overlap from CaN at *m/z* 54.

Experimental

An Agilent 7500 ICP-MS fitted with the T-mode interface was used to determine trace metals in two river water reference materials, JAC0031 and JAC0032. All required analytes were acquired in a single run under a single set of conditions. The stability of the ArN correction by T-mode was assessed by measuring Fe under different nitric acid conditions. Finally, iron was determined in the presence of a high concentration of calcium typical of a hard river water. Instrument operating parameters are shown in Table 1.

Table 1. Operating Parameters of the Agilent 7500 T-mode ICP-MS

Plasma gas flow rate	15.0 L/min
Aux. gas flow rate	1.0 L/min
Carrier gas flow rate	1.05 L/min
RF power	1550 W
Nebulizer	PEEK, Babington type
Spray chamber	Quartz, Scott type
Spray chamber temp.	2 °C
ICP torch injector	Quartz, 2.5 mm i.d.
Sample uptake rate	0.5 mL/min
Sampling cone	Nickel (T-mode)
Skimmer cone	Nickel (T-mode)
Sampling depth	6 mm
Points/mass	3
Integration time/mass	3 secs for Fe and Se 1 sec for others
Replicates	3

Results and Discussion

The certified values of river water standards JAC0031 and JAC0032 and the results obtained with the T-mode system are given in Table 2. JAC0031 is a river water that contains 12.5 ppm Ca, 4 ppm Na, and 2.86 ppm Mg, acidified with nitric acid to 0.1N. JAC0032 is the same sample with the addition of a multi-element spike.

The spiked amount ranges from 1 to 50 ppb, depending on the element. For this initial measurement, no interference correction was used.

The results shown in Table 2 indicate that the T-mode analysis gave good agreement with the certified values for most elements, including Fe, As, and Se, despite the low concentration level. The measured value for Fe determined at mass 54 is much closer to the certified value than the value determined at mass 56, due to interference by CaO at *m/z* 56 - but since no interference correction was used, these data also clearly demonstrate the fundamental interference removal capability of the T-mode system. Based on these encouraging results for ^{54}Fe in the two river water reference materials, the stability of the ArN correction with varying nitric acid content was assessed. The results in Table 3 show the determination of Fe in the samples JAC0031 and JAC0032, after adjustment to 0.2 N with nitric acid, calibrated against standards prepared in 0.1 N nitric acid. If the Fe determination was susceptible to a changing ArN signal, an error from the different nitric acid content might be expected, but the accurate determination of Fe shown in Table 3 indicates that the ArN correction was effective.

The following correction equation, based on ^{15}N, was applied to improve the accuracy of Fe determination in a varying nitric acid matrix:

$$Fe(54) = Count(54) - (ArN^*(54) / N^*(15)) \times N(15)$$

Where ArN*(54) / N*(15) is calculated by measuring a blank solution, the plasma conditions are adjusted to give a constant ArN*(54) / N*(15) ratio at any nitric acid concentration. Table 4 also shows the results obtained for ^{54}Fe in the presence of a high Ca concentration. This is important, as natural waters and hard drinking waters may contain several hundred ppm of Ca. Trace levels of Fe must be determined accurately in the presence of this high Ca concentration, which could not only give rise to a CaO overlap at mass 56, but also a CaN overlap at mass 54. The results shown in Table 4 indicate that the accurate determination of

^{54}Fe is possible, even in the presence of 200 ppm Ca spiked into the reference water samples JAC0031 and JAC0032. While various high Ca matrix samples were introduced during the course of this study, the accurate determination of ^{54}Fe in JAC0032 was reproducible over 9 hours as shown in Figure 4, demonstrating the suitability of this system for routine use with typical drinking water matrices.

Table 2. Determination of Standard River Waters (ppb) JAC0031 and JAC0032

Element	*m/z*	ISTD	**JAC0031** (analytes unspiked)		**JAC0032** (analytes spiked)	
			Certified	**Quantified**	**Certified**	**Quantified**
B	11	7	9.1±0.5	11.7	59±2	64.8
Al	27	59	13.4±0.7	15.3	61±2	67.3
Cr	52	59	0.14±0.02	0.14	10.1±0.2	10.4
Fe	**54**	**59**	**6.9±0.5**	**6.0**	**57±2**	**57.7**
Mn	55	59	0.46±0.02	0.51	5.4±0.1	5.7
Fe	**56**	**59**	**6.9±0.5**	**10.3**	**57±2**	**63.7**
Ni	60	59		0.15	10.2±0.3	10.6
Cu	65	59	0.88±0.03	1.16	10.5±0.2	11.5
Zn	66	59	0.79±0.05	1.15	11.3±0.4	12.4
As	75	115	0.28±0.04	0.28	5.5±0.3	5.4
Se	82	115	(0.1)		5.2±0.3	5.1
Mo	95	115		0.61		0.57
Cd	111	115	(0.003)		1.00±0.02	1.03
Sb	121	115				0.17
Pb	208	205	0.026±0.003	0.057	9.9±0.2	10.2

Table 3. Determination of Acidified Standard River Waters (ppb) JAC0031 and JAC0032, Adjusted to 0.2 N HNO_3

Element	*m/z*	ISTD	**JAC0031** (analytes unspiked)		**JAC0032** (analytes spiked)	
			Certified	**Quantified**	**Certified**	**Quantified**
Fe	54	Co	6.9±0.5	6.41	57±2	58.50

Table 4. Determination of Simulated Hard River Waters (ppb) JAC0031 and JAC0032, Spiked with 200 ppm Ca, 0.1 N HNO_3

Element	*m/z*	ISTD	**JAC0031** (analytes unspiked)		**JAC0032** (analytes spiked)	
			Certified	**Quantified**	**Certified**	**Quantified**
Fe	54	Co	6.9±0.5	6.83	57±2	57.33

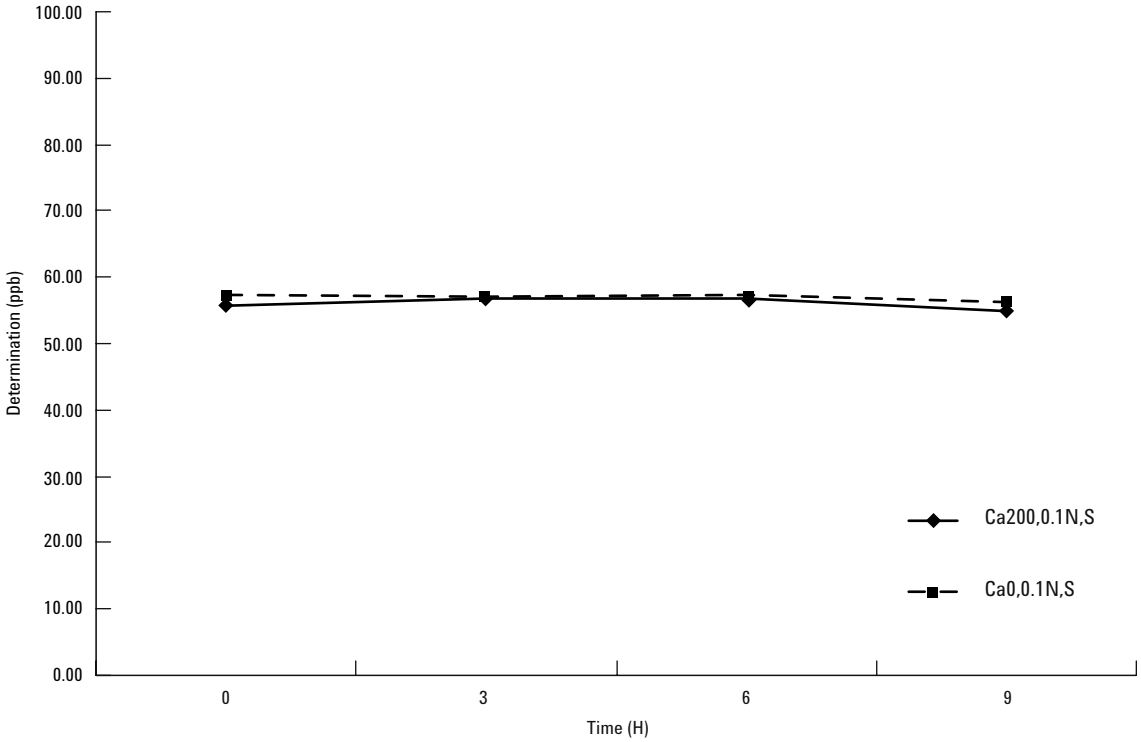

Figure 4. Stability of determination of ^{54}Fe for 9 hours.

Iron Isotope Determination in Ca Rich Samples

If the sample introduction system is not optimized, $^{40}Ca^{16}O$ is not efficiently broken down in the plasma, and high levels of Ca will lead to interferences with Fe at *m/z* 56. A measure of the effectiveness of the Agilent 7500 Series sample introduction system design is to examine the effect of Ca levels on Fe isotope ratios. Table 5 summarizes data from a series of isotope ratio measurements of Fe. In the experiment, natural Fe standards at 0.5 ppm (to ensure good counting statistics for the minor isotopes) were spiked with 5, 10, 20 and 100 ppm Ca. Even small levels of CaO species would cause a significant biasing of the Fe isotope ratio measurements. As the data illustrates, up to 20 ppm Ca the interference is negligible, and even at 100 ppm Ca, the effect is minimal (no interference correction was applied). This reduction of Ar-based and M-O polyatomics by the T-mode with correct plasma optimization enables the isotopic measurements of Fe in real-life clinical and biological samples.

Table 5. Fe Isotope Ratio Measurements

	54/Total	%RSD	56/Total	%RSD	57/Total	%RSD	58/Total	%RSD
No Ca	5.84	0.67	91.75	0.05	2.12	0.54	0.28	0.78
5 ppm Ca	5.83	0.46	91.74	0.04	2.14	0.41	0.28	0.62
10 ppm Ca	5.81	0.42	91.74	0.03	2.16	0.40	0.28	0.63
20 ppm Ca	5.81	0.55	91.69	0.05	2.21	0.44	0.28	0.67
100 ppm Ca	5.70	0.39	91.45	0.02	2.56	0.34	0.29	0.5
Certified Fe Isotope	5.85		91.75		2.12		0.28	
Main overlaps	CaN		CaO		CaOH		CaOHH	

Conclusion

An Agilent 7500 Series ICP-MS fitted with the T-mode interface is a simple mechanism for the effective reduction of argon related molecular ion interferences. While reducing the ArN and ArO interferences on Fe, the high temperature plasma reduces the formation of refractory polyatomics, such as CaN, which cannot be achieved under normal or cool plasma conditions. This means that T-mode is suitable for low-level Fe measurements in high Ca matrices such as river water.

In addition, trace levels of all the regulated elements in both river and drinking water can be determined routinely. With detection limits for Fe at the single ppb level and at sub ppb level for As and Se, the T-mode system has found widespread acceptance in Germany and Japan for the analysis of drinking waters.

Finally, accurate Fe determination in varying levels of calcium matrices or varying nitric acid concentrations is also possible using this novel interference reduction system.

Printed in the USA
May 25, 2001
5988-2993EN

Agilent Technologies

Determination of Mercury in Drinking Water Samples by ICP-MS Using EPA Method 200.8

Application Note

Environmental

Abstract

The quantitative determination of mercury in drinking water samples, simultaneously with 20 other elements (as described in the EPA Method 200.8) is presented. To avoid the Hg memory effects normally experienced with conventional nebulizer/spray chamber sample introduction systems, gold was added off-line to all standards and samples to act as a complexing agent. The addition of gold and the design of the 4500 ICP-MS assure fast washout time and allow the determination of all elements, including mercury, in drinking water by a single ICP-MS run.

Agilent Technologies
Innovating the HP Way

Introduction

EPA Method 200.8[1] describes the multi-element determination of trace metals waters and wastes by inductively coupled plasma-mass spectrometry (ICP-MS). This method provides procedures for determination of dissolved elements in ground waters, surface waters and drinking water. It may also be used for the determination of total recoverable element concentrations in these waters as well as waste waters, sludges and solid waste samples. EPA Method 200.8 is applicable to 21 elements, including mercury, enabling all required elements to be analyzed by a single technique - ICP-MS. This application note describes the additional sample preparation necessary for the successful determination of mercury.

As stated in the method, samples may be analyzed directly by pneumatic nebulization without acid digestion if the samples have been properly preserved with acid and have turbidity of < 1 NTU at the time of analysis. This total recoverable determination procedure is referred to as *direct analysis* (section 1.4 - EPA Method 200.8).

For the *direct analysis* of water samples which do not require digestion/extraction prior to analysis, and for which turbidity is < 1 NTU, the combined concentrations of inorganic and organo-mercury species in solution can be determined provided gold is added off-line to both samples and standards alike (section 1.6 - EPA Method 200.8)[1].

System Design

The 4500 ICP-MS was developed with the routine user in mind: innovative hardware and software design has resulted in the automation of routine tasks such as the optimization of ion lenses, plasma conditions, and adjustment of the ICP torch position. By making optimization independent of the operator, performance becomes more consistent, even in a multi-user environment.

A programmable computer-controlled peristaltic pump system allows for a selection of the optimum rinse time to accommodate both high sample throughput and effective elimination of memory effects. An example of a peristaltic pump program is shown in Figure 1.

The pump speed and time both before acquisition (sample uptake) and after acquisition (rinse) can be set by the user. Pumping the sample into the system at high speed reduces sample uptake time, and a stabilization time allows the system to stabilize at the normal acquisition uptake rate prior to the commencement of data acquisition. The system can also be programmed to rinse longer after standards than samples, minimizing total rinse time. An second optional rinse following acquisition is also available, enabling the use of two different rinse solutions for special applications.

Memory Interferences

Memory interferences, commonly referred to as *memory effects* arise when analyte signal is enhanced due to contribution

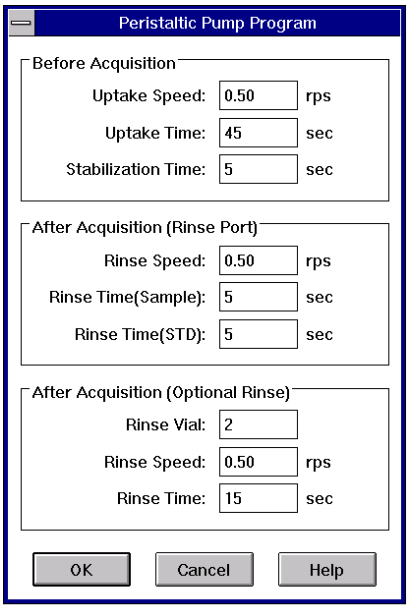

Figure 1.
Peristaltic Pump Program

from a previous high concentration sample. Memory effects can result from the adsorption/desorption of the analyte anywhere in the sample introduction system: Peristaltic pump tubing, nebulizer, spray chamber, torch or interface.

In acidic solution, mercury has a tendency to be retained on the glassware, particularly on the injector tip of the torch and in the spray chamber. As a result, the analyst has to program long washout times, and the aspiration of a sample containing very high levels of mercury will require the sample introduction system to be dismantled and thoroughly cleaned. The off-line addition of gold to the sample solution dramatically reduces washout times, since gold complexes with mercury presumably forming an amalgam, allowing it to be washed effectively from the system. The addition of gold to both standards and samples enables determination of mercury in the same analysis as for the other 20

elements in the EPA Method 200.8, allowing all elements to be measured in a single run by I CP-MS alone.

Instrumentation

The instrument used for this work was a 4500 ICP-MS fitted with a Babington-type nebulizer, glass spray chamber and quartz one-piece torch. An ASX-500 autosampler (CETAC Technologies Inc., Omaha, NE), was also fitted.

Reagents, Standards and Labware

The importance of good quality of reagents used was discussed in the Agilent Application Note (publication No. 5964-4277E)[2]. For this work, a Milli-Q SP point-of-use deionized water system (Millipore, Bedford, MA) was used to prepare all standards.

Plasma gas flow rate	15.0 L/min	
Aux. gas flow rate	1.0 L/min	
Carrier gas flow rate	1.17 L/min	
RF Power	1300 W	
Nebulizer	PEEK, Babington - type	
Spray chamber	Glass, double pass	
Spray chamber temp	1 deg C	
ICP torch injector	Quartz, 2.5 mm	
Sample uptake rate	0.4 mL/min	
Sampler cone	Nickel	
Skimmer cone	Nickel	
Sampling depth	6.4 mm	
Acquisition parameters	**Quantitative**	**Monitoring**
Points/mass	3	6
Integration time/mass	0.99 sec	0.6 sec
Total acquisition time/replicate	36 sec	139 sec
Replicates	3	1
Total acquisition time/sample	109 sec	139 sec

Table 1.
4500 ICP-MS Operating Parameters

Fresh mercury standards were prepared daily from a 10 mg/L (ppm) stock solution. Gold was added off-line to all standards and samples at the level of 100 mg/L (ppb), along with the internal standard (Tb at 50 µg/L). Both standards were prepared from

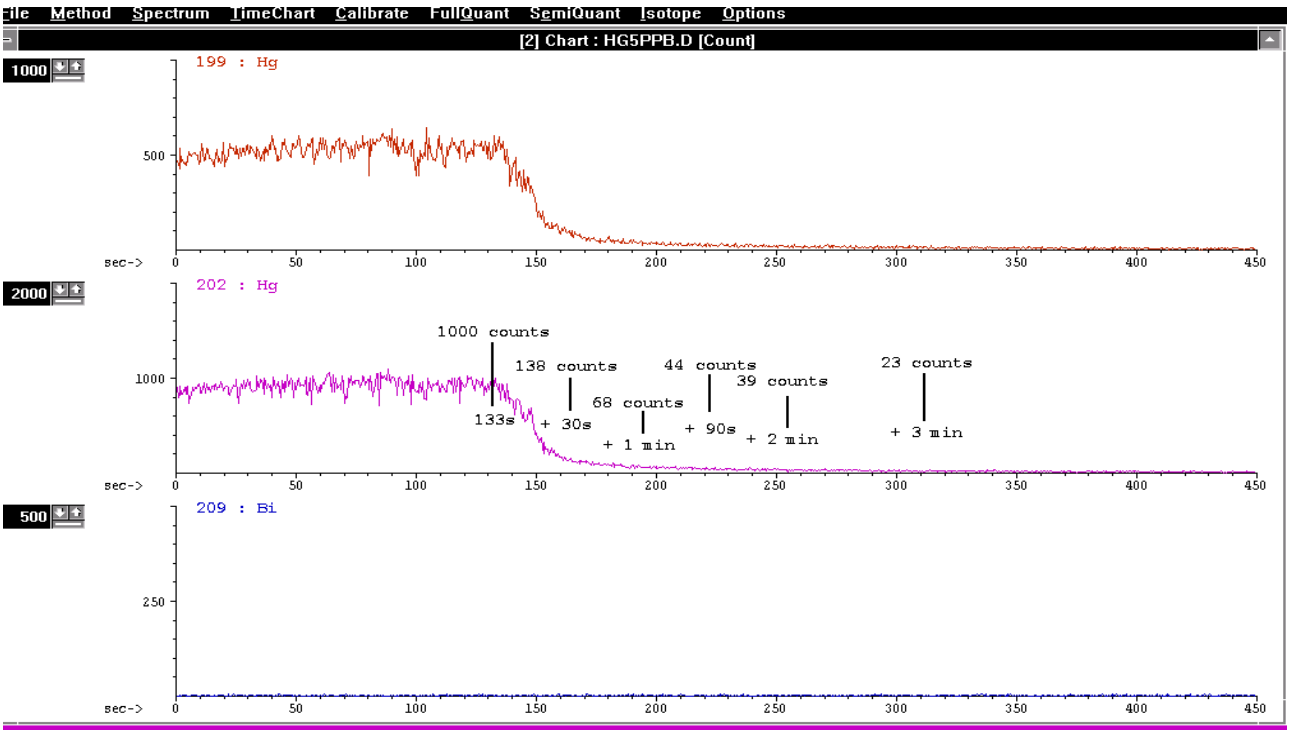

Figure 2.
Monitoring of the Mercury Washout Time

1,000 mg/L stock solutions (Inorganic Ventures, Lakewood, NJ). All standards and samples were acidified with 1% (v/v) ultrapure nitric acid (Optima Grade - Fisher Scientific, Pittsburgh, PA)

Experimental

To study the effect of gold addition on mercury washout, a 5 µg/L Hg solution was spiked with 100µg/L Au and aspirated by the 4500 ICP-MS. The washout of the mercury signal with a gold wash solution was measured by monitoring the signal in time resolved mode. Two mercury isotopes were monitored, plus bismuth for the purpose of background monitoring. Figure 2 shows the washout characteristics observed for a ^{5} ppb mercury standard solution. For ^{202}Hg, the signal counts measured at readings signal counts 30 seconds apart are shown. Two orders of magnitude washout for mercury was achieved in less than one minute, demonstrating much better washout than in acidic solution without the addition of gold. Operating parameters for both the washout study and routine quantitative analysis are given in Table 1.

A graphical representation of the acquisition method printed from the Agilent ChemStation software is shown in Figure 3. This method was applied for all 21 elements listed in EPA Method 200.8, including Mercury. A mercury calibration containing standards at 0, 2 and 5 ppb Hg was constructed and is shown in Figure 4. Terbium (mass 159) was used as the internal standard (IS). As can be seen, an excellent fit was obtained, and from the slope of the curve, detection limits in the low ng/L range can be estimated.

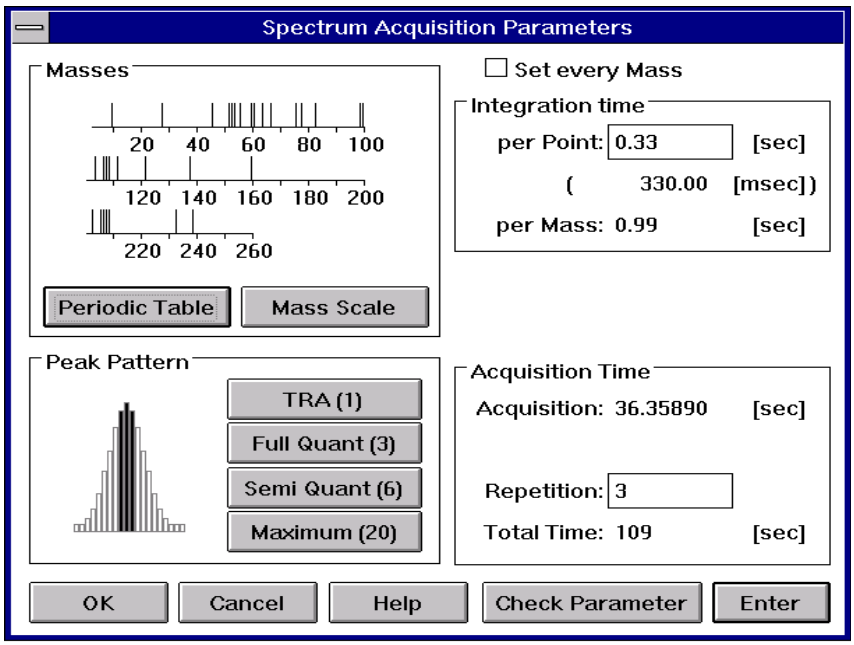

Figure 3.
Acquisition Parameters for Quantitative Determination of 21 Elements (including Mercury) by ICP-MS According to EPA Method 200.8

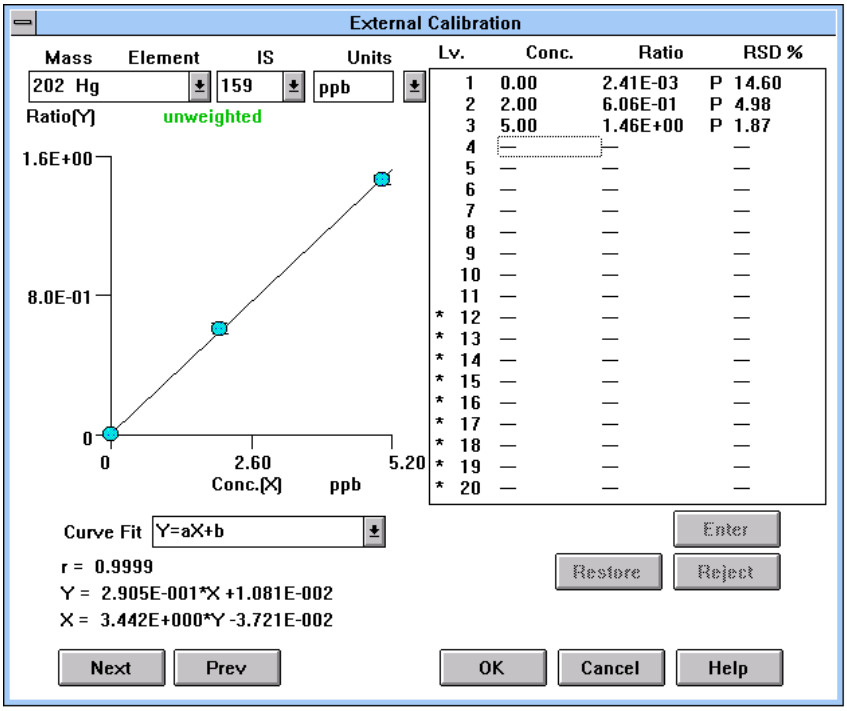

Figure 4.
Mercury Calibration Curve (Tb used as internal standard)

EPA Method 200.8 (section 7.4.1) specifies the maximum concentration of the calibration standard to be 5 ppb.

Conclusions

The determination of mercury has been shown to be easily i ncorporated into the standard multi-elemental analysis protocol of water samples using ICP-MS. Mercury carry-over was readily eliminated by the off-line addition of gold. This procedure allowed the analysis of mercury in the same run as the other analytes, enabling the measurement of all required elements by a single instrument. The addition of gold to the samples at the time of collection will minimize losses of mercury in sampling vessels.

References

[1] EPA Method 200.8 EMMC version. Revision 5.4. EMSL Cincinnati OH 45268 - May 1994

[2] Analysis of Drinking Water and Waste Water by ICP-MS Using EPA Method 200.8. Agilent Application Note (publication No5964-4277E), December 1997

Printed 4/2000
Publication number 5965-5383E

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The Determination of As in Samples Containing High Concentrations of Chloride by ICP-MS

Technical Note

Michiko Yamanaka

Introduction

One of the main advantages of ICP-MS over ICP-AES is its relative freedom from spectral interferences. There are, however, a few cases where spectral overlap is a problem. One example is the determination of As in samples containing high (%) levels of chloride. The polyatomic ion $^{40}Ar^{35}Cl$ interferes with ^{75}As, and in addition $^{40}Ar^{37}$ Cl interferes with ^{77}Se. The interference of ArCl on Se is not a problem since a different Se isotope can be selected. As, however is monoisotopic, so no alternate isotope is available. One method to overcome such polyatomic overlaps is to resolve the interference using high resolution. In this case, however, a resolving power of >7500 is required to effectively separate As and ArCl. At this resolution, ion transmission is only ~1% of the transmission at unit mass resolution and so detection limits are compromised.

The 4500 ICP-MS offers the precise, routine determination of As even in very high concentrations of chloride by the use of mathematical correction. Elemental (interference correction) equations resident in the 4500 ICP-MS ChemStation software correct for the interferences on both As and Se simultaneously. Most importantly, the inherent ion signal stability of the 4500 ICP-MS allows for very precise correction, making it possible for the 4500 ICP-MS to determine As at the ppb and sub-ppb level even in 5% HCl. This technical note examines the effect of increasing Cl concentration on the observed As signal, and determines the ability of the 4500 ICP-

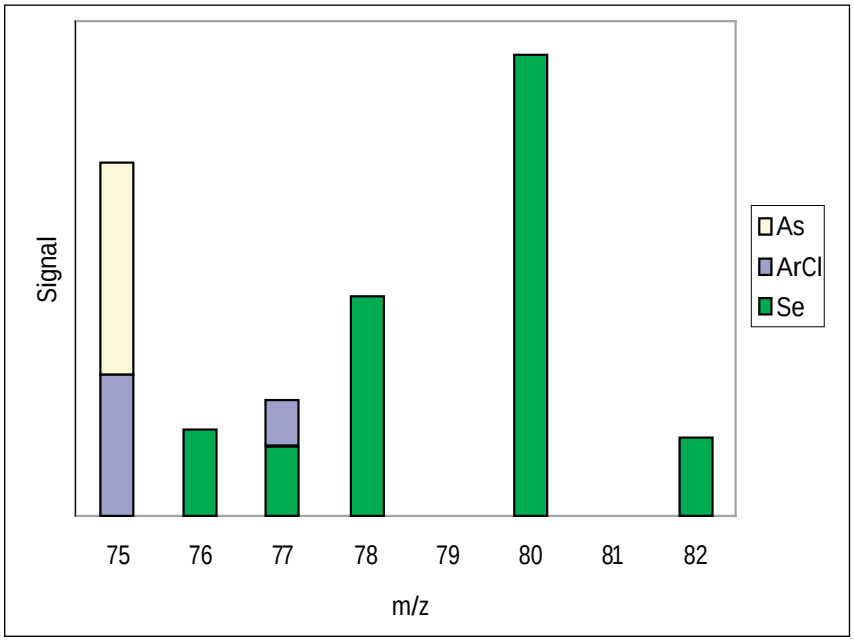


Fig. 1
Isotopic patterns of As, Se and ArCl

MS to measure As in HCl. The derivation and use of elemental equations is also explained.

Elemental equations

In samples containing chloride, the ion signal measured at mass 75 is the sum of ^{75}As and $^{40}Ar^{35}Cl$. This is shown in Fig. 1, which depicts the elemental ratios of As, Se and ArCl. This diagram is for graphical representation only; the intensities of the bars are arbitrary and do not relate to concentration values of each individual species. Also, Ar_2 species have been omitted for clarity. The ratio of the ArCl species at masses 75 and 77 is the same as the ratio of the Cl isotopes at masses 35 and 37. Therefore, the signal intensity of ArCl at mass 75 can be derived from the ArCl signal at mass 77. However, Se also has an isotope at mass 77, so the presence of Se will increase the observed signal at mass 77. Thus the contribution of Se to the total signal intensity at mass 77 must also be calculated using an alternate Se isotope. For Se correction, mass 82 is normally chosen, since the 78 and 80 isotopes suffer interference from Ar_2. In practice, this correction is simply and automatically performed by the ChemStation software using elemental equations. In this example, the equation given in EPA method 200.8 (trace metals in drinking water and wastewater by ICP-MS) was used and is shown in Fig. 2.

Influence of Cl Concentration on As Signal

To study the effectiveness of using elemental equations to correct for Cl interference, a series of 1 µg/l (ppb) As solutions were spiked with Cl at 0, 100, 200, 500 and 1000 mg/l (ppm). The solutions were measured for As, both with and without the use interference correction. A plot of observed As concentration vs. added Cl is shown in Fig. 3. Without correction, the observed As signal increased with added Cl, as expected. At 1000 mg/l Cl, the apparent increase in As was approx. 1 µg/l. Using interference correction, however, no increase in As concentration was reported, demonstrating the effectiveness of interference correction. The 4500 ICP-MS generates a very stable ion signal from both elemental and polyatomic ions, allowing precise, reproducible correction for ArCl. Ion signal stability (typically <1%RSD over 2 hours) is due mainly to the mechanical and electronics design of the 4500 ICP-MS, but also partly due to the precise temperature control of the spray chamber (+/-0.1 °C), enabling a very stable sample aerosol to be generated.

As signal intensity (mass 75)

$= 1.000 * (^{75}C) - 3.127[(^{77}C) - (0.815) * (^{82}C)]$

$= 1.000 * (^{75}C) - 3.127 * (^{77}C) + 2.549 * (^{82}C)$

(^{M}C): ICP-MS signal (counts) at mass M

Fig. 2
Elemental equation for As

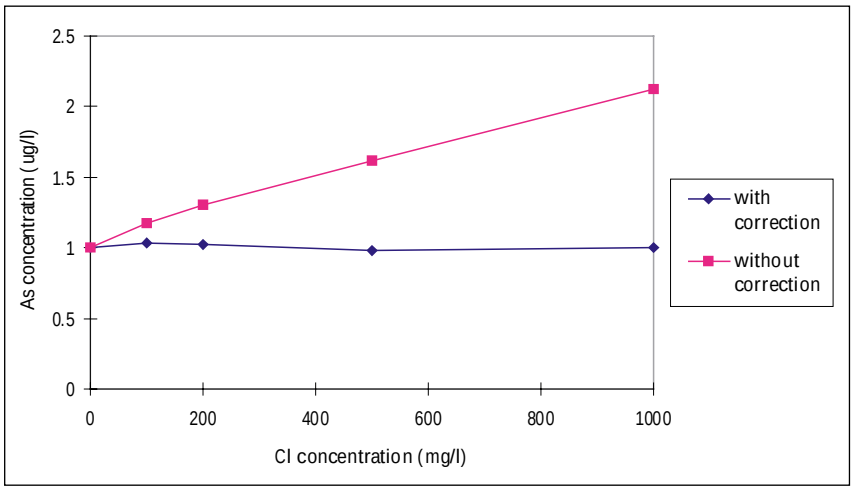
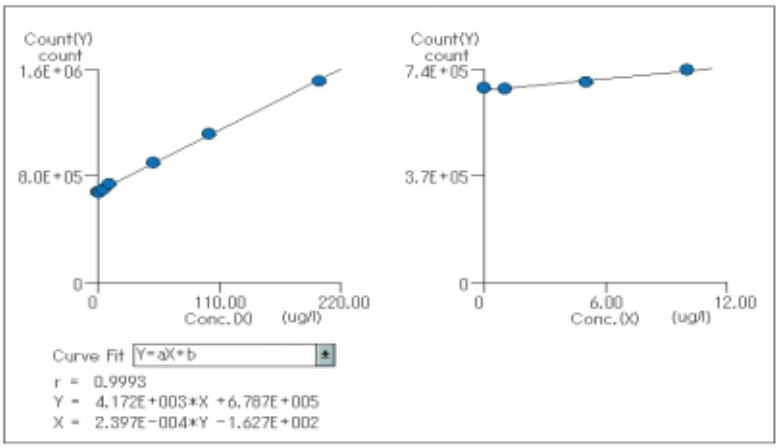


Fig. 3
Influence of Cl on As signal

Determination of As in Cl Matrix

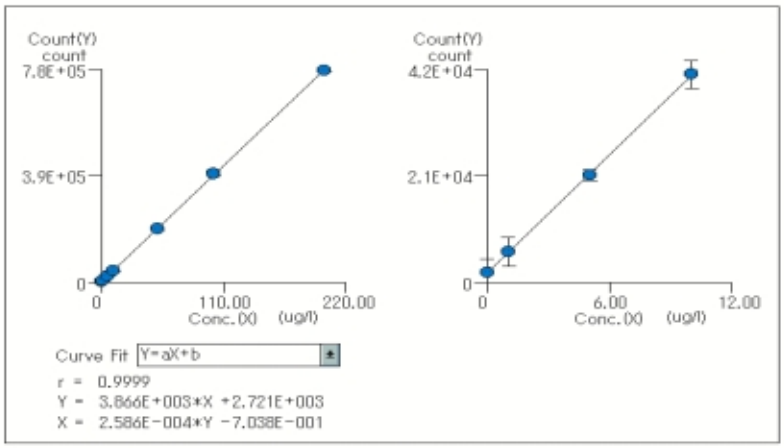
To study the effectiveness of applying interference correction to the quantitative determination of As in a chloride matrix, a series of standard solutions in a 5% HCl matrix were prepared. The As concentrations were 0, 1, 5, 10, 50, 100 and 200 µg/l. The standards were measured using the 4500 ICP-MS - the operating parameters used are given at the bottom of this page. No internal standards were used. Calibration plots for As were constructed, both with and without interference correction selected. The data was



Concentration range:

0-200 µg/l **0-10 µg/l**

Fig. 4-1
As calibration plots in 5% HCl without interference correction



Concentration range:

0-200 µg/l **0-10 µg/l**

Fig. 4-1
As calibration plots in 5% HCl with interference correction

not blank subtracted. The plots shown in Fig. 4-1 were generated without applying interference correction. Although the corration was good, the high background due to ArCl can be seen. The scale is expanded on the right to show the low concentration points. Below 10 µg/l, the calibration plot becomes essentially flat. In contrast, when interference correction is applied (Fig. 4-2), excellent correlation and linearity were obtained, even at the 1 µg/l level. Clearly, the combination of interference correction and good ion signal stability (elemental and polyatomic) allow the 4500 ICP-MS to precisely determine As even in a chloride matrix. The ability to detect As at sub-ppb levels in the presence of chloride is particularly important to the study of toxic metals in foods, biomedical, environmental and clinical applications. In addition, the demonstrated ability of interference correction to compensate for polyatomic overlap at low ion concentrations can be applied to other classic ICP-MS interferences. Other interferences will be studied in future Agilent Technical Notes.

Operating conditions

RF power	: 1.3 kW
Sampling depth	: 8 mm
Plasma gas	: 16 l/min.
Auxiliary gas	: 1.0 l/min.
Carrier gas	: 1.15 l/min.
Nebulizer	: Concentric type

Printed 4/2000
Publication number 5963-9561E

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The Determination of Selenium by ICP-MS, Using the ShieldTorch

Application Note

Ed McCurdy
Glenn Woods

Selenium is an element of considerable interest in environmental and biomedical applications. Several instances of selenium deficiency and toxicity in agricultural livestock have been reported, whilst there is increasing interest in Se as it relates to human physiology.

Se is an essential trace element in humans, but there is a narrow range between Se deficiency and Se toxicity. An excess of Se leads to seleniosis, whilst deficiency has been implicated in coronary heart disease, arthritis, cirrhosis and cancer. Se is available in several forms as a dietary supplement, many of which are derived from yeasts.

The determination of Se by ICP-MS has been one of the most enduring challenges for the technique, due to a combination of factors.

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Firstly, Se is typically present at relatively low levels in natural (uncontaminated) biological materials and the total concentrations present may comprise several different forms of Se, which can have an impact on certain methods of analysis.

Secondly, whilst the Ar plasma is probably the most capable source yet devised for atomic spectrometry, it does suffer from certain limitations with respect to the ionisation environment and the background spectrum. In the case of Se, these two factors conspire to give a low analyte signal level and a relatively high blank signal.

Se has a high first ionisation potential, which means that a smaller proportion of the Se atoms in the plasma are converted into ions. The signal for Se is around 10% of the signal which would be obtained for a fully ionised element, so the signal to noise is about 10x poorer than it could be. Furthermore, all of the Se isotopes are potentially overlapped by polyatomic interferences from plasma or matrix-based peaks, which restricts the choice of Se isotopes which can be used for quantitation. The available Se isotopes and their respective potential overlaps are shown in Table 1.

Se Isotopic Mass	Isotopic Abundance (%)	Potential Overlap(s)
74	0.89	$^{36}Ar^{38}Ar$
76	9.36	$^{38}Ar^{38}Ar$ and $^{36}Ar^{40}Ar$
77	7.63	$^{40}Ar^{37}Cl$
78	23.78	$^{38}Ar^{40}Ar$
80	49.61	$^{40}Ar^{40}Ar$
82	8.73	^{82}Kr and $^{81}Br^{1}H$

Table 1.
Selenium Isotopic Abundances and Potential Interferences

Clearly, many of these potential interferences are Ar-based, which makes them difficult to avoid in an Ar plasma. Several techniques have been suggested to alleviate the problems associated with these overlaps, each of which has its own advantages and limitations.

The various techniques which have been used are discussed in the following paper, together with a novel approach which relies on the selective ionisation of the analyte in a plasma environment which is optimised for the reduction of the Ar-based polyatomic ions.

Hydride generation can be used for Se measurements, either utilising a stand-alone hydride generation instrument, or using a hydride generation accessory as the sample introduction method for ICP-MS. The transport efficiency of hydride generation is certainly improved over the use of a conventional spraychamber, but there are several chemical limitations to the technique, most notably that all of the Se must be converted to a form which will form hydrides. In addition, with hydride generation-ICP-MS, the main problem of Ar_2 overlaps on 4 of the Se isotopes is not addressed.

Alternative plasmas have been used, particularly He-based microwave induced plasmas (MIP). This solution offers the dual benefit of a more highly ionising plasma environment (as He has a higher ionisation potential than Ar), together with the removal of the Ar-based polyatomic species. However, these systems are expensive to run, and are not widely available or established as commercial instrumentation.

In common with most polyatomic interferences, the Ar-based overlaps on the Se isotopes can be separated from the analyte peak through the use of a high-resolution magnetic sector mass spectrometer. In addition to offering a very expensive and non-routine solution to the problem, high-resolution mass spectrometers do not address the fundamental problem of the presence of the interfering peak. In order to separate a polyatomic from an adjacent analyte peak, a theoretical resolution is normally calculated, based on equal heights for the 2 peaks and separation only to the 10% valley definition. However, in the case of a trace analyte (such as Se) adjacent to a major interference (such as Ar_2), these calculations are not appropriate. A much higher resolution setting will typically be required and the transmission and sensitivity will therefore be severely compromised. Typically, operation at resolutions of several thousand (as needed even for the separation of equal height peaks) will result in a reduction in transmission of around 95%, i.e. only around 5% of the original signal remains.

Techniques have been investigated for the removal of various polyatomic species using selective collisions with a gas which is introduced into the mass

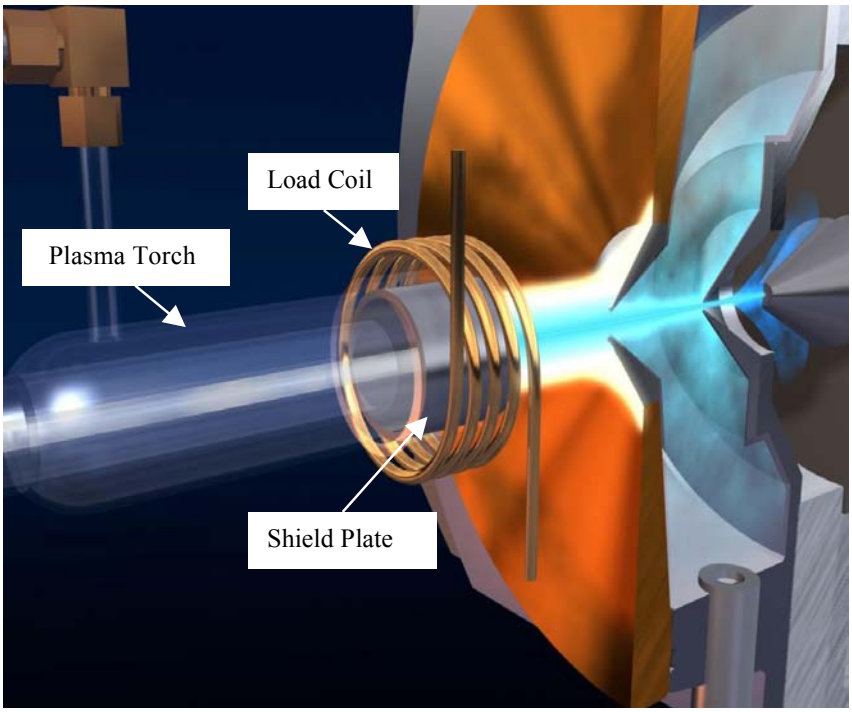

Figure 1.
Schematic of Shield Torch System on the 4500 ICP-MS Series

Forward Power	1000 W
Sampling Depth	13 mm
Carrier Gas Flow	1.2 L/min
Blend Gas Flow	0.8 L/min
Ion Lens Tuning	Typical Cool Plasma Settings
Acquisition Time	21 seconds/repeat
Number of Repeats	3 (10 for LOD blank

Table 2.
4500 ICP-MS Operating Conditions for Se Analysis

spectrometer vacuum chamber. These techniques have not, as yet, proved robust in their ability to decompose specific polyatomics without the introduction of numerous additional clusters ions, which can result in an increase in the overall complexity of the spectrum. Furthermore, collision cells have been shown to be highly susceptible to contamination from matrix components, resulting in poor tolerance to natural samples. Whilst an individual poly-atomic ion can be attenuated, albeit usually with severe attenuation of the analyte signal, it has been demonstrated that different optimum conditions are required for each poly-atomic ion, so the technique is appropriate only on a single-element batch processing basis.

A simple and elegant solution to the problem of Se analysis has been developed by Agilent Technologies application staff, working with the ShieldTorch System on the 4500 ICP-MS.

The ShieldTorch system comprises a grounded metal plate which lies between the plasma RF load coil and the plasma torch, as shown in Figure 1. This has the effect of removing the capacitive coupling between the coil and the plasma, so the plasma is held at the same potential (ground) as the mass spectrometer interface.

Combined with changes to the operating parameters of the plasma (gas flows and sampling depth), which reduce the temperature of the central channel of the plasma, this leads to a background spectrum which is virtually free from Ar-based peaks. Since Se has a relatively high first ionisation potential, it might be expected that reducing the plasma temperature would dramatically reduce the Se signal. However, it is straightforward to optimise the 4500 ICP-MS to give minimal Ar_2 signal whilst retaining good sensitivity for Se. The principal operating parameters are outlined in Table 2.

Under these conditions, the Ar_2 background is much reduced, as shown in Figure 2, whilst Se can be measured at the low ppb level, also shown in the same Figure. From these spectra, it is clear that the Se isotopic pattern matched the theoretical isotopic abundance, indicating that the Ar_2 polyatomics have been effectively removed. This analytical methodology was applied to the measurement of Se in HCl. With the robustness of the higher-power Cool Plasma of the 4500 ICP-MS, this change in matrix did not require any further optimisation or re-tuning of the plasma parameters or ion lenses.

For the quantitative measurement of low concentrations of analytes,

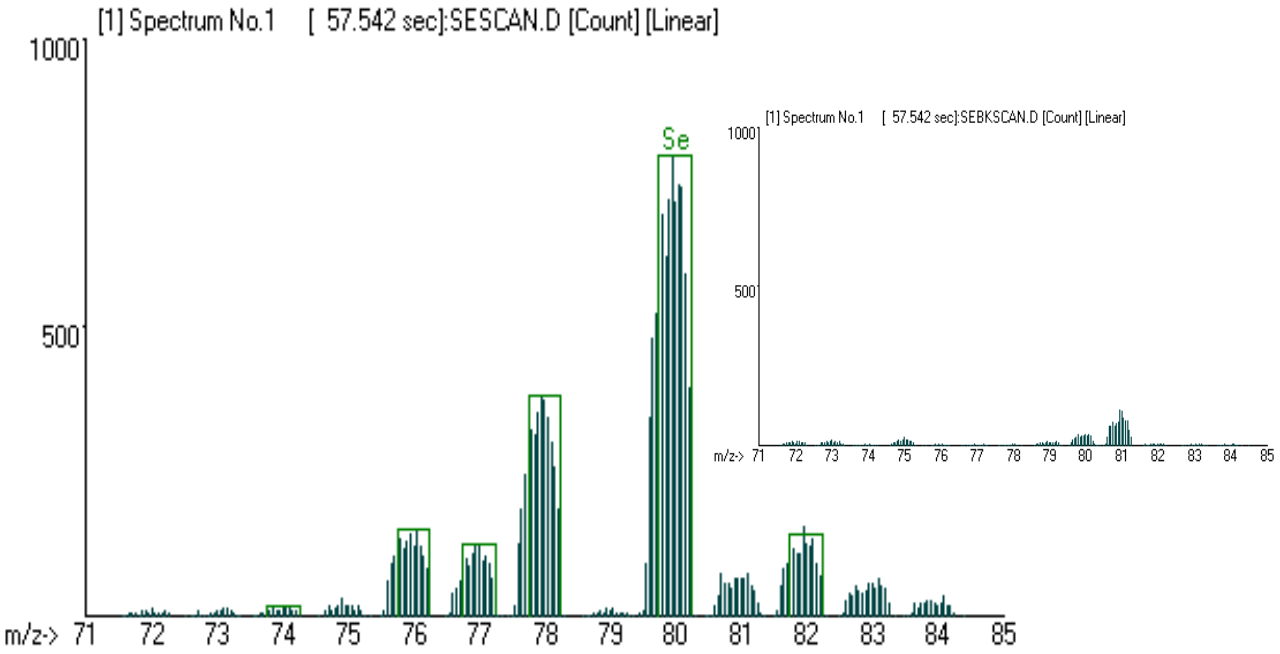

Figure 2.
Selenium Isotopic Pattern in 10ppb Standard and Blank (inset)

Isotope	Dwell time/point (s)	Dwell time/mass (s)
76	2.0	6.0
77	2.0	6.0
78	0.4	1.2
80	0.2	0.6
82	2.0	6.0

Table 3.
Integration Times Used for Selenium Analysis

it is normal to concentrate the integration time on the peaks of interest. For this reason, a peak jumping acquisition was used, where the quadrupole settles only at the top of each set mass. This ensures that the best signal to noise is achieved, although the spectral information is much more limited. The integration times used for the Se isotopes in this study are shown in Table 3. Different integration times were used, in approximately inverse proportion to the isotopic abundance of the individual isotopes, so approximately equal counts (in raw counts) were collected for each.

The calibrations obtained for the Se isotopes at mass 76, 78, 80 and 82, measured in a matrix of 4% HCl, are shown in Figures 3 to 6. In each case, the calibration was in the range from 0ppb to 5ppb. The linearity for each calibration was 1.000.

No blank subtraction was used, no interference correction was required and no internal standard was added.

In HCl, a further possible polyatomic peak might be encountered, due to the formation of $^{40}Ar^{37}Cl$, which would overlap the Se isotope at mass 77. Whilst there are 4 Se isotopes which are free from interference (as shown in Figures 3 to 6), it would be useful if a further isotope could be measured as well. A greater number of available isotopes increases the possibility of carrying out stable isotope tracer analyses in biological systems, as well as extending the possibility of conducting isotope dilution analysis to improve the accuracy of measurements at ultra-trace levels.

The fit of the Se isotopic template to the spectrum for 10ppb Se in 4% HCl is shown in Figure 7. Inset in this spectrum is the spectrum for the blank 4% HCl, on the same intensity scale. The residual ArCl peak at mass 77 is equivalent to about 3ppb, suggesting that the ^{77}Se isotope might be analytically useful at the sub-10ppb level, in addition to the other 4 Se isotopes shown in Figures 3 to 6.

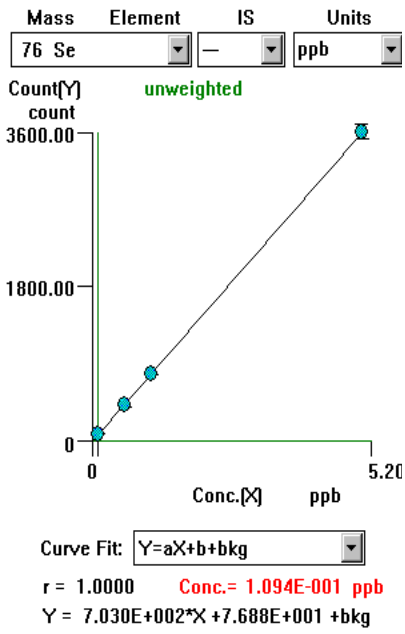

Figure 3.
Calibration for Selenium 76

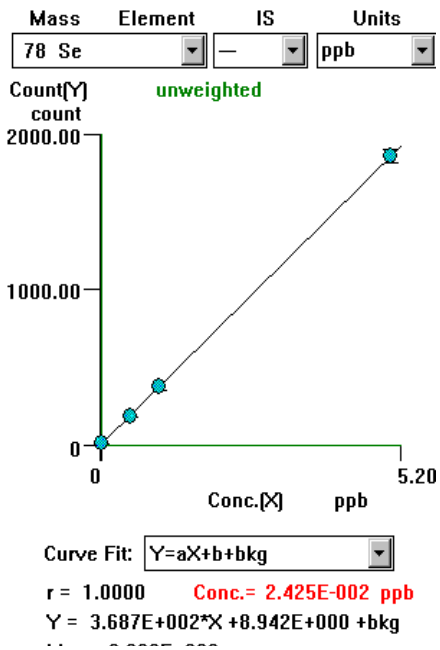

Figure 4.
Calibration for Selenium 78

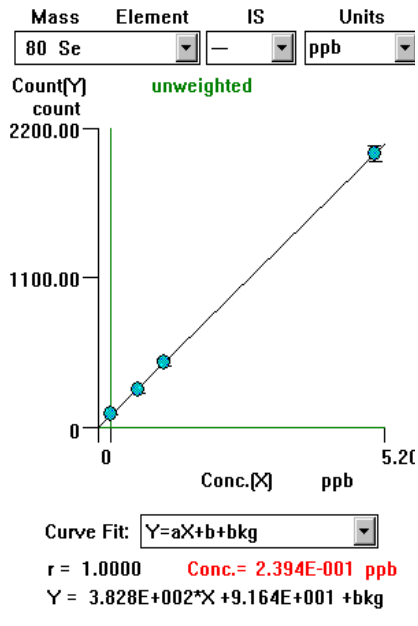

Figure 5.
Calibration for Selenium 80

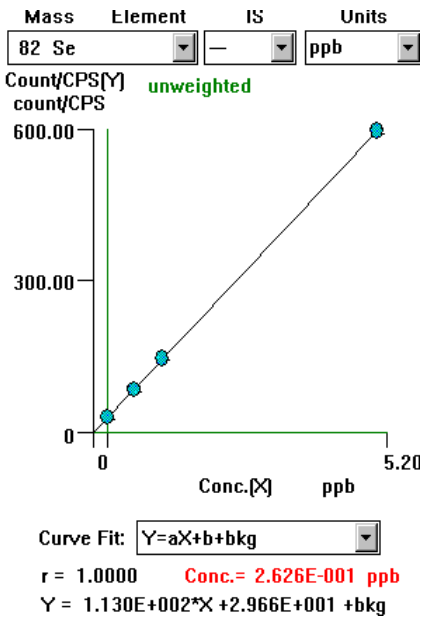

Figure 6.
Calibration for Selenium 82

The calibration for Se at mass 77 is shown in Figure 8. As with all of the other calibrations shown in Figures 3 to 6, this calibration was generated using the method of standard additions, so the intercept on the y-axis indicates the contribution from the ArCl background. Even in the presence of this background, an acceptable calibration was obtained at the sub-5ppb level.

The 4% HCl Blank was repeated 10 times and the detection limit for each Se isotope was calculated based on the multi-point calibration in 4% HCl. The 3 sigma detection limits calculated are shown in Table 4. Note that these are conservative detection limits, as they are based on the analysis of a matrix blank (4% HCl) analysed as a real sample, immediately following the analysis of the calibration standards. Detection limits a factor of 2-5 lower than these values have been obtained under optimum analytical conditions. Also shown in Table 4 are the background equivalent concentrations for each of the Se isotopes, indicating that backgrounds well below 100ppt were achieved for the 3 Se isotopes.

To be useful in real analysis, it must be demonstrated that good precision can be obtained at analytically useful concentrations. The calibration graphs show the good precision obtained on each calibration standard and Table 4 shows the actual precision obtained at the 1ppb level.

The analysis of Se is of increasing interest in human nutrition and toxicology. The ability to measure trace Se concentrations without resorting to separate analytical techniques is beneficial. The

Shield Torch System of the 4500 ICP-MS allows operation of the ICP-MS under optimum conditions for Se analysis, without the limitations in matrix tolerance associated with collision or reaction cells. The possibility of mass spectrometric determination of multiple Se isotopes, allowing stable isotope tracers to be used, is an exciting development in the growing applications for higher power cool plasma analysis using the ShieldTorch System on the 4500 ICP-MS.

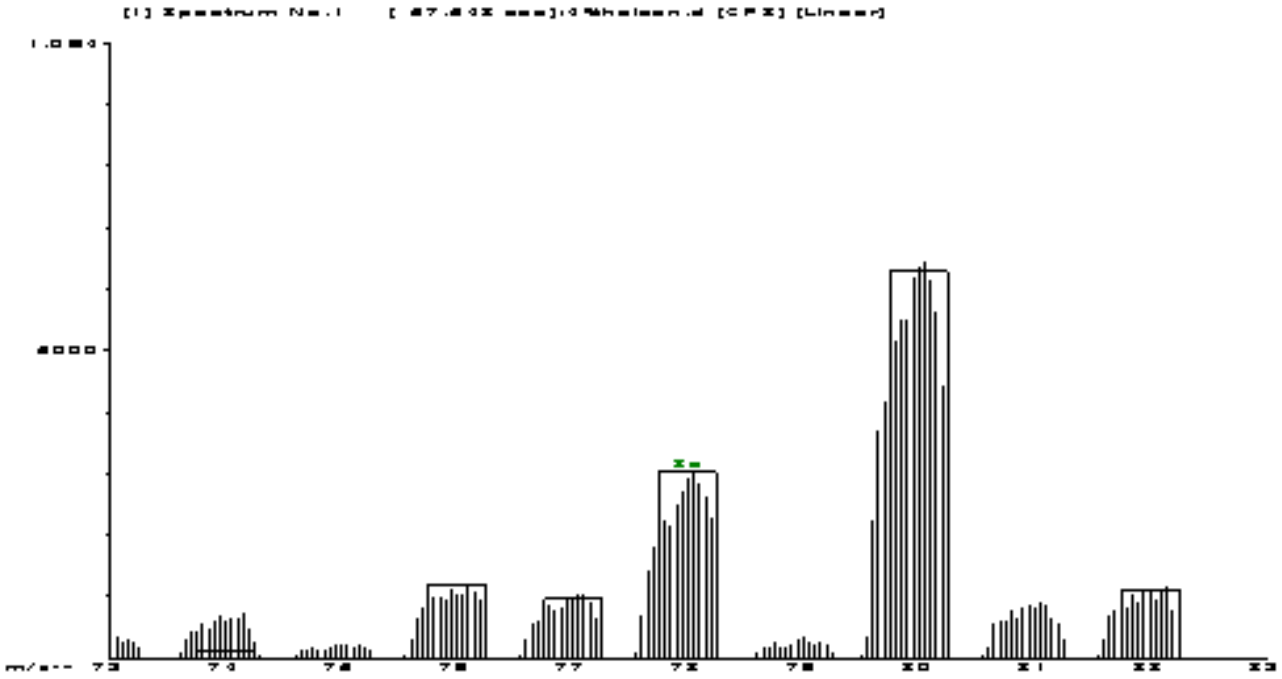

Figure 7.
Selenium Isotopic Pattern in 4% HCl Blank (inset) and 10ppb Standard

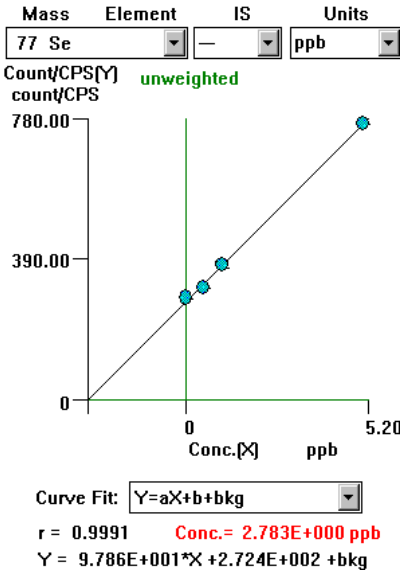

Figure 8.
Calibration for ^{77}Se in 4% HCl

Se Isotope	BEC (ppt)	3s LOD (ppt)	Precision at 1ppb (%)
76 Se	172.8	38.04	2.61
77 Se	2880	81.12	2.47
78 Se	89.5	49.14	5.24
80 Se	29.6	59.82	4.97
82 Se	31.1	59.7	2.35

Table 4.
Summary of Detection Limit Data for Selected Se Isotopes

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Printed 4/2000
Publication number 5968-7598E

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The Determination of Trace Elements in Soils and Sediments by ICP-MS

Application Note

Environmental

Concerns regarding “safe” levels of contaminants in the environment, particularly heavy metals, continue to grow. The requirement for analysis of more elements at ever decreasing concentrations is exposing the limitations of currently used analytical techniques. Further improvements in sensitivity and elemental coverage are required. While GFAAS (Graphite Furnace Atomic Absorption Spectrometry) and ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) are still the most commonly used techniques in environmental elemental analysis, ICP-MS is the only technique that offers the improvements in sensitivity that will be demanded in the near future. The 4500 ICP-MS benchtop ICP-MS offers high throughput multielement analysis at the sub ug/ml (ppb) level with the robustness and ease of operation required for true routine use. In this application brief, the analysis of two typical environmental solids - lake sediment and soil - is described. The samples analyzed were standard reference materials - IAEA (International Atomic Energy Agency) SL-1 (lake sediment), and IAEA SOIL-7 (soil).

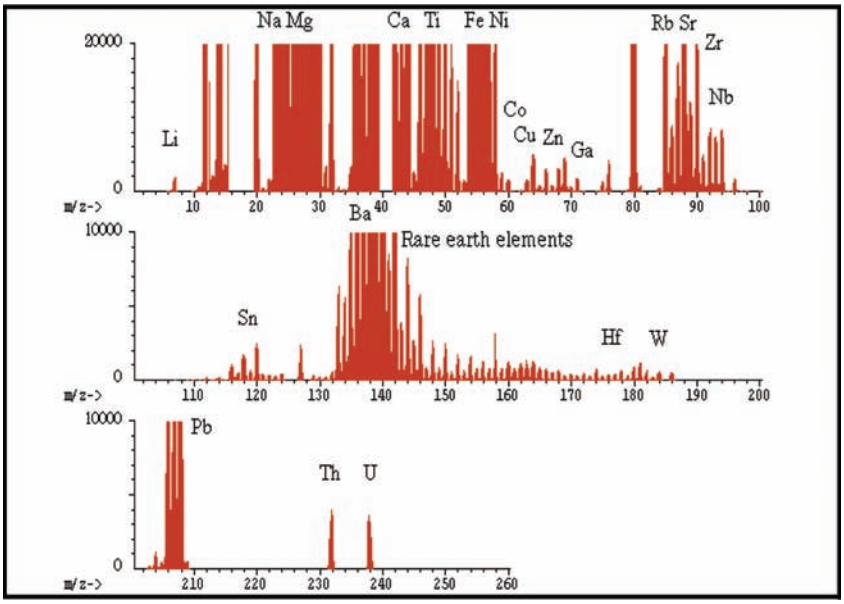

Fig. 1.
Qualitative spectrum of SL-1 (Lake sediment)

Sample Preparation

0.1g of each sample was digested with 1ml of pure water, 0.3ml of hydrofluoric acid (38%) and 0.7ml of nitric acid (68%) using microwave digester for 1 hour. After digestion the sample was diluted to 100ml with deionized water.

Procedure

An internal standard mix containing Be, In and Bi was added to each sample. The sample solutions were quantified by external standardization, by measuring them against multi-element standards.

Operating conditions

RF power	: 1.3 kW
Sampling depth	: 8 mm
Plasma gas	: 16 l/min.
Auxiliary gas	: 1.0 l/min.
Carrier gas	: 1.15 l/min.
Nebulizer	: Babington type

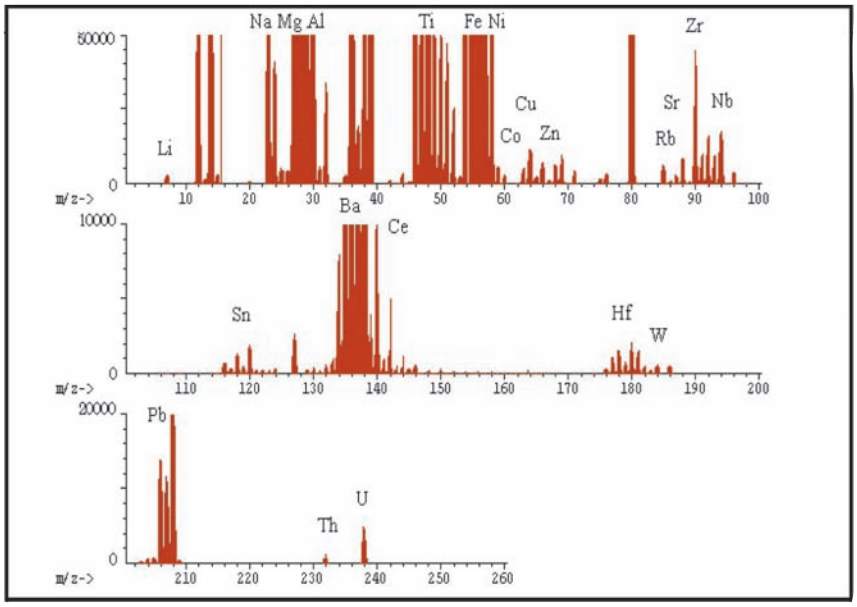

Fig. 2.
Qualitative spectrum of SOIL-7

Results

Lake sediment

Fig.1 demonstrates the qualitative spectrum of SL-1. A large number of elements, ranging from Li at low mass to U at high mass can be clearly observed, even though the total analysis time was only 100 sec.

The quantitative results and the certified values are given in Table 1. The major constituents in this sample are shown in Table 2. After digestion, Fe, Mn, Mg, K and Al were present in solution at levels ranging from a few mg/l(ppm) to 100s of mg/l(ppm), giving rise to the possibility of interference due to spectral overlap. The 4500 ICP-MS's excellent abundance sensitivity and low levels of polyatomic species ensured that the analyte values obtained were in good agreement with certified values.

Element	m/z	Certified	Measurement
V	51	170±15	169
Cr	52	104±9	115
Mn	55	3400±160	3520
Co	59	19.8±1.5	20.2
Ni	60	44.9±8.0	49.6
Cu	65	30±5.0	32.0
Zn	66	223±10	222
As	75	27.5±2.9	29.6
Se	82	*2.9	1.10
Cd	114	0.26±0.05	0.38
Sb	121	1.31±0.12	1.20
Pb	208	37.7±7.4	40.7
Units: mg/kg			

Table 1.
Quantitative values: SL-1
*** Not certified - information only**

Element	Content
Fe	6.7
Mn	0.34
Ti	0.52
Na	0.17
Al	8.9
Ca	0.25
K	1.5
Mg	2.9
S	1.2
Units: %	

Table 2.
Main constituents of SL-1

Soil

Fig.2 shows the qualitative spectrum obtained from SOIL-7. The presence of over 20 elements can be clearly observed, although the elemental composition is quite different to SL-1, even from visual inspection of the qualitative spectra.

Quantitative results and the certified values are given in Table 3 and again very good agreement was obtained. The main components of this sample are also given in Table 4. As can be seen, the sample matrix contains Ca at over 20%, which can affect the determination of Co and Ni, due to interference from CaO. Nevertheless, the 4500 ICP-MS values for Co and Ni agree well with the values supplied, which demonstrates the applicability of this technique to real life sample matrices, even where analytes are present at trace levels in the sample digest.

Element	m/z	Certified Conc.	Certified Confidence interval	Measurement
V	51	66	59-73	67.3
Cr	52	60	49-74	60.7
Mn	55	631	604-650	629
Co	59	8.9	8.4-10.1	8.60
Ni	60	*26	*21-37	24.1
Cu	65	11	9-13	9.85
Zn	66	104	101-113	104.4
As	75	13.4	12.5-14.2	13.8
Se	82	*0.4	*0.2-0.8	3.11
Cd	114	*1.3	*1.1-2.7	1.20
Sb	121	1.7	1.4-1.8	1.60
Pb	208	60	55-71	61.7
Units: mg/kg				

Table 3.
Results of SOIL-7
*** Not certified - information only**

Component	Content
Al^2O^3	8.9
Ca^0	22.9
Fe^2O^3	3.7
K^2O	2.9
MgO	1.9
Na^2O	0.6
SO^3	0.3
SiO^2	38.5
TiO^2	0.5
Loss on ignition (900°C)	20.5
Units: %	

Table 4.
Matrix components of SOIL-7

Agilent Technologies, Inc.
Printed 4/2000
Publication number 5963-7138E

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Specific Determination of Bromate and Iodate in Ozonized Water by Ion Chromatography with Two Detection Methods : Postcolumn Derivatization and ICP-MS Detection

Submitted to : Journal of Chromatography A, 789, 259-265 (1997)

Application Note

Environmental

Michiko Yamanaka

Abstract

A specific determination for bromate, iodate and other halogen anions in drinking water by direct injection using ion chromatography (IC) with either inductively coupled plasma mass spectrometry (ICP/MS), or the postcolumn derivatization is described. The advantages of ICP/MS as an element selective detector was evaluated for bromate and iodate by considering the comparison with the postcolumn derivatization. Samples were directly injected into the IC column, and halogen anions were separated. The eluates were directly introduced into ICP/MS and detected at 79 and 127 amu. The detection limit (*S/N* = 3) for bromate and iodate with injection of 0.5 mL were 0.45 µg Br/L and 0.034 µg I/L, respectively. The IC combined with ICP/MS was applied to the simultaneous determination of bromate, bromide and other halogen anions in raw and ozonized water. Good agreement was obtained for the determined values by IC-ICP/MS and postcolumn derivatization. Furthermore, several bromine species different from bromate or bromide were detected by IC-ICP/MS.

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Introduction

Bromate can be formed by the oxidation of bromide ions during ozonation and possibly by other oxidants in water treatment [1-4]. Bromate has been estimated as a potential carcinogen, and has been classified in Group 2B by the International Agency of Research on Cancer (IARC). The concentration of bromate in drinking water associated with an excess lifetime cancer risk of 10^{-5} corresponds to 3 µg/L [5]. The World Health Organization (WHO) recommended the provisional guideline value of 25 µg/L which is associated with an excess lifetime cancer risk of 7×10^{-5}, because of limitation in available analytical and treatment methods [5].

Ion chromatography (IC) with a pretreatment method [6] or an on-line preconcentration method [7-8] has been reported for the determination of trace bromate. However, the peak of bromate at the detection limit level will often vanish in that of chloride which is always present in water at a level of three orders of magnitude higher. The authors have developed a sensitive and selective ion chromatographic determination method of bromate with postcolumn conversion into tribromide by hydrobromic acid [9]. Sub-µg/L of bromate in water was determined by using the developed postcolumn derivatization. Furthermore, other disinfectant by-products such as chlorite and iodate were also detected with similar detection limits.

On the other hand, inductively coupled plasma mass spectrometry (ICP/MS) combined with liquid chromatography or IC (LC-ICP/MS or IC-ICP/MS) is an effective technique for the speciation study of metallic and organometallic species because of its element selectivity and sensitivity. The combined technique has been also applied to the determination of halogen species, especially, iodine that can be sensitively detected by ICP/MS [10-14].

In the present work, the specific determination of bromate, iodate and other halogen species in drinking water by direct injection using IC with ICP/MS and the postcolumn derivatization is described. The advantages of ICP/MS as an element selective detector was evaluated for bromate and iodate by considering the comparison with the postcolumn derivatization. Furthermore, the IC-ICP/MS system was applied to the simultaneous determination of halogen anions in raw and ozonized water.

Experimental

Reagents

All reagents used were purchased from Wako Pure Chemical Industries (Osaka, Japan). Stock solutions (1000 mg/L as elements) for each anion were prepared by dissolving with pure water and stored in refrigerator. Analytical solutions were prepared by diluting the stock solution to the required concentration just before use. Pure water was obtained from Milli-Q system (Nihon Millipore, Tokyo, Japan).

Instrument

Ion chromatograph used in this experiment was Model IC7000S (Yokogawa Analytical Systems Inc., Japan) equipped with a UV/VIS detector, and ICP/MS was Model 4500 (Agilent Technologies, Inc. USA). Excelpak ICS-A23 and ICS-A13 (7.6 mm x 4.6 mm i.d. each, Yokogawa Analytical Systems Inc.) were chosen as separation columns. ICS-A23 and ICS-A13 were packed with hydrophilic and semi-hydrophilic anion exchange resin with 0.05 mequiv./g of dry, respectively.

IC-ICP/MS

Ion chromatograph and ICP/MS were connected by 500 mm x 0.3 mm i.d. of ETFE tube. Ammonium carbonate was chosen as a mobile phase. Ammonium salt was used to prevent a salt deposition and clogging at sampling orifice of ICP/MS caused by sodium in a mobile phase. The operating conditions of ICP/MS are described in Table 1.

Postcolumn derivatization

Two Excelpak ICS-A13 columns in series were chosen to separate the halogen species according to the previous paper [9]. The operating conditions of the postcolumn derivatization are described in Table 2.

Results and Discussion

Separation of halogen anion

First of all, the separation of halogen anions using ICS-A13 as the separation column according to previous paper [9] was examined to establish appropriate separation conditions. The chromatography behaviour of iodide on anion-exchange resins has been described [15]. In this experiment, however, the peak of iodide showed a broad and tailing shape, while bromate, bromide and iodate showed good peak shapes. It was also noted that the retention time was long (more than 30 min) and depended on its concentration. It was not drastically improved in spite of a series of change of mobile phase.

RF power	1300 W
RF reflected power	<1 W
Plasma gas	16.0 L/min
Auxiliary gas	1.00 L/min
Carrier gas flow	1.06 L/min
Sampling depth	7.5 mm
Mass	79 amu (Br), 127 amu (I)
Integration time	0.5 sec
Number of scans	1

Table 1
Operational conditions of ICP-MS.

Ion Chromatography	
column	Excelpak ICS-A13 x 2
mobile phase	5 x 10^{-3} mol/L Na_2CO_3/1 x 10^{-3} mol/L $NaHCO_3$, 1.0 mL/min
column temp.	40 °C
injection volume	0.1 mL
Reagent preparation	
reagent	5 mg/L $NaNO_2$ in 0.5 mol/L NaBr, 1.0 mL/min
preparation reagent	0.75 mol/L H_2SO_4, 1.0 mL/min
cation hollow fiber	5 m
Postcolumn derivatization	
reaction coil	3 m x 0.5 mm i.d.
reaction temp.	40 °C
detection	UV-268 nm

Table 2
Operating conditions of postcolumn derivatization

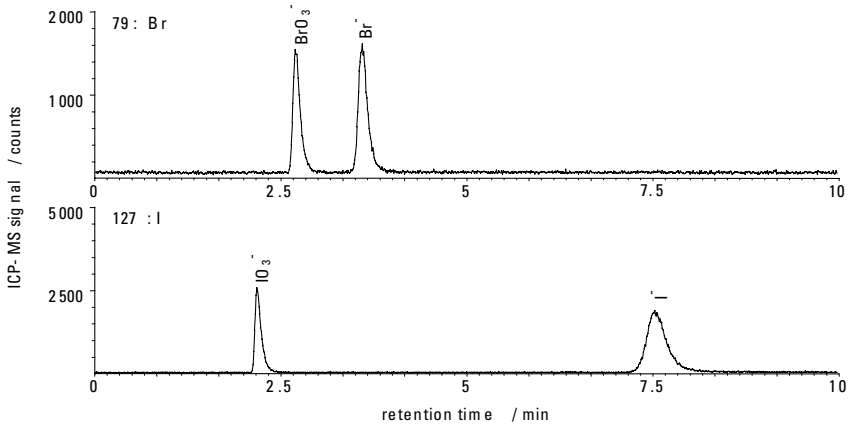


Fig. 1
Chromatograms of halogen anion standards by IC-ICP/MS. Peaks: BO_3 (10 µg/L), Br (10 µg/L), IO_3 (1 µg/L), and I (2 µg/L).
Experimental conditions: column, Excelpak ICS-A23; mobile phase, 0.03 mol/L $(NH_4)_2CO_3$; flow rate, 1.0 mL/min; column temperature, 40 °C; injection volume, 0.5 mL.

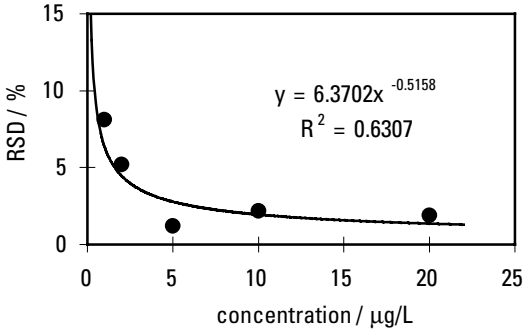
Therefore, ICS-A23 was used on behalf of ICS-A13 because of increase of hydrophilicity of packing materials. The use of the ICS-A23 with 0.03 mol/L ammonium carbonate solution (pH 9.2) made it possible to improve peak shape and retention time of iodide. Fig. 1 shows the chromatograms of halogen anion standards by direct injection with a 0.5 mL sample. Four halogen anions were completely separated within 8 minutes. The analytical time will be reduced by increasing the concentration or pH of mobile phase. For the purpose of this work, that is the simultaneous separation of many halogen species, these separation conditions were a compromise between the number of determinants and analytical time.

Evaluation of IC-ICP/MS

The linearity, detection limits and repeatability for bromate and iodate were determined. The linear range of bromate and iodate was more than 3 orders of magnitude, from 0.5 x 10^{-3} to 1 mg/L and from 0.1 x 10^{-3} to 1 mg/L, respectively. Equally, good linearity for bromide and iodide was also obtained. The detection limits (n = 3) for bromate, bromide, iodate and iodide were 0.45 µg/L, 0.44 µg/L 0.034 µg/L and 0.051 µg/L, respectively. The repeatability (n = 6) for 1.0 µg/L of bromate, 1.0 µg/L of bromide, 0.1 µg/L of iodate and 0.2 µg/L of iodide was 8.1 %, 8.0 %, 6.2 % and 6.8 %, respectively.

In the standard method for water quality, the quantitative limit is determined by the sample concentration which gives 10 % of relative standard deviation (RSD) [16]. The quantitative limits of this method were obtained from the

a) BrO_3^-



b) IO_3^-

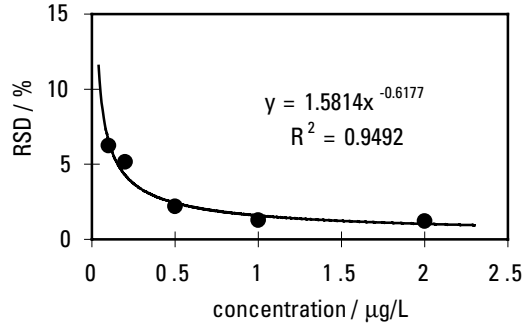


Fig. 2
Relationships between the RSD and sample concentration for bromate a) and iodate b). Experimental conditions are same as those given in Fig. 1.

RSD values which were calculated for each set of 10 measurements of bromate and iodate solutions at various concentrations.
Fig. 2 shows the relationships between the RSD and the sample concentration. The concentrations of bromate and iodate at 10 % of RSD were 0.42 µg/L and 0.051 µg/L, respectively. Fig. 2 also shows the signal stability in the concentrations which give sufficient sensitivity for bromate and iodate.
In both species, RSDs were saturated around 1% even in high concentrations. The saturated RSD is considered to be affected by ICP-MS stability.

Interference by coexistent substance
The interference from coexistent substances such as chloride, sulfate and nitrate has been reported by Creed and others[14]. They reported that bromate can be determined in a chloride matrix with 5-6 orders of magnitude higher. However, a retention time shift for bromate in 1000 mg/L of chloride matrix was observed. So, the interference from coexistent substances such fluoride, chloride, nitrite, phosphate and sulfate was examined. Mixed anion standard solutions ranging in concentration from 5 to 50 mg/L of anions were injected. Peaks of these anions were not observed on the chromatogram. The retention time shift for halogen anions in the concentration below 50 mg/L of anions matrix was not observed. However, one peak was observed at void volume in chromatogram of 79 amu. This peak was recognized to be a polyatomic ion ($^{40}Ar^{39}K+$) formed by combination of potassium in sample solution with argon as the plasma gas, because it appeared at the retention time of potassium that was observed in 39 amu. Conclusively, this peak due to potassium will be neglected on the determination of bromate because it is eluted at the void volume of the anion exchange column and completely separated from that of bromate under these separation conditions.

Application to the determination of halogen anions in the water
The presented method was applied to the determination of halogen anions in several water samples. The chromatograms of the ozonized water by using IC-ICP/MS and the postcolumn derivatization were shown in Fig. 3. The determined concentrations of halogen anions in raw (river) and ozonized water are listed in Table 3. The concentrations of halogen anions determined by both method were relatively in agreement. However, some iodate values obtained using ICP-MS were slightly higher than that of postcolumn derivatization. The disagreement could be due to lack of precision in such low concentration. Furthermore, there could be interference from other iodine-containing species coeluting with iodate, because ICP-MS would detect any species containing iodine, and it would give positive error in the iodate values. Bromate values by ICP-MS were a little lower than that of postcolumn method. The reason is

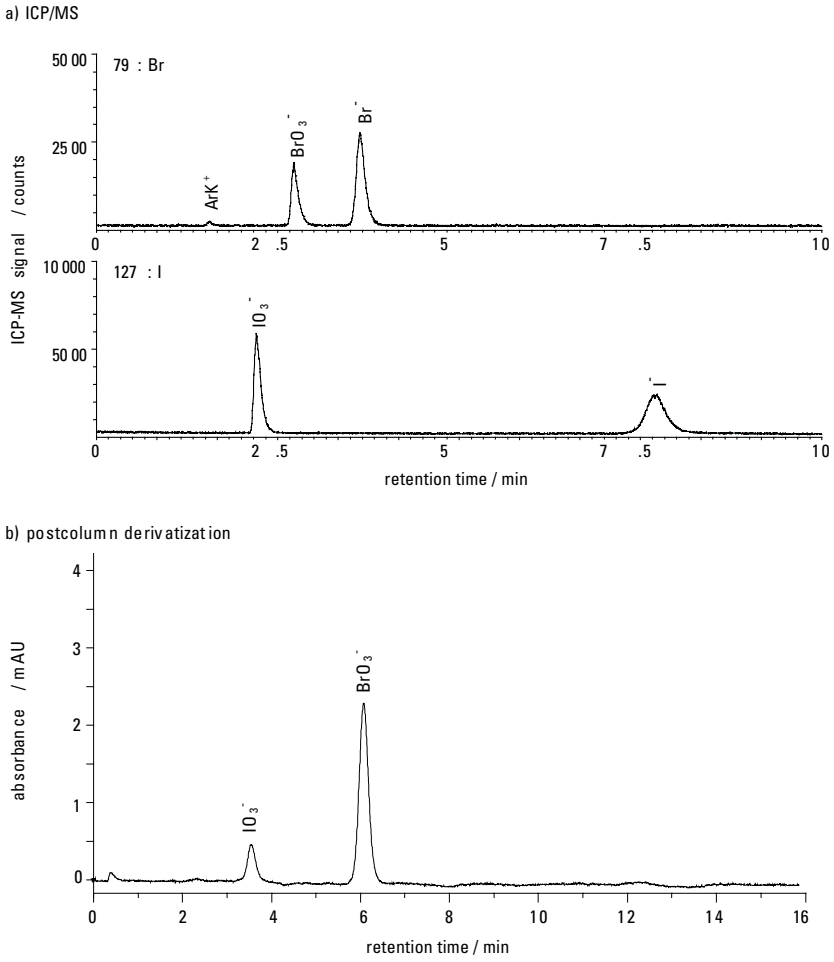


Fig. 3
Chromatograms of ozonized water (sample B) using IC-ICP/MS a) and postcolumn derivatization b). Peaks: a) BO_3 (13.0 µg/L), Br (17.8 µg/L), IO_3 (3.57 µg/L), and I (3.56 µg/L) b) IO_3 (4.13 µg/L), BO_3 (15.7 µg/L) Experimental conditions are same as those given in Fig. 1 and Table 2. Unit of the concentrations is µg/L as species.

	determined concentrations [µg/L as species]					
	IC–ICP–MS				postcolumn	
samples	BrO_3^-	Br^-	IO_3^-	I^-	BrO_3^-	IO_3^-
A. raw water	0.26	28.9	0.44	0.63	0.29	0.09
B. ozonized sample A	13.0	17.8	3.57	3.56	15.7	4.13
C. raw water	1.64	59.1	1.26	2.97	1.65	0.60
D. ozonized sample C	1.88	38.5	5.66	0.14	2.31	4.98
E. ozonized water	1.87	5.73	5.45	0.05	1.85	4.77

Table 3
Comparison of determined concentrations of halogen anions in raw and ozonized water. Experimental conditions are same as those given in Fig. 1 and Table 2.

not clear because bromate values in this postcolumn reaction procedure doesn't suffer from interference from other oxidants [9].

Bromate and iodate with the concentration of µg/L level were detected even in the raw water. Probably, the contamination of river water with trace bromate was caused by a waste water. On the other hand, the existence of iodate in mineral water has been also reported [17]. The concentrations of bromate and iodate in the ozonized water were rather increased than those in the corresponding raw water, while that of bromide was decreased by ozonation. Apparently, bromate and iodate will be formed during ozonation for the water treatment. However, the material balances of bromine and iodine were absolutely incompatible. These results suggest the halo-oxyacids are produced by oxidation of the corresponding halides, but that they are not always produced by the same mechanism.

Sample E gave a distinctive chromatogram at 79 amu (Fig. 4). Several unidentified species different from bromate or bromide were detected. These species are estimated as bromine compounds because no interferences from other elements are observed at 79 amu. The existence of other bromine species suggests that these species could lead to bromate during ozonation. It can also explain that the sum of bromate and bromide was not constant for ozonized water and its raw water (sample D and C) in Table 3. Furthermore, a large unidentified peak with a broad peak shape was also detected at the retention time of about 40 minutes in the chromatograms of ICP/MS at 127 amu. Heumann

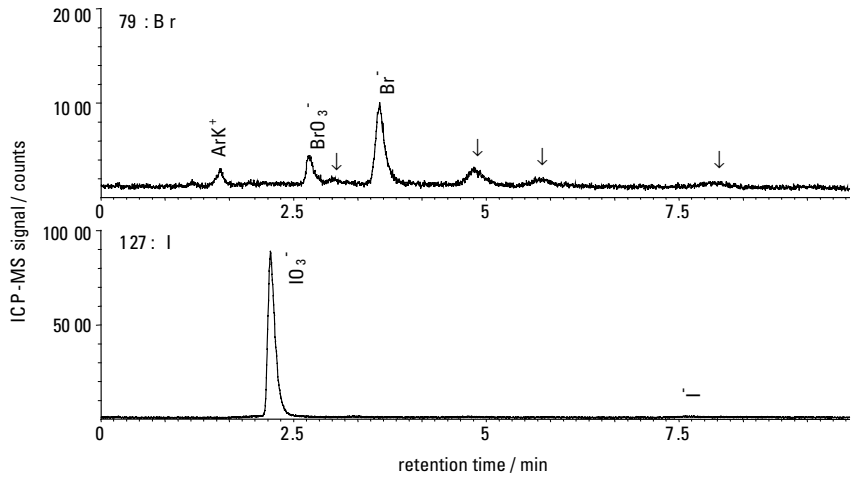


Fig. 4
Chromatograms of ozonized water (sample E). Peaks: BO_3 (1.87 µg/L), Br (5.73 µg/L), IO_3 (5.45 µg/L), and I (0.05 µg/L).
Experimental conditions are same as those given in Fig. 1. Unit of the concentrations is µg/L as species.

et al. reported that organoiodide exists in river water, because the peaks with exactly the same retention time were obtained in both chromatograms of ICP/MS and UV detector at 254 nm [13].

Therefore, the detection of these unidentified peaks by a simultaneous detection using ICP/MS and UV detector was examined. No peaks in the chromatogram of UV at 254 nm were observed at the retention times of these unidentified peaks in the chromatogram of ICP/MS. Furthermore, the retention behaviors of the unidentified peaks were evaluated by adding ethanol to the mobile phase. The retention times were drastically decreased as the concentration of ethanol increased. Clearly, these species were retained by their hydrophobicity, not ionicity. The elucidation of the unidentified peaks detected at 79 amu will be very difficult because of their lower amounts. The unidentified peak detected at 127 amu might be based on inorganic iodine rather than organoiodine but its chemical structure is not still determined. These unidentified peaks might be concerned in the production mechanism of the halo-oxyacids by the ozonation. A further detailed examination would be necessary to elucidate these unidentified peaks.

Conclusions

A specific determination for bromate, iodate and other halogen anions in drinking water by direct injection using IC with ICP/MS and the postcolumn derivatization is presented. Bromate and iodate in ozonized water were determined at the µg/L level without any interference from other anions. The sensitivity of the ICP/MS detector for halogens was also very high similar to that of metals and greater than that of other detectors for halogens. The proposed method will be effective for the simultaneous determination of halogen anions.

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Printed 4/2000
Publication number 5968-3049E

Agilent Technologies
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Meeting UK Drinking Water Inspectorate Requirements for Trace Metals Analysis Using the 4500 ICP-MS

Application Note

Environmental

Glenn D. Woods
Ed McCurdy

Abstract

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has gained wide acceptance for the determination of many trace elements, but is less frequently used for the determination of higher levels of elements, due to its perceived limitations in dynamic range and matrix tolerance.

Several applications require the measurement of trace and minor elements in the same sample, which generally means that laboratories must employ multiple techniques to perform a complete analysis. One such application is the measurement of inorganic components in drinking water, where the analyte concentrations that must be measured range from sub-ug/L (ppb) to 100's of mg/L (ppm). Traditionally, this analysis would have been carried out using a combination of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Graphite Furnace Atomic Absorption Spectrometry (GFAAS) and hydride and fluorescence techniques specifically for As, Se, Sb and Hg.

This paper discusses the validation of the 4500 ICP-MS ICP-MS for this analysis, allowing all of the controlled elements to be measured in a single run, using a single technique. Results are presented from the performance testing of the 4500 ICP-MS for the analysis of 26 trace and minor elements in Drinking Water, using the validation and quality control criteria defined in the NS-30 "A Manual on Analytical Quality Control for The Water Industry" by the UK Water Authority.

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Introduction

Inductively coupled plasma mass spectrometry (ICP-MS) has been used for the determination of many trace and minor elements in various samples from diverse fields including environmental, geological, metallurgical, semiconductor, petrochemical and biomedical. One of the principal benefits which has led to the widespread use of ICP-MS has been its wide elemental coverage and excellent detection limits for a range of elements which are impossible to measure by a single alternative technique. In particular, for the measurement of trace elements at ug/L and ng/L levels, ICP-MS has often been able to replace Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Graphite Furnace Atomic Absorption Spectrometry (GFAAS), hydride generation and fluorescence techniques, allowing all important toxic trace elements to be measured in a single analysis.

Typically, however, ICP-MS has been perceived as inappropriate for the determination of higher levels of analytes, due to limited matrix tolerance and excessive sensitivity, which limits the upper calibration level. Although most ICP-MS instruments have the capability to measure over a wide dynamic range (8 orders of magnitude), this usually requires regular and time consuming cross calibration across two detector modes and still allows measurement only up to a few 10's mg/L.

(1) Drinking Water Quality

In the United Kingdom, drinking water quality is monitored by the Drinking Water Inspectorate (DWI), using the Water Supply (Water Quality) Regulations of 1989. These regulations list a total of 56 parameters, including colour, turbidity, organic compounds, anions and inorganic elements, which must be monitored in drinking water supplies. The list includes 21 inorganic elements, ranging from Ca (maximum concentration or Prescribed Concentration Value (PCV) of 250 mg/l) to Hg (PCV of 1 ug/L). The regulation requires that the detection limit (calculated from 4.65 x the standard deviation of the blank) for each determinand must be less than one tenth of the PCV, so the detection limit of Hg, for example, must be less than 0.1 ug/L.

Under the Drinking Water Regulations, each laboratory is required to performance test the analytical systems for each parameter before that analytical system can be used for routine analysis of compliance samples. The design of the performance testing and calculation of the performance characteristics should be in accordance with the guidance given in the publication "NS-30", a manual on analytical quality control in the Water Industry.

(2) Requirements for Acquisition of Performance Testing Data

Taking into account the DWI requirements and NS-30 guidelines, the following protocol for performance testing is typical for metals analysis:

1) The calibration range should be such that all results fall within the range.

2) The calibration must have at least 3 points plus blank, to demonstrate a straight line.

3) Samples and standards must be prepared fresh, before each batch.

4) A maximum of 2 batches can be analysed on any one day, provided the instrument is switched to overnight conditions between batches.

5) Samples must be analyzed in random order.

6) Samples must be analysed in replicate, in at least 5 batches. In practice, analysis of duplicate samples in 11 batches satisfies the DWI condition on degrees of freedom.

7) A batch of samples must consist of the following:
Blank, Standards (typically at concentrations appropriate to the PCV and the levels found in representative samples) and Samples of the type to be measured routinely.

(3) Requirements for Statistical Validation

After acquiring the concentration data, the results must satisfy the following QC criteria:

1) The maximum tolerable error of individual results should not exceed 1/10 of the PCV or 20% of the result, whichever is the greater.

2) The maximum tolerable standard deviation of individual results should not exceed 1/40 of the PCV or 5% of the result, whichever is the greater.

3) The maximum tolerable systematic error (or bias) of individual results should not exceed 1/20 of the PCV or 10% of the result, whichever is the greater.

4) The estimates of total standard deviation must not be significantly greater at the 95% confidence level than the specified maximum tolerable total standard deviation at the relevant concentration.

5) The recovery of an added spike should not be significantly less than 95%, or significantly greater than 105%.

6) The limit of detection must be lower than 1/10 of the PCV.

The performance testing protocol described in NS-30 validates not only an analytical instrument, but also the entire laboratory protocol. If any aspect of sample or calibration standard preparation is not reproducible, then the error will be observed in the between batch variation. For this reason, it is essential that sample and standard preparation techniques are well developed and carried out reproducibly.

In a large analyte suite, there may be several issues that must be addressed regarding element stability, compatibility and cross-contamination, in addition to straightforward issues of the selection of appropriate glass/plasticware to avoid element leaching or adsorption. As Hg was one of the required analytes, Au was added to the standards and samples at 100 ug/L final concentration to stabilize Hg. In the absence of Au, the Hg signal is found to be unstable and exhibits extended washout times. The internal standard (IS) mixture, which contained Be, Sc, Y, In, Tb and Tl, was added to the standards and samples automatically by means of the on-line IS addition system.

The 4500 ICP-MS was validated for the 21 controlled elements (B, Na, Mg, Al, P, K, Ca, Cr, Fe, Mn, Ni, Cu, Zn, As, Se, Ag, Cd, Sb, Ba, Hg and Pb) in drinking water, according to the NS-30 protocol. At the same time, the 4500 ICP-MS was also validated for a further 5 elements (Li, V, Co, Sr and Sn). Whilst NS-30 lists 21 inorganic components which must be monitored, additional elements may also be measured, provided that the NS-30 protocol has been followed and validation requirements have been met. Thus, each lab can extend the validated elemental range of the technique to meet their own needs and also their customer's specific requirements.

Instrumentation

The ICP-MS instrument used was a standard Hewlett-Packard 4500 ICP-MS, in conjunction with a Cetac ASX-500 random access autosampler. The 4500 ICP-MS was configured with the standard sample introduction system, which consists of a Agilent High Solids nebuliser, quartz spray chamber, quartz one-piece torch and Ni sample and skimmer cones. The standard 4500 ICP-MS sample introduction system is ideally suited to the analysis of high-matrix environmental samples, as discussed below:

• Low Sample Uptake Rate

The sample uptake rate of the 4500 ICP-MS is only 0.1 to 0.4mL/min, compared to between 0.8mL/min and 2.5mL/min, which is typical for conventional ICP-MS or ICP-OES instrumentation. This lower solution flow rate means that matrix loading on the plasma is minimised. In turn, this means that the plasma can dry, decompose, dissociate, atomise and ionise the sample analytes and matrix more efficiently, resulting in reduced spectral interferences and reduced sample matrix effects.

The matrix tolerance capabilities of the Babington-type nebuliser are well known, but commercially available versions of these nebulisers tend to require very high solution flow rates and can be prone to pulsing and poor washout. The Agilent High Solids nebuliser is a modified Babington-type design, manufactured for the 4500 ICP-MS. It features a wide, square section groove cut into an angled front face and optimised to produce a stable aerosol at low sample uptake rates. The design of the groove ensures that no sample solution is trapped on the nebuliser face, which in turn prevents spiking during washout due to sample re-nebulisation.

• Low Polyatomic Ion Formation

Some of the most troublesome interferences in ICP-MS are caused by the overlap of polyatomic ions formed from combinations of oxygen with the argon carrier gas or matrix ions. If the sample introduction area, in particular the spray chamber, is maintained at a constant low temperature (0-2 °C), the water vapour loading in the sample aerosol can be reduced and so the cooling effect of the aerosol on the plasma is reduced. This results in a higher plasma temperature and gives more efficient breakdown of oxide species.

The normal method for monitoring the likely impact of oxide overlaps in the ICP-MS spectrum is by measuring cerium. Of all elements,

cerium has one of the highest metal-oxide (MO) bond strengths. The CeO/Ce ratio can therefore be used as a "worst-case" indicator of the likely interference problems an ICP-MS instrument will suffer in real sample analyses, where high levels of elements such as S, Cl, Al, Mg, Ca, etc. (all of which will form oxide species at much lower levels than Ce) might be encountered.

On the 4500 ICP-MS, the typical CeO/Ce ratio is <0.5%, partly due to the use of a cooled spray chamber, but also as a result of the use of a wide bore injector (2.5mm diameter) in the plasma torch. The wide injector diameter ensures that the sample aerosol is relatively diffuse in the central channel of the torch and so the plasma energy can decompose the sample matrix more efficiently, breaking up any refractory oxide species. Furthermore, the wide torch injector reduces gas velocity through the central channel giving a longer sample residence time in the plasma, which also assists matrix oxide decomposition.

• Linear Calibration

In order to determine high and low level elements in the same run, an ICP-MS instrument should have a wide dynamic range. Most ICP-MS instruments achieve this using a detector that can be operated in both pulse-count and analog mode for the measurement of low intensity and high intensity signals respectively. The 4500 ICP-MS has the capability to construct a single linear calibration line for all elements, from ng/L to 100's mg/L levels, without regular adjustment of detector or tuning parameters. The cross-calibration of the 2 detector modes is achieved using a single solution, analysed once, and the calibration is stable over long periods of analysis.

Standard and Sample Preparation

Calibration stock standards were obtained from BDH and a series of working stock solutions was prepared, each stock containing compatible groups of the analytes. Ca, Na, Mg, K, Al, Fe, Cu and Zn were prepared from 10,000 mg/L stock solutions while the other elements were prepared from 1,000 mg/L single element stocks. Calibration standard solutions were prepared at concentrations appropriate to the levels normally found in the sample types to be tested. Hg and Se were prepared in a separate stock from the other elements. In order to analyze Ag, all samples and calibration standards were prepared in 1% v/v HNO3 and 0.5% v/v HCl solution.

Two tap waters, one river derived and the other borehole derived, were analyzed as samples and two Blanks, two Analytical Quality Control (AQC) solutions and two Spiked solutions were prepared. Two vials were prepared for each blank, sample, spike and AQC. All the standard and sample solutions were freshly prepared for each batch.

The Internal Standard solution, which contained Be, Sc, Y, In, Tb and Tl, was added to the samples and standards by means of the on-line IS addition system of the 4500 ICP-MS. After on-line dilution in the sample stream, the final concentration of the internal standards was approximately 0.1mg/L, with the exception of Be, which was 10x higher to compensate for its low degree of ionisation.

Automatic setup of the pulse count/analog (P/A) factor of the detector was carried out using a tuning solution which contained Ca, B, P, Fe, Ba, Na, Mg, Al, K, Cu Zn and Sr at concentrations between 0.1mg/L and 100mg/L. The appropriate concentration for each element was selected, to give an acceptable count-rate in both detector modes.

Experimental

(1) Instrumental Conditions

After turning on the plasma and allowing 15 minutes for the system to warm up, the instrument was tuned by using 3 of the elements present in the internal standard solution (Be, Sc and Tl).
The instrument was tuned by monitoring mass 9, 45 and 205, to give a sensitivity of around 200,000, 200,000 and 300,000 counts per second, respectively. This represents at least a factor of 10 lower sensitivity than can be achieved when the instrument is tuned for maximum sensitivity. i.e. the system was "detuned". With a system tuned for maximum sensitivity, the higher level analytes such as Na and K would be "over-range", i.e. above the maximum measurable concentration of the instrument.

For the successful analysis of environmental samples by ICP-MS, several sample introduction and plasma parameters must be considered, namely RF power, carrier gas flow, sample uptake rate and sampling depth. Higher plasma temperature and longer residence time of the analytes are critical parameters in order to decompose heavy matrices effectively and to minimize oxide formation. Table 1 shows the parameters used for routine

Parameter	Setting
Forward power	1350W
Peri-pump speed (analysis)	0.1 rps
Peri-pump speed (uptake/rinse)	0.3 rps
Sampling depth	8.5 mm
Carrier gas flow	1.25 L/min
Rinse time	30 sec
Acquisition Time	73 sec
Number of repeats	3

Table 1
4500 ICP-MS Operating Conditions for the Analysis of High Matrix Samples

1. Blank 1	13. Tap water A + Spike (L) 1
2. Blank 2	14. Tap water A + Spike (L) Hg/Se 1
3. AQC (L) 1	15. Tap water A + Spike (L) 2
4. AQC (L) Hg/Se 1	16. Tap water A + Spike (L) Hg/Se 2
5. AQC (L) 2	17. Tap water B 1
6. AQC (L) Hg/Se 2	18. Tap water B 2
7. AQC (H) 1	19. Tap water B + Spike (H) 1
8. AQC (H) Hg/Se 1	20. Tap water B + Spike (H) Hg/Se 1
9. AQC (H) 2	21. Tap water B + Spike (H) 2
10. AQC (H) Hg/Se 2	22. Tap water B + Spike (H) Hg/Se 2
11. Tap water A 1	23. Drift
12. Tap water A 2	24. Drift Hg/Se

analysis of environmental samples using the 4500 ICP-MS.

(2) Sample Analysis

Each analytical batch consisted of the blanks, samples, spikes and AQC's shown below. Following analysis of the calibration standards, the sample batch was analysed in random order. A different random order was used for each batch, ensuring that no bias was introduced by running the test solutions in a constant order. Drift check solutions, which do not form part of the validation sequence, were analyzed at the end of each batch.

The analysis of Hg and Se was separated from the other elements for two reasons:

1) Following successful validation of the method, the mixed calibration standards should be stable for a week. However, Hg would not be expected to be stable over this period, so the Hg standards would need to be prepared fresh daily.

2) The Ca standard solution contained a small amount of Se. This contamination introduced a bias into the Se calibration at low level, so Se was calibrated using a standard mix that contained no Ca.

With regard to Fe, As and V, which are considered difficult elements to analyse by ICP-MS due to polyatomic overlaps from ArN, ArCl and ClO respectively, an interference correction equation was used to correct for background contributions from these polyatomics. Although HNO3 and HCl were intentionally added to all samples in order to stabilise the solutions and allow the analysis of Ag, interference correction worked well and excellent results were obtained for all of these elements.

Results and Discussion

(1) Calibration Curves

Figures 1 shows the calibration curves of some of the major elements, determined at concentrations up to 300mg/L. Figure 2 shows calibrations for some of the trace elements, calibrated at low ug/L levels. Linear calibrations were obtained in all cases.

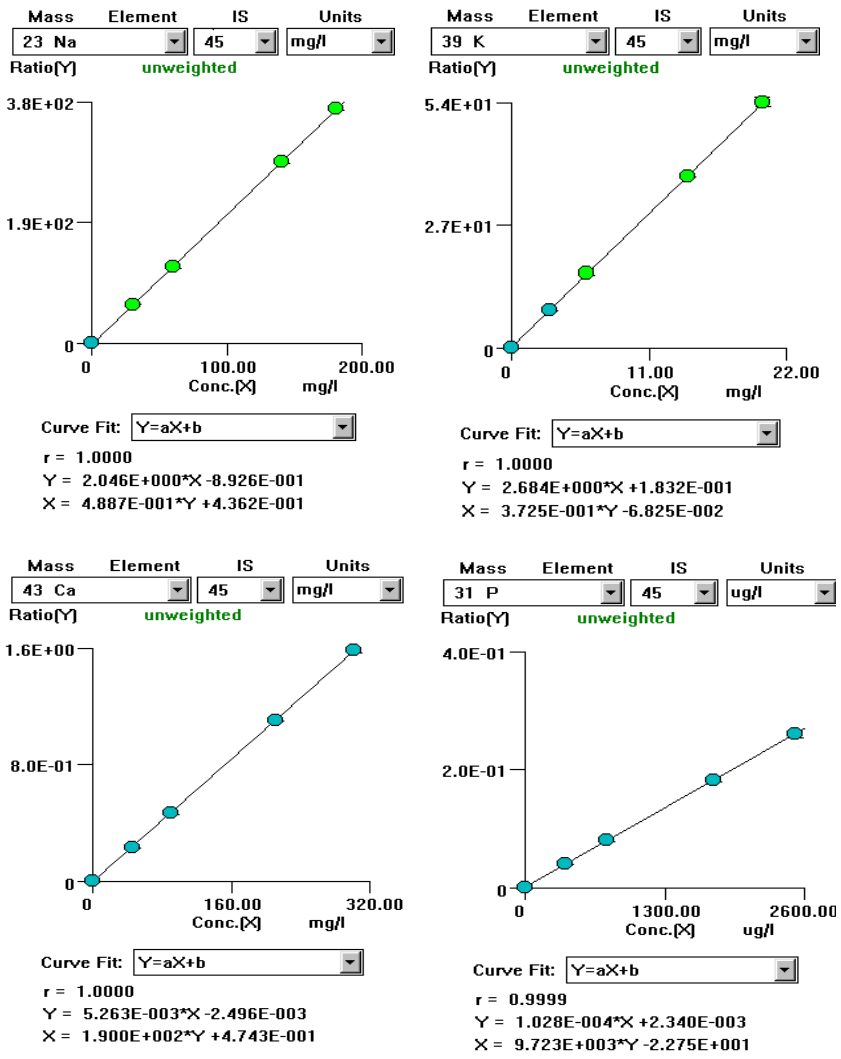


Figure 1
Calibrations for Selected Major Elements

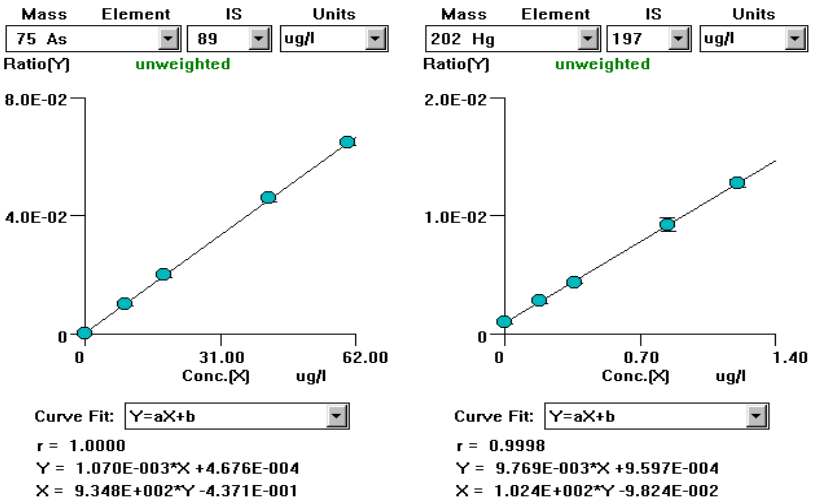


Figure 2
Calibrations for Selected Trace Elements

(2) NS-30 Performance Testing Results

Once quantitative results had been obtained from all of the batches, the statistical processing defined in NS-30 was carried out. Tables 2 and 3 show the calculated results of one high and one low concentration element found in the tap water samples (Na and Hg respectively)

Na (23) IS – Sc	Blank	AQC(L)	AQC(H)	Sample 1	Spike 1	Sample 2	Spike 2
Mean (mg/L)	-0.7005	53.0727	123.1818	34.0273	155.954	44.0409	95.8818
M1	6.0556	16.3264	32.9273	8.1404	67.2455	9.9548	19.7033
M0	0.0002	2.6527	13.0909	0.7782	10.5909	1.4223	5.8182
F value	26122.086	6.1546	2.5153	10.4607	6.3494	6.9992	3.3865
Significant	p=0.001	p=0.01	NS	p=0.001	p=0.01	p=0.01	p=0.05
Sw	0.0152	1.6287	3.6181	0.8821	3.2544	1.1926	2.4121
Sb	1.7400	2.6147	3.1493	1.9186	5.3223	2.0655	2.6349
St	1.7401	3.0805	4.7968	2.1117	6.2384	2.3851	3.5722
F 0.05	1.8307	1.7202	1.6228	1.7522	1.7202	1.7202	1.6435
Calc. F	0.2153	0.6748	0.6065	0.3171	0.6401	0.4045	0.5552
Degree F	10	13	17	12	13	13	16
Bias OK?		*Pass*	*Pass*				
SD OK ?		*Pass*	*Pass*	*Pass*	*Pass*	*Pass*	*Pass*
Recovery		107.55	103.24		101.61		103.68
95% Conf. Limits		2.83	1.55		2.48		2.79
Recovery OK ?					*Pass*		Pass
Limit of Detection	0.077 mg/L						
LOD OK?	*Pass*						

Table 2
NS-30 Performance Test Results for Na

Hg (202) IS – Au	Blank	AQC(L)	AQC(H)	Sample 1	Spike 1	Sample 2	Spike 2
Mean (mg/L)	-0.0011	0.2121	1.0650	0.0105	1.0623	0.0138	0.2295
M1	0.0001	0.0004	0.0011	0.0001	0.0012	0.0002	0.0004
M0	0.0002	0.0003	0.0006	0.0001	0.0009	0.0002	0.0007
F value	1.2233	1.4911	1.8473	2.3750	1.4631	1.2303	1.8405
Significant	NS	NS	NS	NS	NS	NS	NS
Sw	0.0127	0.0163	0.0244	0.0077	0.0292	0.0134	0.0260
Sb	0.0000	0.0081	0.0159	0.0064	0.0140	0.0045	0.0000
St	0.0127	0.0182	0.0291	0.0100	0.0324	0.0141	0.0260
F 0.05	1.5558	1.5705	1.5865	1.6228	1.5705	1.5558	1.5705
Calc. F	0.2562	0.5278	0.2990	0.1589	0.3711	0.3194	1.0840
Degree F	21	20	19	17	20	21	20
Bias OK?		*Pass*	*Pass*				
SD OK ?		*Pass*	*Pass*	*Pass*	*Pass*	*Pass*	*Pass*
Recovery		106.61	106.61		105.18		107.841
95% Conf. Limits		3.57	1.23		1.07		3.4205
Recovery OK ?					*Pass*		Pass
Limit of Detection	0.064 ug/L						
LOD OK?	*Pass*						

Table 3
NS-30 Performance Test Results for Hg

Tables 4 and 5 show the summary results of all elements. In every case, all of the statistical analysis indicated that the 4500 ICP-MS gave acceptable results under the requirements of the protocol defined in NS-30.

Elements	PCV	Units	LOD	AQC	Recovery	Spike	Recovery	95% SD
Li		ug/L	0.328	40	102.52	40	100.00	2.48
B	2000	ug/L	99.453	400	104.41	400	91.34	4.50
Na	150	mg/L	0.077	50	106.15	50	103.68	2.79
Mg	50	mg/L	0.064	10	102.77	10	105.42	2.43
Al	200	ug/L	1.562	200	102.34	200	100.68	2.06
P	2200	ug/L	4.524	500	97.07	500	98.34	3.44
K	12	mg/L	0.021	6	104.97	6	98.91	1.56
Ca	250	mg/L	0.338	100	103.32	100	97.73	2.70
V		ug/L	0.606	10	101.09	10	100.10	2.74
Cr	50	ug/L	1.332	10	104.09	10	98.46	3.64
Fe	200	ug/L	14.692	200	98.84	200	98.94	2.90
Mn	50	ug/L	0.900	50	100.45	50	101.69	2.40
Co		ug/L	0.052	10	102.36	10	105.24	1.02
Ni	50	ug/L	0.869	10	108.91	10	105.58	2.05
Cu	3000	ug/L	1.173	500	101.78	500	100.60	0.71
Zn	5000	ug/L	2.632	500	107.12	500	102.08	1.34
As	50	ug/L	1.067	10	93.13	10	98.51	2.49
Se	10	ug/L	0.837	2	102.77	2	108.95	5.96
Sr		ug/L	1.062	120	104.47	120	90.34	3.29
Ag	10	ug/L	0.055	3	102.47	3	100.17	2.09
Cd	5	ug/L	0.130	1	105.91	1	104.59	3.29
Sn		ug/L	0.107	10	102.86	10	103.53	1.88
Sb	10	ug/L	0.032	2	100.73	2	101.46	1.20
Ba	1000	ug/L	1.029	200	101.93	200	101.03	1.15
Hg	1	ug/L	0.064	0.2	106.05	0.2	107.84	3.42
Pb	50	ug/L	0.130	50	100.52	50	100.00	1.23

Table 4
NS-30 Performance Test Summary Results For All Elements Low AQC & Spike

Elements	PCV	Units	AQC	Recovery	Spike	Recovery	95% SD
Li		ug/L	180	101.14	180	100.15	2.02
B	2000	ug/L	2000	97.77	2000	97.40	1.85
Na	150	mg/L	120	102.65	120	101.61	2.48
Mg	50	mg/L	50	103.95	50	103.80	2.38
Al	200	ug/L	1800	99.81	1800	100.53	1.41
P	2200	ug/L	2200	98.74	2200	103.53	1.70
K	12	mg/L	12	103.97	12	101.31	1.26
Ca	250	mg/L	250	100.96	250	93.62	1.20
V		ug/L	50	98.84	50	97.70	2.26
Cr	50	ug/L	50	99.78	50	96.95	2.36
Fe	200	ug/L	1600	97.78	1600	95.99	2.94
Mn	50	ug/L	120	99.13	120	99.78	1.88
Co		ug/L	50	98.87	50	98.52	0.92
Ni	50	ug/L	50	101.14	50	98.76	1.45
Cu	3000	ug/L	3000	99.73	3000	98.17	0.90
Zn	5000	ug/L	5000	100.72	5000	98.47	1.36
As	50	ug/L	50	93.36	50	96.84	2.38
Se	10	ug/L	10	100.73	10	105.16	3.71
Sr		ug/L	600	100.48	600	94.38	0.76
Ag	10	ug/L	10	103.91	10	99.38	1.63
Cd	5	ug/L	5	103.58	5	102.52	1.71
Sn		ug/L	50	102.88	50	103.04	2.11
Sb	10	ug/L	10	100.50	10	100.65	0.61
Ba	1000	ug/L	1000	100.23	1000	98.48	1.08
Hg	1	ug/L	1	106.50	1	105.18	1.07
Pb	50	ug/L	90	99.70	90	100.26	1.12

Table 4
NS-30 Performance Test Summary Results For All Elements High AQC & Spike

Conclusions

The 4500 ICP-MS was applied to the analysis of drinking water, following the methodology defined in NS-30. Linear calibrations were obtained for trace elements at low and sub-ug/L levels, in the same acquisition as the major elements at 100's mg/L.

All QC criteria were met, and the system was validated for 21 NS-30 elements plus an additional 5 elements. Operating in a reduced sensitivity mode allowed for the measurement of high concentration elements, such as Na and Ca, while the wide dynamic range of the instrument still allowed detection limit criteria for trace elements such as Hg to be met.

Printed 4/2000
Publication number 5968-7602E

Agilent Technologies
Innovating the HP Way

New ASTM Standard:

Recommended operating conditions for the Agilent Capillary Electrophoresis system

Maria Serwe

Environmental

Abstract

ASTM Subcommittee D19.05 on Inorganic Constituents in Water approved a new standard test method for determination of dissolved inorganic anions in aqueous matrices using capillary ion electrophoresis and chromate electrolyte[1]. The Agilent Capillary Electrophoresis system provided equivalent performance during the inter-laboratory study preceding approval (c/w sect. 17.6 in test method). This document (reference B1.16 in test method) describes equivalent method parameters specific for the Agilent system equipped with DAD detection and computer control through Agilent ChemStation.

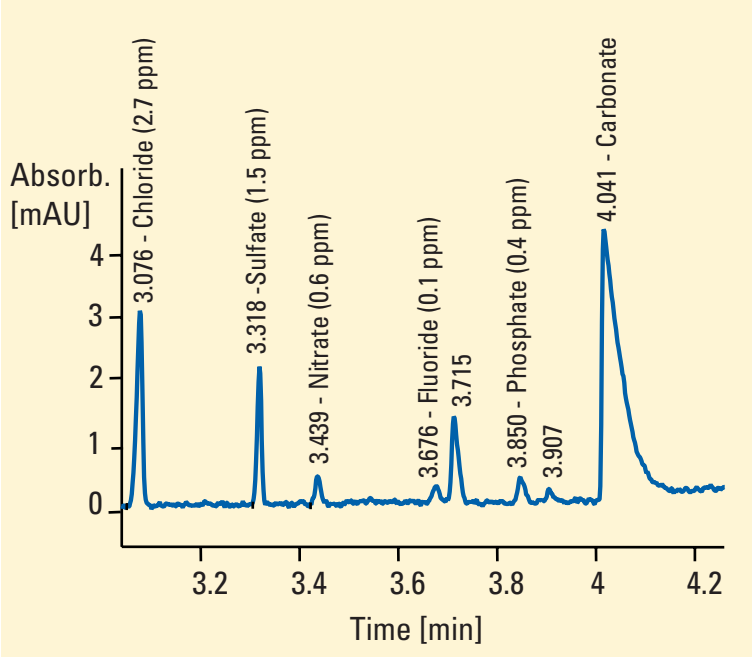


Figure 1
Analysis of waste water from a municipal waste treatment plant

Method Entries

Lift offset	4 mm
Cassette temp.	25° C
Preconditioning	
flush 1.1 min from flush buffer vial into waste vial	
Electric	on
Polarity	negative
Voltage	system limit
Current	0.00 µA
Power	system limit
Low current limit	0.00 µA
Time table	
0.3 min, current = 14.00 µA	
Injection	
by pressure, 50 mbar x 6.2 sec (37 nl)	
UV-detection	
Signal = 470/50 nm, reference = 275/10 nm, response time = 0.2 sec (PW > 0.01 min)	
Integration	
peak top type = center of gravity	
Calibration	
calculate with corrected areas	

Method parameters
The parameters described here are supplementary to the test method (see also reference 2).

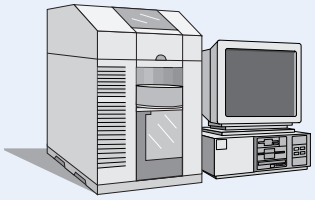
Capillary
Standard bare fused silica capillary (L = 64.5 cm, l = 56 cm, 75 µm id), fitted with a blue alignment interface. A new capillary is prepared by flushing 0.5 N NaOH for 5 min, water for 1 min and run buffer for 3 min (at 1 bar). If the current on a new capillary must be tested (c/w sect. 11.4), a voltage of 18.5 kV should be applied. If the system is idle overnight, leave the capillary in buffer. For long-term storage flush the capillary with water followed by air.

Vials
2-mL glass vials with polyurethane caps are used as buffer or waste container. 1-mL capped polypropylene vials are used as sample container. The buffer vials (inlet, outlet and flush buffer vial) are filled to 1 mL, the waste vial is filled with 0.6 mL buffer. For best migration time stability the run buffer vials should be replaced after 10 runs. It is not recommended to use the replenishment system with the Waters IonSelect™ High Mobility Anion Electrolyte.

Sample preparation
The waste water samples were diluted (1:20) and filtered through a 0.45 µm filter prior to injection.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent ChemStation



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1. D6508-00 (2000)

2. M. Serwe and J. Krol, "Determination of Dissolved Inorganic Anions in Aqueous Matrices Using Capillary Ion Electrophoresis and Chromate Electrolyte" Poster presentation at HPCE 2000 in Saarbrücken, Germany.

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Released 03/2000
Publication Number 5968-8660E

Agilent Technologies
Innovating the HP Way

Analysis of Arsenic, Selenium and Antimony in Seawater by Continuous-Flow Hydride ICP-MS with ISIS

Application Note

ICP-MS

Environmental

Steve Wilbur

Analysis of arsenic and selenium in seawater at trace levels presents a number of challenges. While ICP-MS is generally considered to be a highly sensitive, interference free technique for analysis of trace metals in environmental samples, matrix effects can result in unacceptably high detection limits for these two elements. These matrix effects are based on two phenomena; 1) ionization suppression in the plasma of high ionization energy elements such as As (9.81 EV) and Se (9.75 EV) in the presence of a significant excess of easily ionizable elements such as Na (5.14 EV) and 2) spectral interferences by argon based polyatomic species such as ArCl and ArAr. For example, ArCl interferes with the only isotope of arsenic and all the significant selenium isotopes suffer from polyatomic interferences of Ar, Cl, or Br. Optimum sensitivity therefore requires some mechanism for separating the analyte from the matrix and reducing or eliminating the argon based polyatomic species. Since arsenic, selenium and a number of other elements (Sb, Te, Bi, Ge, Sn, Pb) are known to form gaseous hydrides under specific reducing conditions, these elements can be removed from the matrix (for example the Na and Ca) and analyzed as gasses in a flowing stream of argon. Reduction or elimination of argon polyatomics can be achieved in the Agilent 4500 or 7500 ICP-MS systems through the use of the ShieldTorch™ and cooler plasma conditions. As a result, by combining hydride generation with cool plasma/ShieldTorch, it is possible to lower the background equivalent concentrations for all As and Se isotopes to low ppb to mid ppt levels in seawater samples.

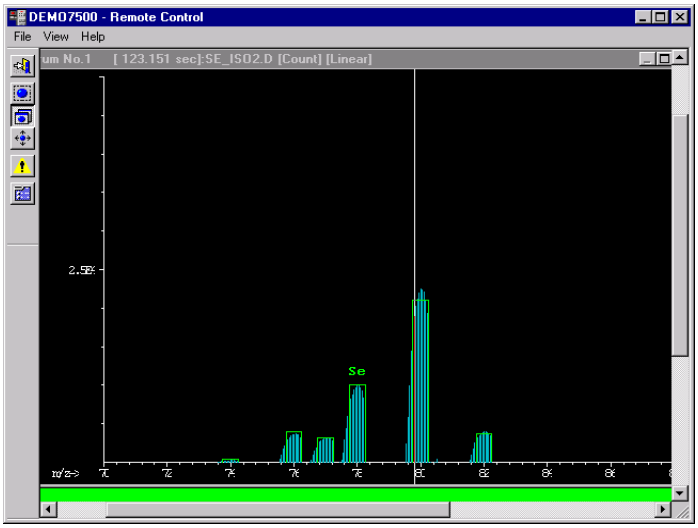

Figure 1. Full-scan Mass Spectrum of Selenium (20 ppb) Showing Excellent Agreement with Expected Isotope Ratios.

Table 1. Common Interferences on As and Se by Normal Plasma, Direct Nebulization ICP-MS

Selenium Isotopes	% Abundance	Major Interferent(s)	% Abundance of Interferent Mass(s)
74	0.89	Ge	35.94
76	9.36	ArAr	0.671
77	7.63	ArCl	24.13
78	23.78	ArAr	0.125
80	49.61	ArAr, BrH	99.202, 50.682
82	8.73	Kr, BrH	11.6, 49.303
Arsenic Isotope			
75	100	ArCl, CaCl	75.48, 43.45

Reaction Chemistry:

For optimum sensitivity, accuracy and precision, both As and Se must be pre-reduced to the most efficient oxidation state for hydride formation. This is achieved through the use of a pre-reduction step. In the case of Se, pre-reduction to the +IV state can be achieved by the use of HCl plus heat. Arsenic requires a stronger reducing environment, in this case a solution of KI plus ascorbic acid is used to reduce As to the desired +III state.

Standards and Reagents:

Tune solution:

20 ppb solution of Se or As pre-reduced as follows.

Pre-reductant Stock for As and Sb (KI + ascorbic acid)

Dissolve 5 grams each KI and ascorbic acid in 100 mL DI water in a polyethylene bottle. Cap and shake to dissolve solids.

Reductant ($NaBH_4$ solution)

Weigh 0.5 g high-purity NaBH4 and 0.125 g NaOH into a 250 mL polyethylene bottle. Bring to volume (250 mL), cap and shake to dissolve solids. Prepare fresh daily.

Calibration standards:

While plasma matrix effects are all but eliminated by using hydride generation, the efficiency of the pre-reduction and reduction steps can be affected by matrix. Therefore, best results will be obtained using matrix-matched standards. In the case of seawater, calibration by method of standard additions gives good results. The standard addition calibration can then be converted to an external standard calibration for analysis of subsequent seawater samples. Replicate 10 mL aliquots of CASS 3 or NASS 5 [1]were spiked with a multi-element calibration stock containing selenium and pre-reduced as described below. Spike levels were 0, 0.01, 0.05, 0.1, 0.5, 1.0 and 5 ppb.

Pre-reduction of samples and standards:

- Arsenic (Antimony and Bismuth)

10 mL of sample (seawater) is added to a 50 ml polypropylene centrifuge tube. 1 ml of the KI/ascorbic acid pre-reduction reagent is added with swirling. 3 mL of concentrated tracemetal grade HCl is added with swirling. The tube is capped loosely and allowed to set for 15 minutes after which it is brought to a final volume of 25 ml with 18 MOhm deionized water.

- Selenium (and Telurium)

10 mL of sample is added to a 50 mL polypropylene centrifuge tube. 10 mL of concentrated, tracemetal grade HCl is slowly added with swirling. The tube is loosely capped and heated in a heat block or boiling water bath at 100 degrees C. for 10 minutes. After allowing to cool, the sample is brought to 25 mL final volume with 18 Mohm DI water.

[1] *National Research Council, Canada*

Tuning

Optimized tuning involves maximizing the analyte signal(s) while minimizing the interferences. Since the interferences are primarily due to argon-based polyatomics, the use of ShieldTorch with slightly reduced forward power to minimize the ionization of Ar is quite effective. The following conditions were set to allow the measurement of Se at m/z 78 or 80 and also work quite well for As.

Basically, a combination of forward power, sample gas flow (carrier plus makeup), and sample depth which minimizes m/z 78 and 80 in the blank and maximizes those masses in the tune solution (20 ppb Se, pre-reduced as described) is desired.

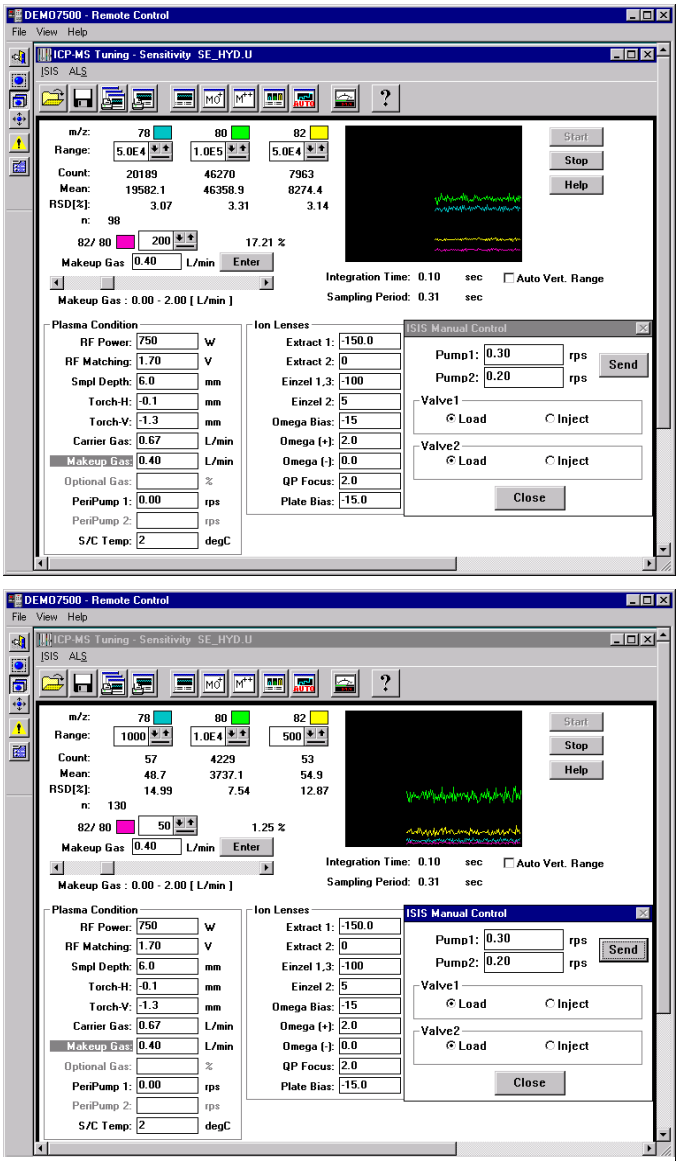

Figure 2. Tune Screens, 20 ppb Se Standard and Prep Blank

As a first step, while aspirating a prep blank under hydride generating conditions, try to reduce the background at m/z = 80 to 10-20,000 counts per 0.1 sec. This is accomplished by cooling the plasma using a combination of RF power and carrier/makeup gas. See figure 2 for sample tune condiitions. Some of the background may be due to trace Se in the reagents used for pre-reduction or hydride formation. Now aspirate the 20 ppb Se tuning solution. Set the acquisition masses at 78, 80 and 82. Set the displayed ion ratio to calculate the ratio of 82 to 80. The natural isotope ratio of Se82 to Se80 is 8.73/49.61 or 17.59%. Therefore as the displayed ratio approaches 17.6%, the background at m/z 80 due to ArAr is minimized. Try to maximize the signal at 78 while maintaining as close to 17.6% for 82/80 as possible.[2]

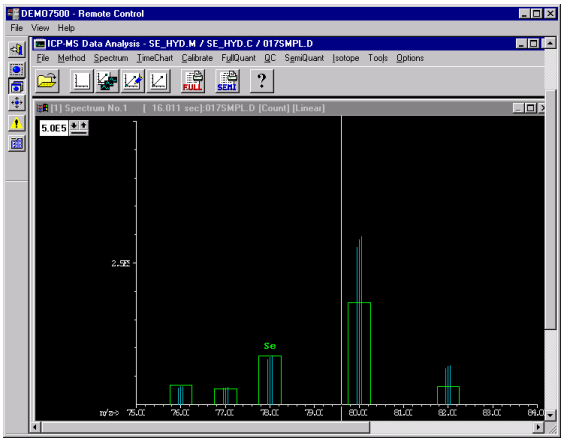

Figure 3. Se spike in CASS 3 Showing Interferences at m/z 80 and 82 from BrH

[2] *Note: This must be done on a clean Tune solution, not a sea water spike since BrH from the sea water can cause high background at both 80 and 82. Occasionally, krypton (m/z 82) in the argon supply can be sufficiently high to adversely affect the ratio as well.*

ISIS Program

The ISIS program builder was used to create the ISIS program below. Typical values for ISIS parameters for hydride generation are shown. As a rule, sensitivity increases with sample flow at the expense of sample consumption. The ratio of sample to reductant is important and should be optimized as well. Normal rinseout times are very fast due to the high sample flows utilized.

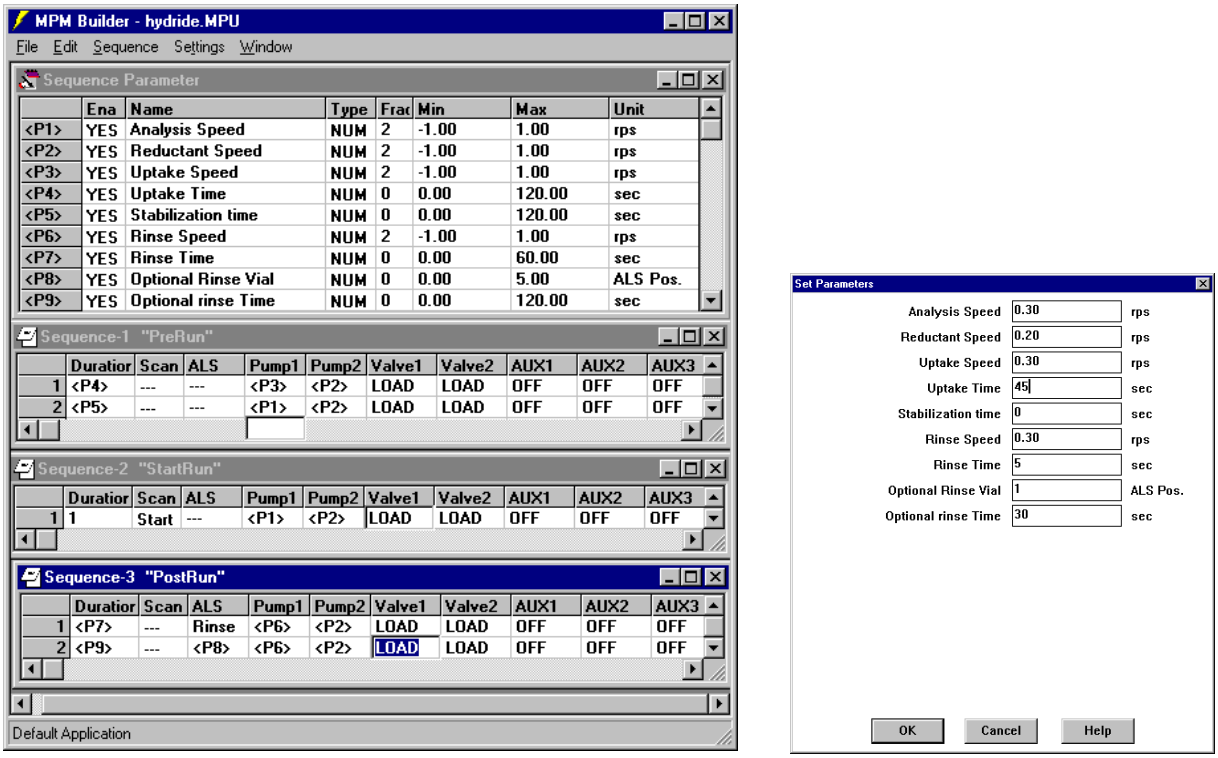

Figure 4. ISIS Program Builder and Method ISIS Parameters Showing Prerun, Startrun and Postrun Programs and Setpoints for ISIS Pumps

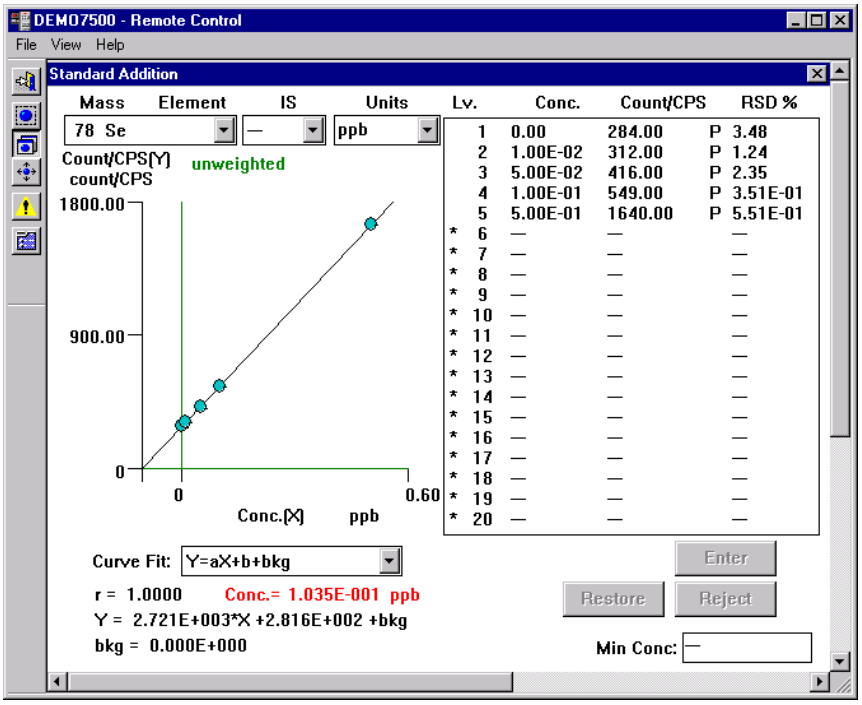

Figure 5. Selenium Standard Addition Calibration in DI Water at 0, 10, 50, 100 and 500 ppt.

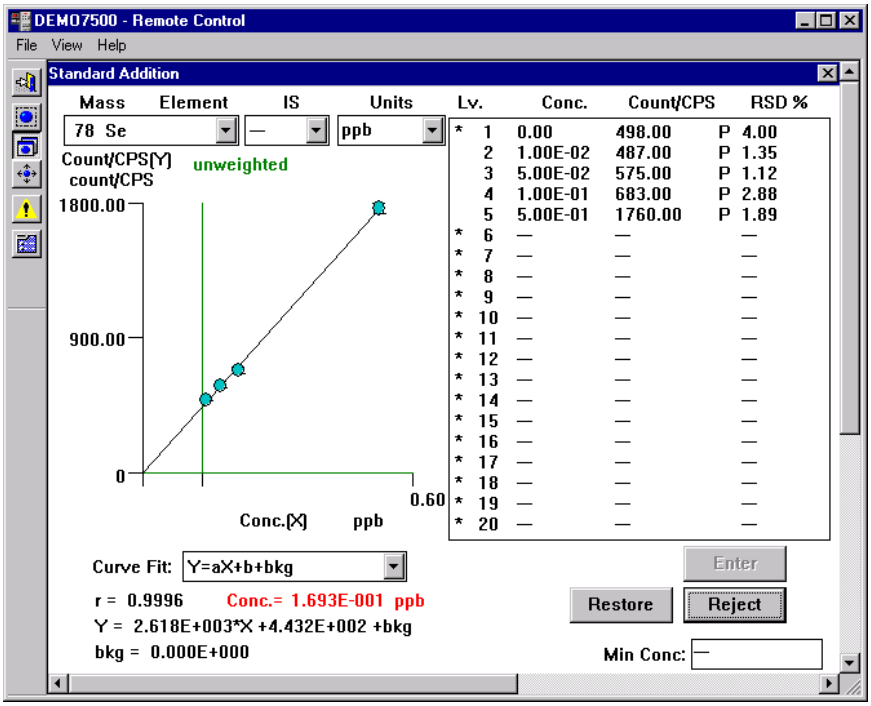

Figure 6. Standard Addition Calibration in CASS 3 Standard Reference Seawater. Calibration Levels; 0, 10, 50, 100, and 500 ppt.

Analysis of Certified Reference Materials

Seawater certified reference materials CASS 3 and NASS 5 were analyzed for As, Se and Sb. Standard addition calibrations were prepared by spiking 10 aliquots of sample with a mixed calibration solution containing the elements of interest. Standard addition calibrations were prepared and then converted to external calibrations for subsequent sample analysis. 3 sigma MDLs were calculated from seven replicate analyses of the unspiked seawater samples using the converted external calibrations. Spike recoveries were also calculated for samples spiked at 0.05 and 2.5 ppb for both elements.

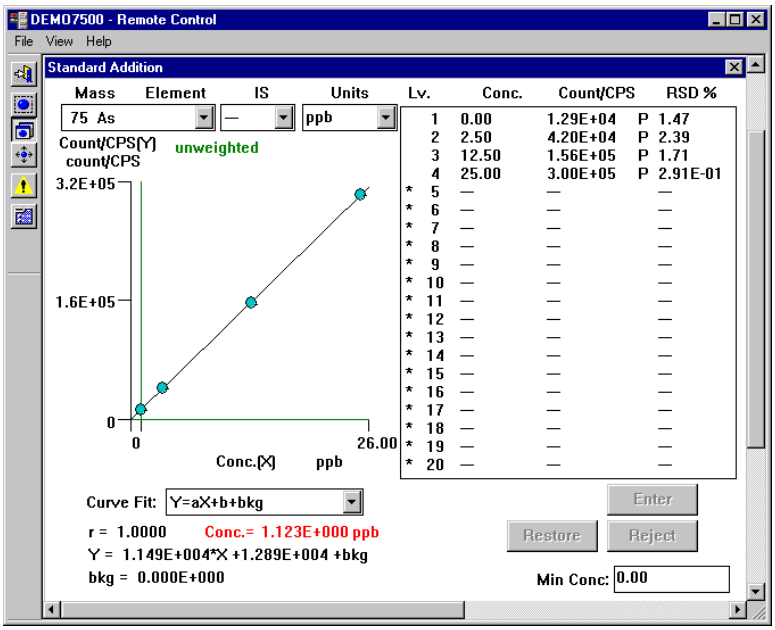

Figure 7. Arsenic in CASS 3 by Standard Addition

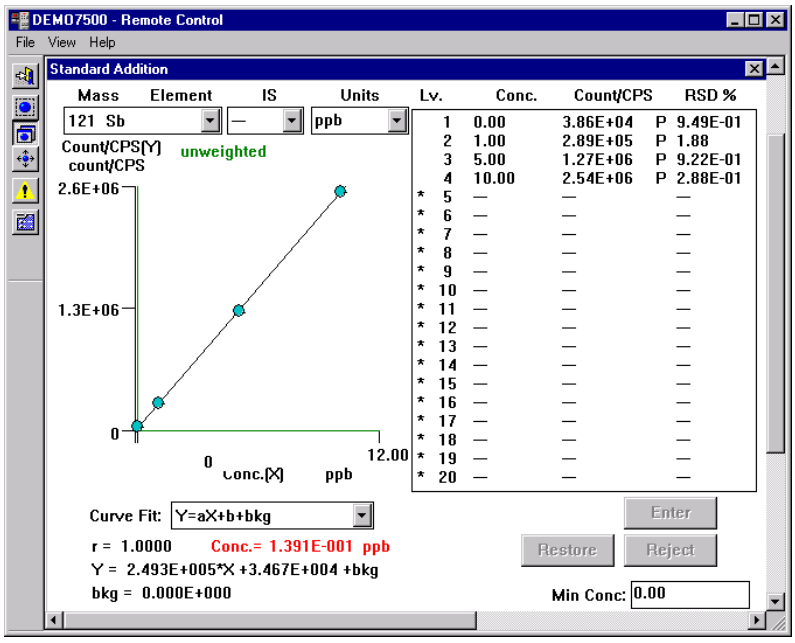

Figure 8. Antimony in CASS 3 by Standard Addition

Summary

The use of online, continuous-flow hydride generation coupled to the Agilent 4500 or 7500 ICP-MS offers a fast, sensitive, routine analytical technique for the analysis of the hydride forming elements such as As, Se and Sb in difficult matrices such seawater. The process can be fully automated for multiple samples using the Cetac ASX-500 autosampler and the Agilent Integrated Sample Introduction system (ISIS). 3-sigma detection limits are typically 10 – 30 ppt for these elements, which is below ambient levels for all three. However, slightly elevated background equivalent concentration for Se can make ambient-level Se analysis borderline at best.

The use of purified reagents may help to reduce the BEC for Se to levels closer to the calculated detection limit. When compared to direct nebulization ICP-MS analysis of 10X diluted seawater, detection limits are improved from 10 to 50 times with no long-term matrix effects on the ICP-MS interface or ion lenses.

Table 2. Results of Analysis of Certified Reference Seawater Materials

Sample	Element /Isotope	Measured Value	Certified Value	Spike Amount	Blank Measurement	% Recovery(3)	MDL (4)
CASS 3	As/75	1.12 ppb	1.09	N/A	-	102	0.03
CASS 3	Se/78	0.682(1)	0.042(2)	0.5	0.193	97.6	0.01
CASS 3	Sb/121	0.34	not certified	0	-	-	0.02
NASS 5	Sb/121	2.87	not certified	2.5	0.34	101	0.02
NASS 5	As/75	1.21 ppb	1.27	N/A	-	95	0.03

(1) raw measured concentration, not corrected for prep(reagent) blank

(2) total selenium is listed but not certified in CASS 3

(3) recovery calculated against certified value where available and against matrix spike recovery where certified value is not available.

(4) 3-sigma using seven replicates

Printed in Japan (03/00)
5980-0243E

Elemental Characterization of River Water using the Agilent 7500a ICP-MS

Application Note

ICP-MS

Environmental

Tetushi Sakai and Chris Tye

Abstract

The quality of river water is often used as a measurement of the overall "environmental health" of a given region. Rivers provide a means of disposal of waste in industrialized countries, yet can also be a source of potable water for domestic use. Monitoring the levels of toxic elements in river water is therefore of utmost importance.

The analysis of river water requires the measurement of trace and minor elements in the same sample, which generally means that laboratories must employ multiple techniques to perform a complete analysis. Inorganic components in river water samples range from sub-μg/L (ppb) to 100's of mg/L (ppm). A combination of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) is usually used for this type of analysis.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has gained wide acceptance for the determination of many trace elements, but perceived limitations in dynamic range and matrix tolerance mean that it is less frequently used for the determination of higher levels of elements. This Application Note summarizes the validation of the Agilent 7500a ICP-MS for river water analysis, allowing the measurement of all elements in a single run, using a single technique.

Introduction

Ambient concentrations of elements in river water can span from ultra-trace levels, for most heavy metals, through to tens or even hundreds of ppm for elements such as sodium, magnesium, potassium and calcium. River water itself can vary in major element composition depending on the underlying geology. Instruments used to characterize the elemental composition of river water should ideally:

- offer the ability to measure many elements in a single acquisition.
- be capable of quantifying species over the complete anticipated concentration range.
- be tolerant to gross changes in major species.

ICP-MS is well suited to environmental samples such as river water analysis because it is a multielement technique,

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offers excellent detection limits and large linear dynamic range.

The Agilent 7500a ICP-MS offers all of the features expected of a fourth generation commercial instrument, but also has excellent tolerance to changes in total dissolved solids. The design features that make the Agilent 7500a particularly suitable for this type of analysis include:

- a nebulizer that operates at low sample flow rates (typically 0.4 mL/min), reducing the sample load on the plasma.
- a thermoelectrically cooled spray chamber that removes much of the water vapor, increasing the plasma temperature.
- an ICP torch that ensures that the sample aerosol is resident in the plasma for sufficient time to ensure complete matrix decomposition. This is typically monitored using the accepted "plasma robustness" indicator of the CeO/Ce ratio. The Agilent 7500a typically has a CeO/Ce ratio of 0.4-0.5%.
- an optimized interface design that ensures minimal sample matrix is passed into the high-vacuum part of the instrument, dramatically reducing the requirement for routine maintenance of the interface cones, the ion lenses and the interface pump oil.
- a simultaneous Dual Mode detector with an exclusive high speed amplifier providing 9 orders of linear dynamic range.

The Agilent 7500a is designed for maximum flexibility and routine ease of use. With its rugged sampling interface, Omega ion lens system, true hyperbolic quadrupole mass analyzer and simultaneous detector, the 7500a offers the performance and flexibility to handle the widest range of sample types and applications.

Table 1 Agilent 7500 Operating Parameters

Plasma gas flow rate	15.0 L/min
Aux. gas flow rate	1.0 L/min
Carrier gas flow rate	1.22 L/min
RF Power	1600 W
Nebulizer	PEEK, Babington - type
Spray chamber	Glass, double pass
Spray chamber temp	2°C
ICP torch injector	Quartz, 2.5 mm
Sample uptake rate	0.4 mL/min
Sampler cone	Nickel
Skimmer cone	Nickel
Sampling depth	6 mm
Points/mass	3
Integration time/mass	3 sec for Be, Cr, As, Se, Hg and U 1 sec for others
Replicates	3

Results

The operating conditions used for this study are shown in Table 1.

Table 2 summarizes the results from an analysis of two Japanese river water standards, JAC 0031 and JAC 0032, using the Agilent 7500a. These standards are useful for understanding the accuracy of a given measurement device; JAC 0031 consists of neat river water, while JAC 0032 is the same water spiked at known levels with different elements. Spike values range from 1 to 50 ppb depending on the element.

The Agilent 7500a demonstrates, by default, low levels of polyatomic species and good matrix tolerance. Consequently, all elements could be determined in the river water standards, without the need for extensive interference correction equations.

The results agree very well with the expected value for all elements. Of particular note is that the recoveries were good across a wide range of concentrations. For instance calcium was measured at over 12 ppm in the same acquisition cycle that mercury was quantified at less than 10 ppt.

ArO at well known polyatomic species can influence the Fe data at low levels, and although the spike recoveries in JAC 0031 are good, agreement with the expected value is affected. If Fe is a regulatory requirement, then the 7500 optional T-mode interface can be used to reduce Ar based polyatomic species even further to improve accuracy and consistency for Fe data at low ppb levels.

The spike recoveries from the analysis of JAC 0032 again return very good agreement with the certified values.

Table 2 Analysis of Two Certified River Standards using the Agilent 7500a

			JAC 0031 (unspiked)		JAC 0032 (spiked)		
Element	m/z	ISTD	Certified	Measured	Certified	Measured	Unit
Be	9	7	---------	<0.001	---------	<0.001	ppb
B	11	7	9.1±0.5	10.2	59±2	60.7	ppb
Na	23	7	4.2±0.1	4.28	4.5±0.1	4.52	ppm
Mg	24	7	2.83±0.06	2.75	2.86±0.04	2.77	ppm
Al	27	7	13.4±0.7	13.5	61±2	62.4	ppb
K	39	7	0.68±0.02	0.65	0.67±0.01	0.64	ppm
Ca	43	89	12.5±0.2	12.3	12.5±0.2	12.3	ppm
V	51	89	---------	7.17	---------	6.96	ppb
Cr	52	89	0.14±0.02	0.15	10.1±0.2	9.70	ppb
Cr	53	89	0.14±0.02	0.17	10.1±0.2	9.76	ppb
Mn	55	89	0.46±0.02	0.49	5.4±0.1	5.35	ppb
Fe	56	89	6.9±0.5	4.30	57±2	51.0	ppb
Co	59	89	---------	0.018	---------	0.019	ppb
Ni	60	89	---------	0.10	10.2±0.3	9.52	ppb
Cu	65	89	0.88±0.03	0.98	10.5±0.2	10.8	ppb
Zn	66	89	0.79±0.05	0.77	11.3±0.4	11.2	ppb
As	75	89	0.28±0.04	0.26	5.5±0.3	5.22	ppb
Se	82	89	(0.1)	<0.1	5.2±0.3	4.82	ppb
Sr	88	89	---------	20.0	---------	19.9	ppb
Mo	95	89	---------	0.53	---------	0.55	ppb
Cd	111	115	(0.003)	<0.02	1.00±0.02	0.98	ppb
Sb	121	115	---------	0.074	---------	0.16	ppb
Ba	137	115	---------	0.87	---------	0.90	ppb
Hg	202	205	---------	<0.007	---------	<0.007	ppb
Pb	208	205	0.026±0.003	0.037	9.9±0.2	10.0	ppb
U	238	205	---------	<0.002	---------	<0.002	ppb

Another well known river water reference material is SLRS-3 from the National Research Council in Canada. SLRS-3 has very low certified levels of many elements and provides a good test of the trace level measurement capabilities of an instrument. The results are summarized in Table 3. Again the results confirm excellent agreement with the expected values of all elements from single figure ppt amounts (beryllium) through to ppm (sodium, magnesium and calcium). The data highlights one of the major strengths of the Agilent 7500a, the accurate quantification of elements from ultra trace to major concentrations under a single set of tuning conditions.

To illustrate the robustness of the sample introduction system over extended measurement periods, a sample of SLRS-3 was analyzed repeatedly over a period of 6 hours. Figures 1a, 1b and 1c summarize the results grouped according to concentration for clarity. Figure 1a is the data from those elements at 500 ppb and above, Figure 1b 5 ppb to 500 ppb, and Figure 1c everything at a concentration less than 5 ppb.

As all of the graphs show, the Agilent 7500a offers good stability over the six-hour period, for all elements, without any systematic change in measured value. Obviously, precision is a function of signal and there is slightly more variation at low concentration values when compared to high, however, the data highlights the excellent long-term stability of the instrument. This stability is derived from a fusion of design features within the instrument. The robust sample introduction system, mass flow controlled plasma gas and rugged interface combine to provide highly

Table 3 Analysis of SLRS-3 River Water Standard using the Agilent 7500a

			SLRS-3		
Element	m/z	ISTD	Certified	Measured	Unit
Be	9	7	0.005±0.001	0.004	ppb
B	11	7	---------	7.14	ppb
Na	23	7	2.300±0.200	2.37	ppm
Mg	24	7	1.600±0.200	1.50	ppm
Al	27	7	31±3	30.0	ppb
K	39	7	0.700±0.100	0.61	ppm
Ca	43	89	6.000±0.400	5.53	ppm
V	51	89	0.3±0.02	0.30	ppb
Cr	52	89	0.3±0.04	0.31	ppb
Cr	53	89	0.3±0.04	0.30	ppb
Mn	55	89	3.9±0.3	3.74	ppb
Fe	56	89	100±2	88.6	ppb
Co	59	89	0.027±0.003	0.025	ppb
Ni	60	89	0.83±0.08	0.76	ppb
Cu	65	89	1.35±0.07	1.40	ppb
Zn	66	89	1.04±0.09	1.02	ppb
As	75	89	0.72±0.05	0.71	ppb
Se	82	89	---------	<0.1	ppb
Sr	88	89	(28.1)	30.1	ppb
Mo	95	89	0.19±0.01	0.29	ppb
Cd	111	115	0.013±0.002	<0.02	ppb
Sb	121	115	0.12±0.01	0.14	ppb
Ba	137	115	13.4±0.6	12.8	ppb
Hg	202	205	---------	<0.007	ppb
Pb	208	205	0.068±0.007	0.078	ppb
U	238	205	(0.045)	0.038	ppb

stable source of sample ions into the mass spectrometer.

Three sigma detection limits were calculated from the standard deviation of ten replicate measurements of a blank solution. These values are shown in Table 4 and illustrate the potential quantification limit for the matrix.

Discussion

The Agilent 7500a ICP-MS is a full-featured high-performance benchtop instrument for the routine determination of elements in a variety of sample types.

With the optimized sample introduction system, 27.12MHz RF generator and robust sample introduction system, the instrument offers very low levels of polyatomic species and extremely good stability. The combination of optimized components yields a high ion transmission at low sample flow rates; therefore preventing contamination of the mass spectrometer components. The off-axis lens system and true hyperbolic quadrupole provide extremely high efficiency ion transmission resulting in excellent signal to noise, and thus low detection limits. The wide dynamic range detector allows measurement of signals over a wide dynamic range allowing all elements to be quantified in a single acquisition and improving throughput.

The results indicate that the Agilent 7500a meets or exceeds all of the criteria required for the analysis of river water.

Table 4 Three Sigma Detection Limits

Element	m/z	DL(3sigma)	Unit
Be	9	0.001	ppb
B	11	0.1	ppb
Na	23	0.0002	ppm
Mg	24	0.00005	ppm
Al	27	0.06	ppb
K	39	0.003	ppm
Ca	43	0.01	ppm
V	51	0.003	ppb
Cr	52	0.01	ppb
Cr	53	0.02	ppb
Fe	54	2	ppb
Mn	55	0.03	ppb
Fe	56	0.3	ppb
Co	59	0.003	ppb
Ni	60	0.02	ppb
Cu	65	0.01	ppb
Zn	66	0.01	ppb
As	75	0.007	ppb
Se	82	0.1	ppb
Sr	88	0.0008	ppb
Mo	95	0.006	ppb
Cd	111	0.02	ppb
Sb	121	0.0007	ppb
Ba	137	0.003	ppb
Hg	202	0.007	ppb
Pb	208	0.001	ppb
U	238	0.002	ppb

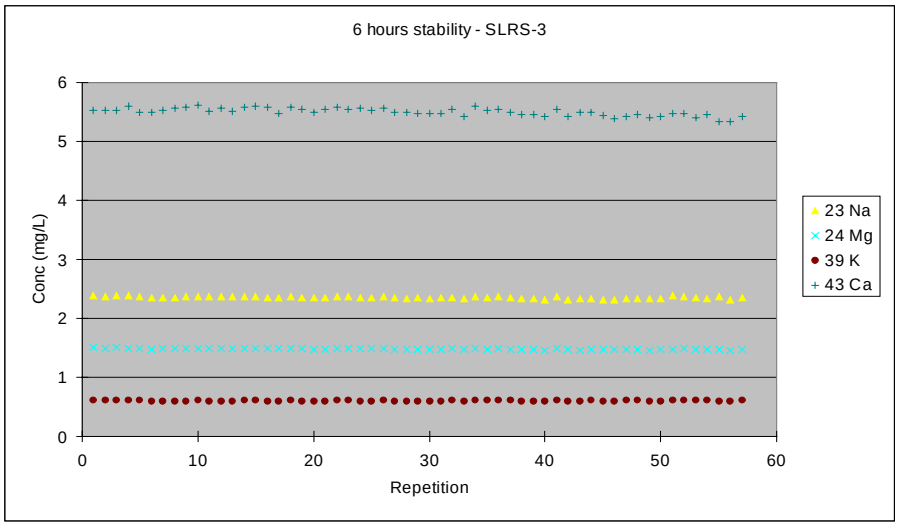

Fig. 1a Agilent 7500a Long Term Stability of Major Elements in SLRS-3

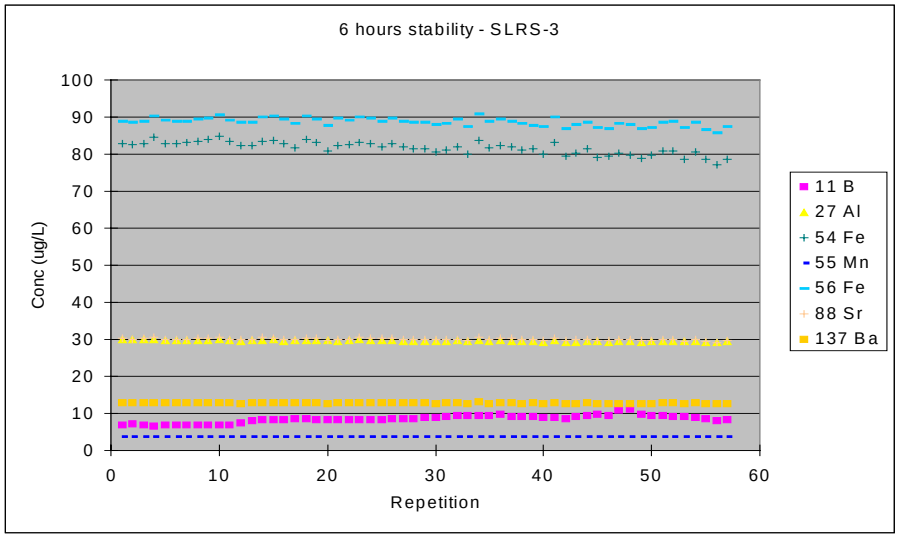

Fig. 1b Agilent 7500a Long Term Stability of Minor Elements in SLRS-3

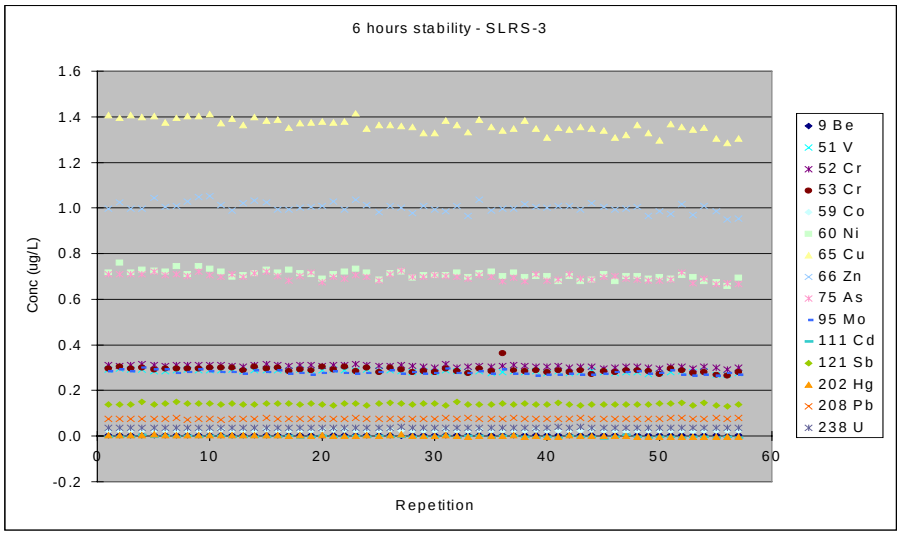

Fig. 1c Agilent 7500a Long Term Stability of Trace Elements in SLRS-3

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Printed in Japan (02/00)
5980-0242E

Indirect Determination of Fluoride Traces in Natural Waters by Ion Chromatography and ICP-MS Detection

Application Note

ICP-MS

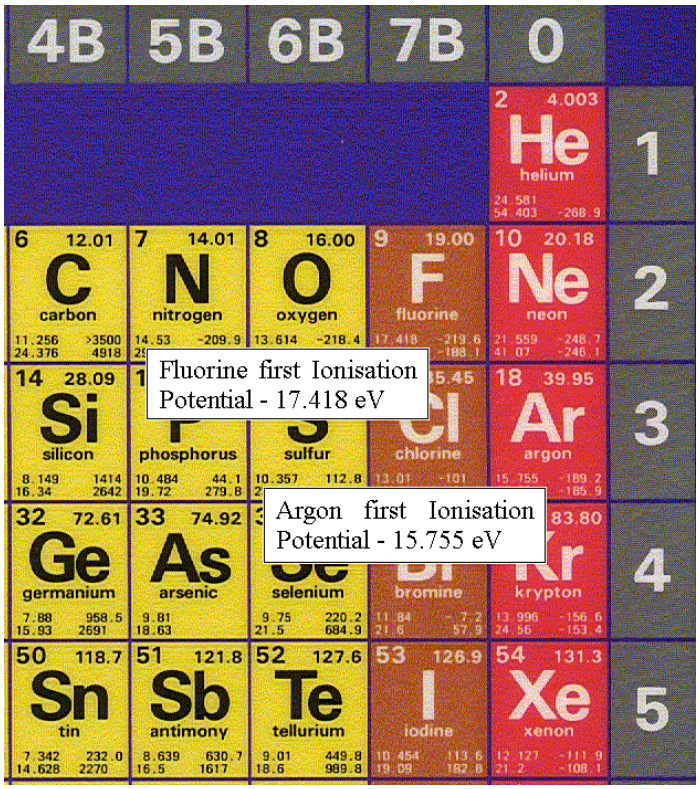
Environmental

Maria Montes Bayon, University of Oviedo, Spain

Abstract

ICP-MS has been widely accepted as a powerful analytical technique for trace element determination in a wide variety of sample types. The technique is rapid, measures virtually all elements in a single acquisition and has limits of detection typically at or below the ng/L (ppt) level. Even initially problematic elements, such as K, Ca, Fe, As and Se, are now routinely measured using the power and flexibility of the Ar ICP to preferentially remove troublesome spectral overlaps.

However, there are some analytical challenges which cannot be overcome by plasma optimisation, most notably the analysis of elements which are not ionised in the Ar plasma. This Application Note presents a novel method for the indirect determination of one such element, fluorine, where the preliminary data indicates that the ICP-MS measurement is not only possible, but offers significant advantages over traditional analytical methods.



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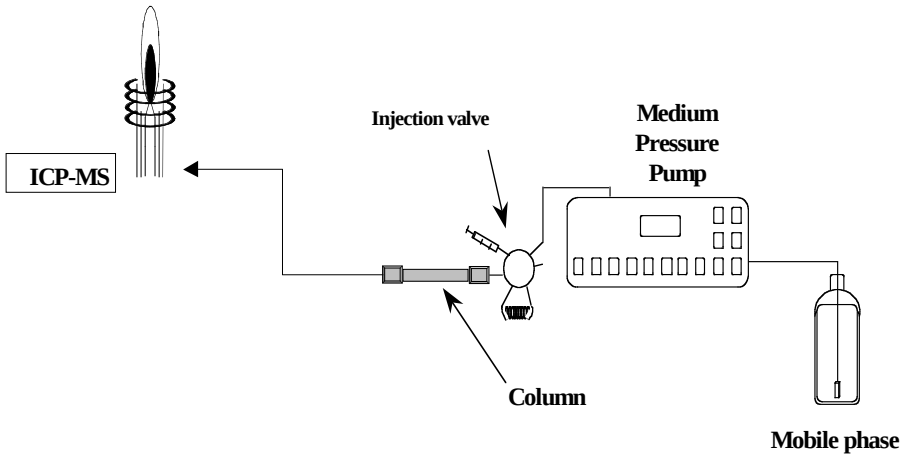

Figure 1 Experimental Set-Up for Fluoride Determination

Table 1 Typical Operating Conditions

Instrument	HP 4500
Rf Power	1300 Watts
Nebuliser	Meinhard
Spray Chamber	Scott type, double pass, room temperature
Sampling depth	5.7 mm
Gas Flow Rates:	
Cool	15 $L.min^{-1}$
Auxiliary	1 $L.min^{-1}$
Carrier	1.17 $L.min^{-1}$
Oxide level (CeO^+/Ce^+)	<0.5 %
Doubly charged level (Ce^{2+}/Ce^+)	<1%

Measurement of Fluoride

During the last decade, the majority of fluoride determinations have been performed using techniques such as potentiometry with fluoride Ion Selective Electrodes (ISE) , ion-chromatography with conductivity detection and, most recently, capillary ISEs has been the preferred technique, but is limited to determinations in the limited to determinations in the mg/L (ppm) range.

The chromatographic separation of Al-fluoride species was first described by Bertsch and Anderson, who determined the stability constants of several AlFx species. In acid aqueous solution, aluminium ions are present as [Al (H2O)6]3+ which can react with F- to form the AlF2+ complex.

Optimum PH for the complex formation seems to be between 2-4, therefore in the present work pH 2.6-3 was selected, where the complex AlF2+ proved to be stable.

Samples and standard solutions were adjusted to pH=3 with nitric acid and spiked with Al3+ requiring at least a 5-fold weight excess of Al to fluoride to assure that only AlF2+ was formed. The samples were diluted by weight, transferred to 10ml polypropylene test tubes and immersed in a water bath at 50ºC for 60 minutes, to ensure quantitative formation of the AlF2+ complex. Under these conditions, several parameters were evaluated to obtain selective separation of the complex AlF2+ in a 5 cm long ion exchange Dionex Ion Pac Column HPIC-CG2. HNO3 was found to be an effective eluent for the separation of AlF2+ from the excess of Al3+. Different molarities of nitric acid, from 0.15 M to 0.75 M, were tested and the conditions chosen for future studies were 0.45 M nitric acid at a flow rate of 0.5 mL/min. The AlF2+ complex was measured indirectly by ICP-MS by collecting data for Al at mass 27.

Figure 1 shows the instrumental set-up of the IC-ICP-MS system. The exit tube from the column was connected directly to the concentric nebulizer of the ICP-MS, which can accept flow rates anywhere from 20uL/min to over 2mL/min.

ICP-MS operating conditions are summarised in Table 1.

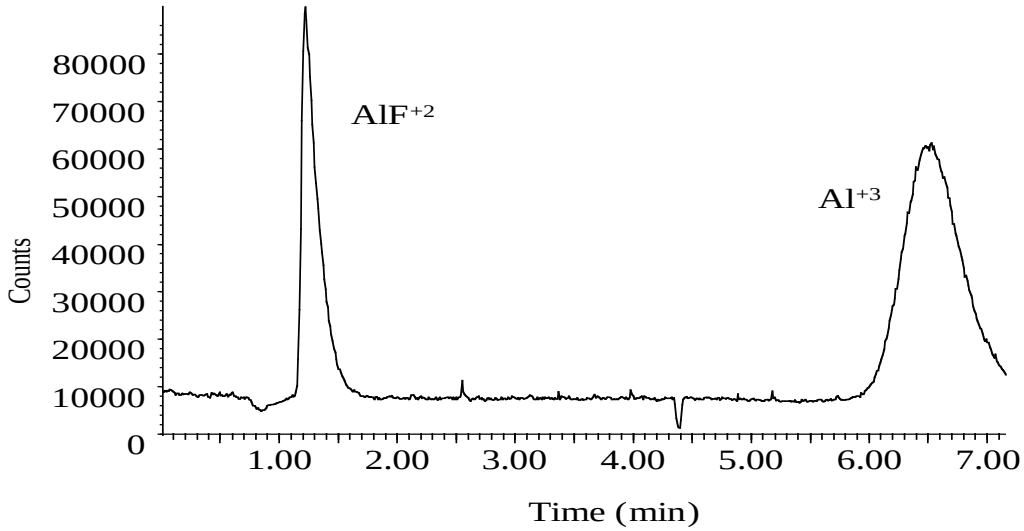

Figure 2 Chromatogram Corresponding to 20 ng.g^{-1} F^{-}

Table 2 Analytical Characteristics

Analytical Characteristics	ICP-MS Detection
Detection Limit	0.1 ng.g^{-1}
Precision	4 % (1)
Linear Range	up to 100(2) ng.g^{-1}
Regression coefficient (r) (n=7 points)	0.9993

(1) on 5 injections of 20 ng.g^{-1} fluoride **(2) using 500 ng.g^{-1} aluminium**

The chromatogram obtained under these conditions for 20 ng/g F^- in the presence of 100 ng/g of Al is shown in Figure 2.

As can be observed, two aluminium containing peaks are detected. The first peak could be ascribed to the AlF^{2+}complex as its peak height/area was found to be proportional to the concentration of fluoride in the sample.

Analytical Performance Characteristis

Analytical performance characteristics are summarised in Table 2. The linear dynamic range for fluoride determination depends on the aluminium excess added to the sample. It was observed that, for a given aluminium concentration, the upper linear limit for fluoride determinations was about one fifth of the total aluminium concentration. Aluminium concentrations higher than 500 ng/g were not tested, to avoid contamination effects in the ICP-MS. In practice, it should be straightforward to dilute samples to a level of fluoride where Al addition would be at an acceptable level, as shown in this study. Alternatively, ISE could be used as a screening tool, after which the IC-ICP-MS method could be used to determine those fluoride levels which were found to be below the limit of detection for ISE.

The detection limit obtained by the IC-ICP-MS method was 0.1 ng/g, calculated as three times the standard deviation of the blank, divided by the slope of a linear calibration between 0-5 ng/g. As can be observed, the detection limit using ICP-MS detection is one of the lowest ever reported for the determination of fluoride.

Figure 3 shows a typical calibration curve obtained from 5 to 50 ng/g (ppb)

of fluoride and containing 200 ng/g aluminium in each standard solution The linear calibration demonstrates that the peak area of the complex is proportional to the fluoride concentration. Determination of Fluoride in Fresh and Sea Water Samples.

Under the optimum separation conditions using HNO_3 for elution, the retention time for Al^{3+} is under 7 minutes, allowing a sampling rate of 6-7 samples per hour. To evaluate the use of the proposed ICP-MS method in routine operation, it was applied to the determination of fluoride in natural and drinking waters from a variety of sources and with different saline concentration.

At this time, no stable aqueous fluoride reference material was available, so it was decided to compare the proposed methodology with the fluoride ion selective electrode (FISE). Since many natural water samples contain fluoride levels below the limit of detection of FISE, a spike-recovery exercise was also undertaken.

In order to minimise aluminium addition to the samples and contamination of the ICP-MS, up to 200 fold dilution of some drinking and sea-water samples was necessary. The results obtained are summarised in Table 3.

Some of the water samples contained fluoride levels too low to be measured by FISE (at around 150 ng/g), so comparison between the FISE method and the IC-ICP-MS method was not possible. However, where FISE was able to measure the levels present, good agreement with the IC-ICP-MS results was obtained. In the other cases tested, the spike recovery exercise indicated that the IC-ICP-MS method gave good recoveries (within 100±10%), showing the applicability of the proposed methodology to perform fluoride determination at extremely low levels in natural water samples.

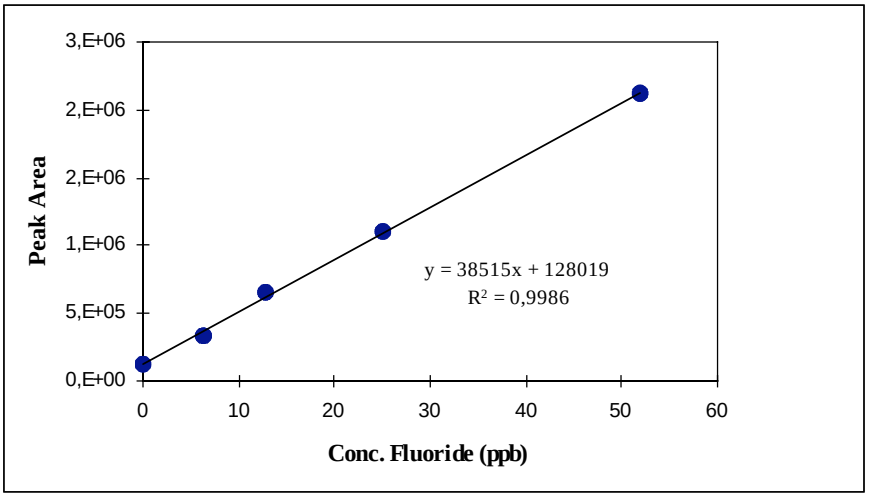

Figure 3 Typical Calibration Curve of Fluoride

Table 3 Results Obtained for Fluoride in Water Samples Using ICP-MS

Water Sample (dilution factor)	Conc. found(n=3) ICP-MS($ng.g^{-1}$)	Conc. found FISE ($ng.g^{-1}$)	Spiked amount ($ng.g^{-1}$)	Recovery (%)
Fontecelta (200)	8050±80	7700	4300	104
Font-Vella (10)	182±2	-	205	97.8
Tap-water (10)	161±1	-	210	90
*Sea-water(100)	1030±60	1080	1080	97.5

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Analysis of Mercury in Wastewater by ICP-MS using the Agilent 7500i

Application Note

ICP-MS

Environmental

Steven Wilbur

The analysis of wastewater for mercury by ICP-MS can present a number of challenges. First, mercury has a relatively low response factor since it is only about 40 percent ionized in a typical argon plasma. It is also subject to ionization suppression in the presence of easily ionized matrix elements that can reduce the response even further. Secondly, because of its high vapor pressure, it can be subject to severe memory effects. Finally, the most abundant Hg isotope available for quantitation is 202Hg, which is only 29.9% abundant.

In order to analyze mercury efficiently in high matrix samples like wastewater, the ICP-MS must be able to maximize the transfer of energy to the analyte atoms. This is achieved in the Agilent 7500i ICP-MS by minimizing the matrix load on the plasma, so ensuring a high and stable plasma temperature. The high plasma temperature also ensures good matrix decomposition, which reduces the impact of the matrix on the interface, ion lenses, vacuum pumps and mass analyser. The use of constant-flow nebulization with the Agilent Micro Flow 100 nebulizer significantly reduces memory effects for Hg, by reducing the total sample flow to the nebulizer and spray chamber. A low sample flow rate, removal of water vapour and use of a wide-bore injector in the plasma torch all contribute to a reduced total matrix load on the plasma, increasing the available energy for analyte ionization. The Agilent 7500's good stability and low random background also allow accurate and precise measurements to be made at very low concentrations.

Acquisition Parameters

Tune conditions are displayed in Figure 1 and Figure 2.

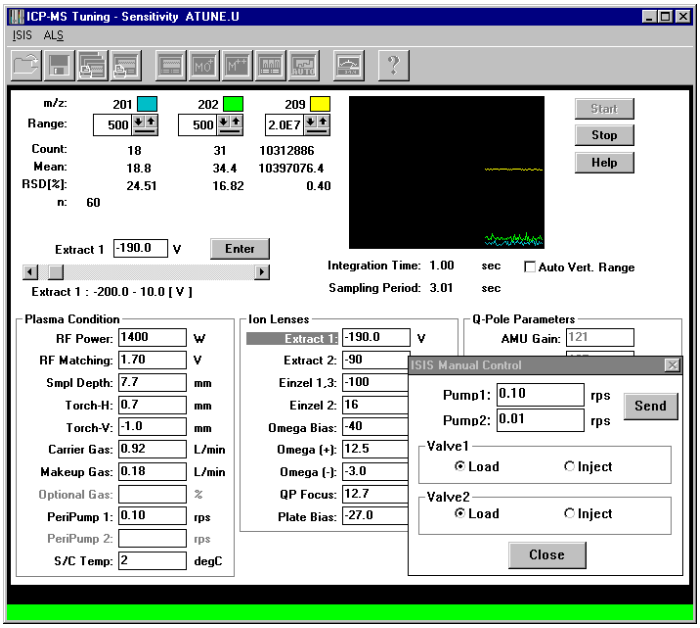

Figure 1 Tune Screen Showing Calibration Blank: counts at m/z 201 and 202 are due to very low background of Hg present in the blank, m/z 209 is bismuth internal standard, (1 sec. integrations)

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Hg measurements were made using 3 sec. integrations per peak. Acquired masses 199, 200, 201, 202 and 209. All 4 mercury masses were summed (at m/z 202) using a correction equation, in order to improve counting statistics.

ISIS parameters

30 seconds uptake at 0.5 rps, analysis speed = 0.1 rps. Constant flow nebulization at ~100 uL/min, using Agilent Micro Flow 100 nebulizer. Rinse 30 seconds at 1 rps.

Calibration

Calibration standards were prepared in DI water acidified to 2% with nitric acid and containing 100 ppb Au to stabilize the mercury at low concentrations in solution. Standards were prepared at 10, 20, 50, 100, 200 and 500ppt Hg. The calibration curve obtained is shown in Figure 3.

Method Detection Limits

3 sigma detection limits were calculated from 7 replicate analyses of the Standard Reference Wastewater. It is shown in Table 2.

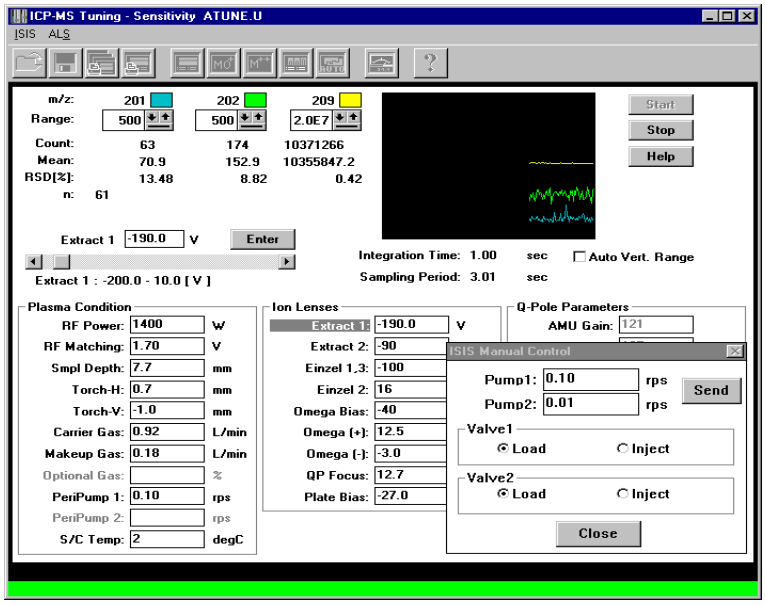

Figure 2 Tune Screen Showing High Purity Standards Certified Wastewater Spiked with 50 ppt Hg (1 sec. integrations). Note signal increase at m/z 201 and 202

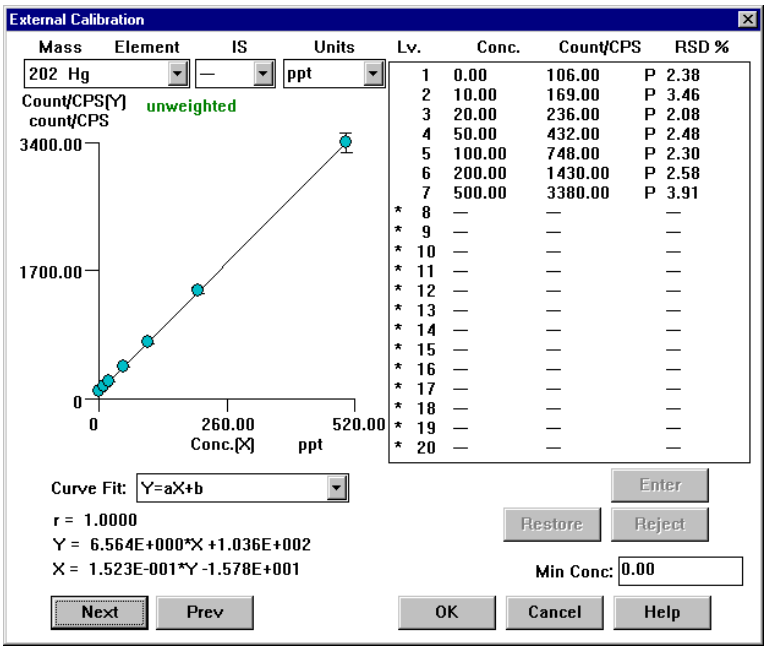

Figure 3 External Calibration, Hg in 2% HNO_3, 10 - 500 ppt

Table 1 Spike Recoveries from Undiluted Wastewater Standard [1]

[Unspiked Sample]	Spike Amount	[Spiked Sample]	% Recovery
14.11 ppt	50 ppt	65.9 ppt	103.6 %
14.11 ppt	100 ppt	118.8 ppt	104.6 %

[1]) High Purity Standards Certified Wastewater – Trace Metals, Lot # 590209

Uptake and Rinseout

By using ISIS in the Rapid Sample Uptake mode, it is possible to transport the sample from the autosampler to the nebulizer input tee very rapidly and at high flow. Since the nebulizer is operating at constant flow, the excess sample or rinse flow is split to the drain line and does not overload the spray chamber or plasma. Rapid uptake and constant-flow nebulization both serve to reduce mercury memory effects. The data in Figure 4 were acquired with the ISIS sample pump at uptake speed using the Agilent 7500 time resolved mode of acquisition. The nebulizer pump was operated at constant flow of ~100 uL/minute. Acquisition was begun with the sample probe in the blank solution containing 2% nitric acid and 100 ppb Au. After 60 seconds, the probe was moved to a 1000 ppt Hg standard solution containing 100 ppb Au. Following an additional 120 seconds, the probe was returned to the blank solution.

Summary

The high sensitivity, low random background and excellent matrix tolerance of the Agilent 7500i ICP-MS, coupled with the low flow and high efficiency of the Agilent Micro Flow nebulizer, permit the analysis of the very difficult element mercury in wastewaters. The 7500i system is capable of excellent linearity, precision and accuracy at sub-ppb concentrations in waters and wastewaters. In this range of concentrations, mercury can be analyzed simultaneously with the other important trace elements, without impacting uptake and rinseout times to an unacceptable degree, thus eliminating a separate analysis for mercury.

Table 2 3 Sigma Detection Limits (ppt) for Mercury Isotopes m/z 199, 200, 201 and Sum of 199, 200, 201 and 202, Calculated from 7 Replicate Analyses of HPS Wastewater Certified Reference Material

File:	Date/Time:	Mercury /199	Mercury /200	Mercury /201	Sum (199,200,201,202)
009SMPL.D#	2/17/2000 11:50	11.19	10.78	12.68	16.53
010SMPL.D#	2/17/2000 11:55	10.84	10.16	10.3	15.54
011SMPL.D#	2/17/2000 11:59	10.34	10.9	12.48	16.23
012SMPL.D#	2/17/2000 12:04	11.82	11.09	12.93	16.41
013SMPL.D#	2/17/2000 12:09	10.54	10.68	11.53	15.88
014SMPL.D#	2/17/2000 12:13	10.62	9.245	10.43	15.72
015SMPL.D#	2/17/2000 12:18	10.3	11.13	11.42	16.09
	3 Sigma MDL (ppt)	**1.676**	**2.077**	**3.040**	**0.938**

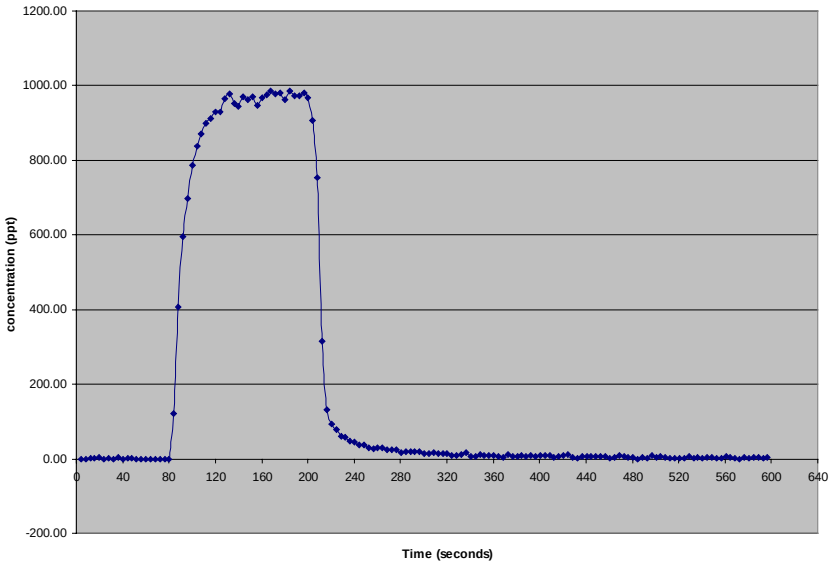

Figure 4 Uptake and Rinseout Profile
blank, 60 sec -> 1000 ppt standard, 120 sec -> blank

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Water

Other Applications

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Time-of-Flight LC/MS Identification and Confirmation of a Kairomone in *Daphnia magna* Cultured Medium

Application

Natural Product Chemistry

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Abstract

***Daphnia* kairomones induce morphological change to green alga. An active compound (8-methylnonyl sulfate), which was originally isolated and determined from *Daphnia pulex* body, was identified from a cultured medium of *Daphnia magna* by liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS)with electrospray ionization after concentration by the Methylene Blue method.**

Introduction

A pheromone is a chemical substance that triggers a variety of behavioral responses in another member of the same species. On the other hand, a kairomone is a chemical substance released by an organism that affects other organisms in a food chain series. It was reported that a unicellular green alga achieved morphological change into 2-, 4- and 8-colonies when the water was cultured with *Daphnia*. However, neither isolation nor elucidation of active compounds has been completed due to the very low concentration of the compounds in the cultured medium. A unique approach was tried: the active compounds were isolated from commercially available frozen *Daphnia pulex* body (10 kg) and the structure of the compounds determined with a combination of purification, chemical synthesis, and bioassay. The synthesized aliphatic sulfates undoubtedly showed activity to induce morphological changes of phytoplankton at an optimum concentration of low ppb (10^{-6} g/L). Because the *Daphnia* kairomones are anion surfactants, they are quantitatively detected with the Methylene Blue method [1]. The concentration of total anion surfactants in *Daphnia* cultured medium was determined at 8.0 ppb. However, this method quantified only the total amount of the surfactants and each active compound was not chemically identified.

The active compounds were isolated from *D. pulex*, but *D. magna* was used for the assay. The frozen *D. pulex* is commercially available; however, *D. magna* is larger in size than *D. pulex* and easy to assay albeit difficult to cultivate at kg-scale. It is possible for each species to release different compounds. Consequently, we report identification and confirmation of kairomones in *D. magna* cultured medium [2].

It is actually impossible to detect these aliphatic sulfates directly using HPLC with commonly used detectors such as ultraviolet absorption or fluorescence. As for LC/MS, electrospray ionization (ESI) in negative ion mode is best matched for these

compounds because all target sulfates ($R\text{-}OSO_3^-M^+$) or amidosulfates ($R\text{-}NHSO_3^-M^+$) ionize well and can easily dissociate to $R\text{-}OSO_3^-$ or $R\text{-}NHSO_3^-$ in aqueous solution, respectively. LC/TOF-MS benefits from the increased identification capability of compounds in comparison to a quadrupole analyzer due to its accurate mass measurement capability.

Therefore, in this study we chose LC/TOF-MS with ESI to directly detect and identify the active compounds in the *D. magna* cultured medium. Furthermore, the orthogonal spray position of this LC/MS ion source resists ion suppression from the sample even though it contains significant matrix components.

Experimental

Compounds 1, 6, and 7 were commercially available from Sigma-Aldrich Japan K.K. (Tokyo, Japan), Kanto Chemical, Co., Inc. (Tokyo, Japan), and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), respectively. Compounds 2, 3, and 5 were synthesized. Compound 4 was isolated from the cultured medium.

The instrument performed the internal mass calibration automatically and constantly, using the second electrospray nebulizer with an automated calibrant delivery system that introduced a low flow of a calibrating solution containing the internal reference mass compounds (*m/z* 112.9856 and 1033.9881). The instrument software constantly corrects the measured masses of all the spectra using the known masses as reference.

Five liters of *D. magna* cultured medium (250 adult bodies per liter dechlorinated tap water, 1 week) was concentrated to 100 mL and treated by the Methylene Blue method. Subsequently, the Methylene Blue reagent was removed by cation exchange resin (DOWEX 50WX8-100). The concentrated sample was dried and dissolved in 25 mL of Milli-Q water for LC/MS analysis. When treatment was complete, the cultured medium was concentrated to 200 times of the original volume. Authentic standards, shown in Table 1 (each at 100 ppb, except for 4, because it was isolated from the cultured medium and no authentic standard was obtained), and the concentrated cultured medium sample were analyzed by LC/ TOF-MS with the ESI source under the same conditions.

LC/MS Method Details

LC Conditions	
Instrument:	Agilent LC 1100
Column:	ZORBAX Eclipse XDB-C18 50 mm × 2.1 mm 3.5 µm (p/n 971700-902)
Column temp.:	40 °C
Mobile phase:	A: 10 mM ammonium acetate aqueous solution B: acetonitrile
Gradient and flow rate:	30% B at 0 min (0.3 mL/min) 30% B at 3 min (0.3 mL/min) 95% B at 8 min (0.3 mL/min) 95% B at 8.1 min (0.5 mL/min) 95% B at 12 min (0.5 mL/min) 30% B at 12.1 min (0.5 mL/min) 30% B at 17 min (0.5 mL/min)
Injection volume:	10 µL
MS Conditions	
Instrument:	Agilent 6210 TOF LC/MS
Source:	Negative ESI
Drying gas flow:	10 L/min
Nebulizer:	350 kPa
Drying gas temp.:	350 °C
V_{cap}:	5000 V
Fragmentor:	250 V
Scan mode:	*m/z* 50-1100, 10,000 transitents/scan, 0.89 scan/sec.
References mass:	*m/z* 112.9856 and 1033.9881

Results and Discussion

Six sulfates (1, 2, 3, 5, 6, and 7) and one amidosulfate (4) were separated by using the volatile buffered mobile phase (ammonium acetate and acetonitrile) for LC/MS. Thus even though 2 and 3, and 5 and 6 were two pairs of isomers they each could be distinguished chromatographically. Analysis time was substantially reduced to less than 10 min by using a short column (50 mm) packed with small particles (3.5 µm). Automatic continuous mass-axis correction with the two known reference compounds gives extremely accurate mass measurement. This provides fewer potential empirical formulas not only for the synthetic compounds but also for the unknown compounds in the *Daphnia* cultured medium.

To yield both the deprotonated molecule and the *m/z* 97 fragment ($HOSO_3^-$), in-source collision-induced dissociation (CID) was used. By setting the MS fragmentor to 250 V, familial fragments (*m/z* 97) in the mass spectra of all the aliphatic sulfates were observed together with each

deprotonated molecule. The extracted ion mass chromatogram of the *m/z* 97 fragment is a selective indicator of the targets, as shown in Figure 1. The measured mass error of the deprotonated molecule ($[M\text{-}H]^-$) in each standard compound is less than 0.4 mDa, as shown in Table 1.

Table 1. Measured Mass Accuracy of Authentic Standards

No.	$[M–H]^-$	Calcd. *m/z*	Measured *m/z*
1	$C_8H_{17}O_4S$	209.0853	209.0857
2	$C_9H_{19}O_4S$	223.1009	223.1011
3	$C_9H_{19}O_4S$	223.1009	223.1013
4	$C_{11}H_{24}NO_3S$	250.1482	250.1484
5	$C_{10}H_{21}O_4S$	237.1166	237.1167
6	$C_{10}H_{21}O_4S$	237.1166	237.1164
7	$C_{12}H_{25}O_4S$	265.1479	265.1481

D. magna cultured medium was concentrated by the Methylene Blue method, and subsequently the Methylene Blue reagent was removed with cation exchange resin. The method was modified to effect a 200-fold concentration of the Methylene Blue complex with anion in an organic layer. The concentrated cultured medium was analyzed by LC/TOF-MS with the ESI source under the same conditions.

The mass chromatogram of the *m/z* 97 fragment ion is a selective indicator of sulfate targets and is especially useful for identification of compounds containing sulfate in complex matrices as in the cultured medium (Figure 2). The retention time of the mass chromatogram of both *m/z* 97 and 237 in Figure 2 matches that of standard 5 in Figure 1. This strongly suggests that the cultured medium contains compound 5.

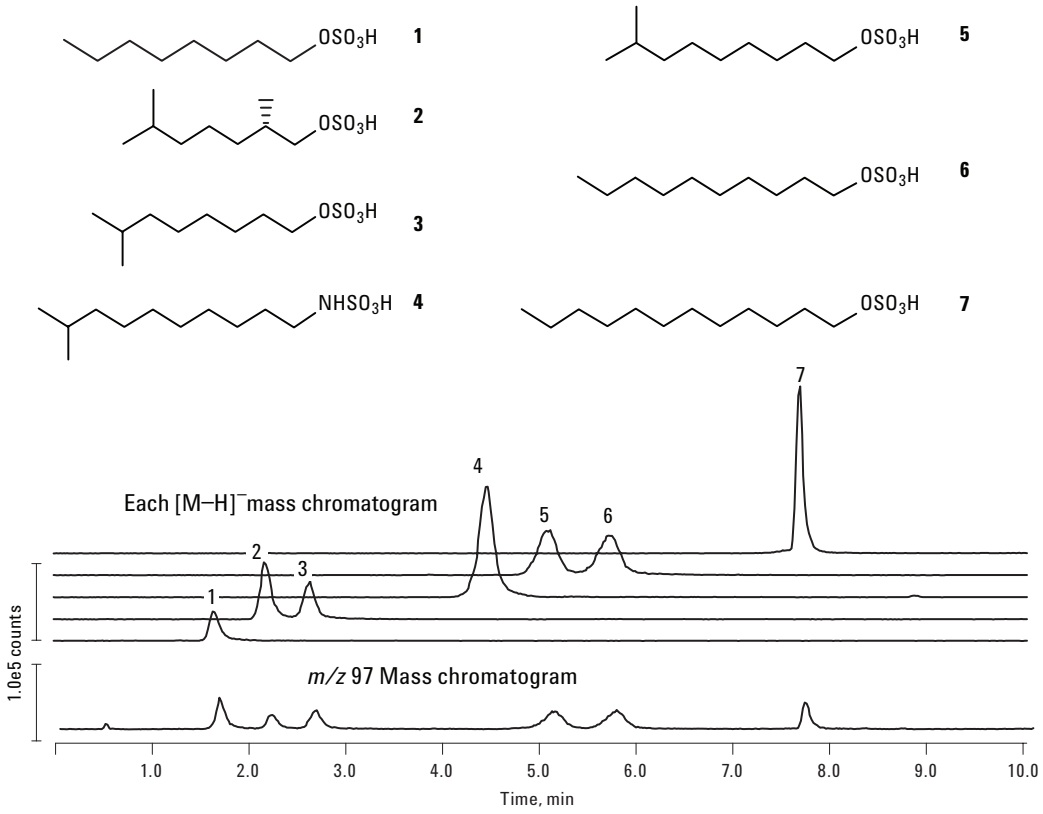

Figure 1. Structures and mass chromatograms for the $[M–H]^-$ ions of authentic standards and the familial fragment ion for sulfate.

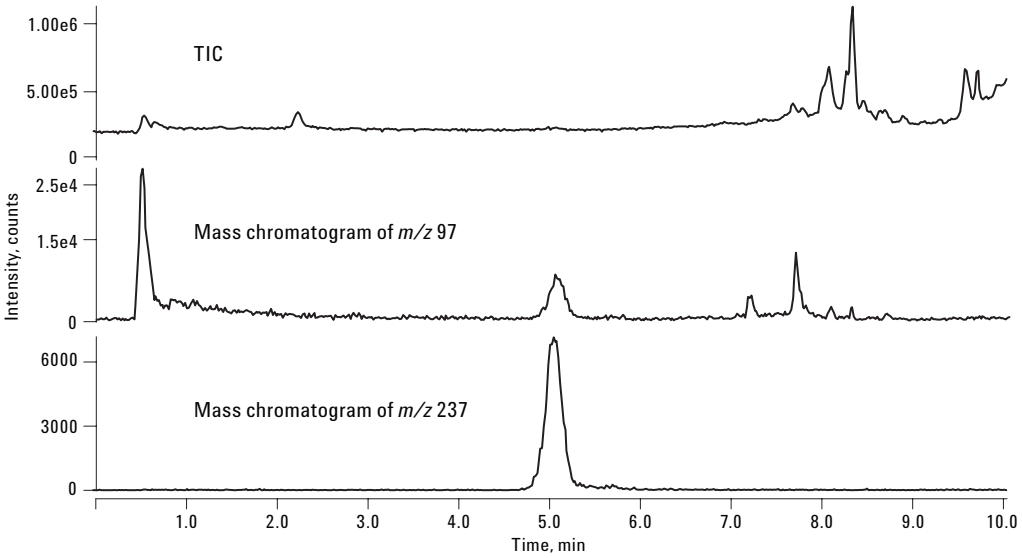

Figure 2. Total ion chromatogram (TIC) and mass chromatogram of *Daphnia* cultured medium.

Using the mass spectral data described below, 5 was identified and confirmed in the *D. magna* cultured medium. Accurate mass measurement of the deprotonated molecule ($[M–H]^-$ in negative ion mode) can give both the molecular weight of the compound and its empirical formula. Low-level error has significant implications when trying to propose possible empirical formulas of unknowns. Actually, at 10 ppm accuracy, *m/z* 237.1169 (Figure 3) provides only three possible empirical formulas, with the elemental composition restricted to combinations of $C_{0\text{-}20}$, $H_{0\text{-}45}$, $N_{0\text{-}5}$, $O_{0\text{-}5}$, and $S_{0\text{-}5}$: $C_{10}H_{21}O_4S$ (error 0.3 mDa), $C_{11}H_{17}N_4S$ (error –1.0 mDa), and $C_{14}H_{13}S$ (error 2.3 mDa).

Accurate mass measurement of fragment ions also provides the atoms that those portions of the molecule contain (valuable structural information). At 10 ppm accuracy, *m/z* 96.9602 provides only one possible empirical formula with the same condition described above: HO_4S (error 0.1 mDa). Accuracy of 20 ppm for *m/z* 96.9602 provides three possible empirical formulas with elemental composition restricted to the same combinations: HO_4S, C_4HOS (error –15 mDa) and HO_2S_2 (error 18 mDa).

The detected and confirmed 8-methylnonyl sulfate (5) is similar to one of the commonly used surfactants, sodium dodecyl sulfate (SDS, sodium salt of 7). All other active kairomone compounds described here also behave as surfactants due to both polar and nonpolar sites in the molecule. Large amounts of surfactants have been produced as detergents and partially released to the environment. Thus, it is a concern that environmentally released concentrations of surfactants acting as the kairomone would indirectly confuse the food chain in lakes and marshes and cause significant ecological disruption.

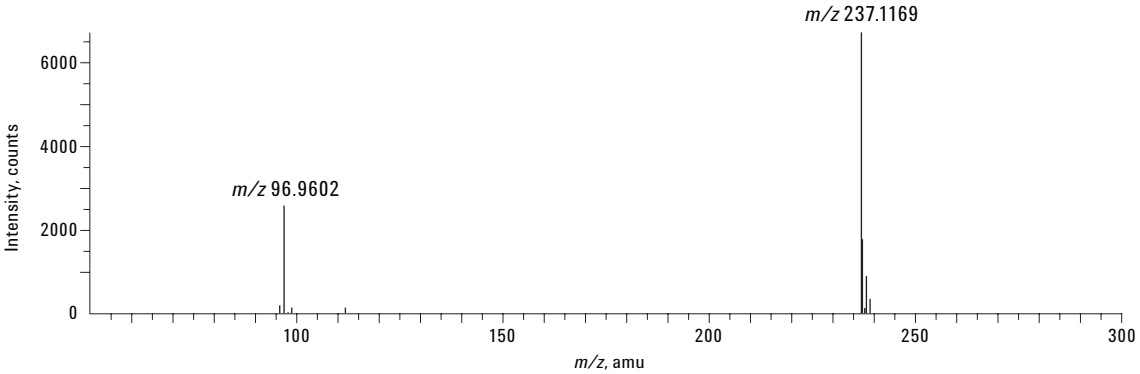

Figure 3. Mass spectrum of the peak at 5.1 min in the *Daphnia* cultured medium in Figure 2.

Conclusions

The kairomone is identified by the combination of LC/TOF-MS with ESI with sample preparation by the simple Methylene Blue method. The identification is confirmed by comparison of the retention time and mass spectrum of the synthetic standard compound with those of the actual sample. The mass error is 0.3 mDa between the actual sample result and the calculated *m/z*. The method needs no derivatization and shows low background due to using ESI in negative ion mode. Target compounds containing the SO_4 moiety were selectively screened by an MS instrument parameter (fragmentor voltage) adjustment. A fully automated introduction system of reference compounds for mass axis calibration gives very stable and reliable mass accuracy results.

Although the presence of other kairomone compounds may be assumed, this is the first direct chemical detection of the *Daphnia* kairomone from a cultured medium.

Reference

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Printed in the USA
April 17, 2008
5989-8387EN

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Analysis of ionic tenside surfactants in wastewater by HPLC

Rainer Schuster

Environmental

Abstract

The detergents used for cleaning floors, worktop surfaces and laundry in the home and hygene industry are the main source of the ionic species of surfactants known as tensides.

Sample preparation

Tensides can be extracted from either surface water or waste water by a liquid–solid technique. Narrow bore technology for lowest solvent consumption and highest sensitivity, with automated diode-array detection for evaluating peak purity and identity.

Separation

Figure 1 shows a normal flow rate elution on a 10 cm Hypersil ODS column with 2.1 mm internal diameter, 5 µm particles. A simple linear gradient and a constant oven temperature of 40 °C achieve good resolution.

- UV absorbance detection or
- Diode-array detection—for peak purity check and peak identity confirmation using UV absorbance spectra.

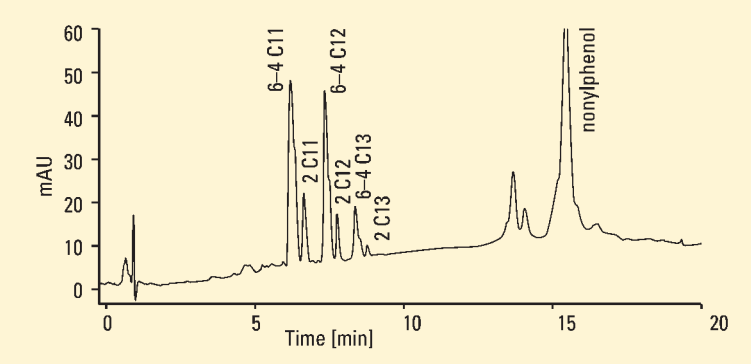

Figure 1
Separation of 10 µl injection of a Marlon A ionic tenside surfactant standard

Conditions

Column
250 x 2.1 mm Hypersil ODS C18, 5 µm
Mobile phase
A: 0.005 M KH_2PO_4
B: acetonitrile
Gradient
0 min 26% B
20 min 100% B
Flow rate
0.25 ml/min
Temperature
40 °C
Detection
222 nm (20 nm bandwidth)
reference 450 nm (100 nm bandwidth)

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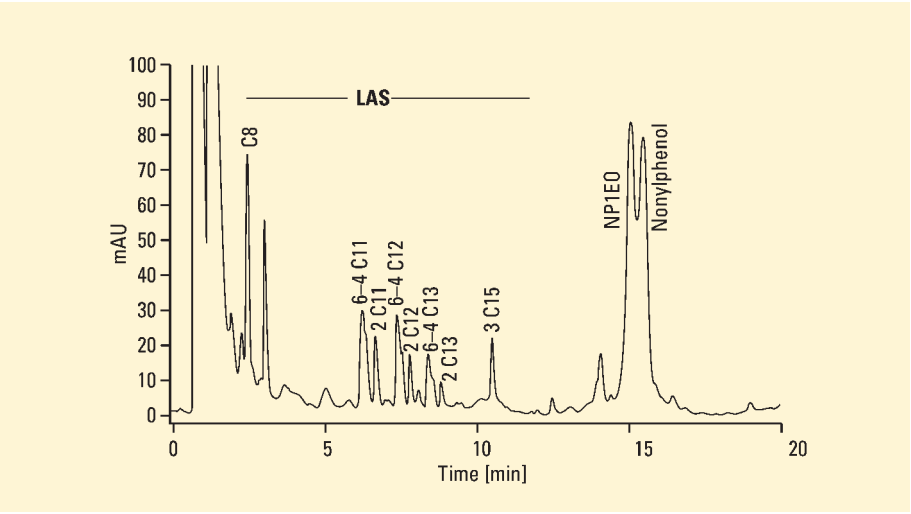

Figure 2
Analysis of linear alkylbenzenesulfonates (LAS), alkylphenol polyethoxylates (APEO) and nonylphenol in waste water

References

1.
Rainer Schuster, "Analysis of ionic tenside surfactants in wastewater by HPLC and diode-array detection," *Hewlett-Packard application note*, publication number 12-5091-1818E,

Conditions

Column
250 x 2.1 mm Hypersil ODS C18, 5 µm
Mobile phase
A: 0.005 M KH_2PO_4
B: acetonitrile
Gradient
0 min 26% B
20 min 100% B
Flow rate
0.25 ml/min
Temperature
40 °C
Detection
222 nm (20 nm bandwidth)
reference 450 nm
(100 nm bandwidth)

Equipment

Agilent 1100 Series
- vacuum degasser
- quaternary pump
- autosampler
- thermostatted column compartment
- diode array detector
- fluorescence detector

Agilent ChemStation + software

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Released 10/97
Publication Number 5966-1877E

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