Consolidated GC-MS/MS Analysis of OCPs, PAHs, and PCBs in Environmental Samples

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Key Words
Organochlorine pesticides, polychlorinated biphenyls, polyaromatic hydrocarbons, soil samples, water samples, building material, repeatability, linearity

Goal
To describe the analysis of semivolatile compounds in various environmental matrices, showing the productivity and high quality results of the GC-MS/MS system.

Introduction
Organochlorine pesticides (OCP), polyaromatic hydrocarbons (PAH), and polychlorinated biphenyls (PCB) are compound classes that are highly familiar to routine environmental or contract testing laboratories. Various approaches are taken to address these compound classes in the diverse matrix environment experienced by these laboratories.

Gas chromatography-mass spectrometry (GC-MS) is well suited for the analysis of OCPs, PAHs, and PCBs. Single quadrupole GC-MS has offered the opportunity for the environmental laboratory to increase selectivity for these analytes over that of classical detectors, such as UV and fluorescence detectors in HPLC and ECD and FID detectors in GC. This has allowed for limited optimization of sample preparation procedures to increase time to result and ultimately reduce laboratory costs.

Triple quadrupole GC-MS/MS provides a significant increase in selectivity when compared to single quadrupole GC-MS. This selectivity has a profound effect on the ability to cut through chemical background (interference), which enhances the capability and productivity of an environmental testing laboratory. This technique is being increasingly utilized by many environmental laboratories, especially those looking for a competitive edge. Unfortunately, for laboratories newer to GC-MS/MS, the adoption of this technique presents a challenge in realizing the productivity advantages offered without significantly impacting continued laboratory operations.[1,2]

This application note describes a high performance, highly productive analysis of OCPs, PAHs and PCBs in environmental samples through a consolidated GC-MS/MS method using the Thermo Scientific™ TRACE™ 1310 GC and the TSQ™ 8000 triple quadrupole GC-MS/MS. Also described is the use of smart software tools that are integrated into the method development and analysis workflow to minimize the time needed to implement and maintain the methodology in routine.

Experimental Conditions
Sample Preparation
Water samples
To 1 L of sample, n-hexane was added and the mixture was shaken. After the separation of water and organic phases, the organic phase was removed and dried with anhydrous Na_2SO_4. An aliquot of the organic extract was evaporated to a volume of 3–4 mL and then evaporated under a gentle nitrogen stream to the final volume.

Solid samples
Into a glass jar, 10 g of the sample (soil, sediment, or building material) was weighed, then anhydrous Na_2SO_4 and 40 mL of extraction solvent mixture (hexane and acetone) were added. The glass jar was sealed with a Teflon® seal and sonicated for 20 min. An aliquot of the sample extract was placed into a Kuderna – Danish apparatus, and another 40 mL of extraction solvent mixture was added to the sample and the extraction was repeated. An aliquot of second extraction was added to the first extraction aliquot. The extract was evaporated to a volume of 3–4 mL and then evaporated under a gentle nitrogen stream to the final volume.

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Method Setup

A method was developed for the Thermo Scientific TRACE™ 1310 Gas Chromatograph and the TSQ 8000 Mass Spectrometer (Table 1).

Table 1. Recommended instrument conditions

TRACE 1310 GC

Injection Volume:	1 µL
Liner:	Siltec baffled liner (P/N 453T2120)
Carrier Gas:	He, constant flow, 1.15 mL/min
Column Type:	20 m, 18 mm ID, 0.18 µm df, TG-XLBMS (P/N 26079-5780)
Column Oven:	Initial 60 °C, hold 1 min. Ramp 30.0 °C min to 200 °C. Ramp 10.0 °C min to 320 °C. Hold 2.0 min.
Transfer Line:	320 °C

TRACE 1310 GC PTV program

Injector Temperature:	80 °C, Splitless Injection 1 min
PTV Inject:	80 °C, 0.1 min. 600 °C/min to transfer step
PTV Transfer:	320 °C, 5 min, 870 °C/min to clean step
PTV Clean:	325 °C, 15 min, clean flow 25 mL/min

TSQ 8000 Mass Spectrometer in EI mode

Source Temperature:	350 °C
Ionization:	EI, 70 eV
Emission Current:	50 µA
Resolution:	Q1 normal
Collision Gas:	Argon

Method Adoption: Mass Spectrometer Acquisition Method and Quantitation Method

With the TSQ 8000 GC-MS/MS system, the operator can perform automated SRM method development, shortening the method development time considerably.

AutoSRM accelerated the method development process. The optimized parameters were tracked in a clear and simple way. The program started from a Full Scan analysis, and the peaks were identified with a library search. Clicking on each peak revealed a list of most intense ions, and those were selected to be pushed into a working list and used for the second injection, the product ion scan.

The results were again plotted into a chromatogram and, by clicking on the peaks, the product ions were listed into a table of decreasing intensity. Finally those product ions were pushed into a working list, and the final optimization started where the ions were subjected to increasing collision energy. This was plotted into a graphical representation and into a third working list. Selecting this working list created a SRM method for all compounds, which was linked to a full instrument method. Furthermore, the transitions and the retention times were exported into a compound data base, linking the method automatically to a quantitative method in Thermo Scientific TraceFinder™ software.

A comprehensive explanation is found in application brief *AB52998: Introducing AutoSRM: MRM Simplicity for High Performance Results.*[3]

Tuning

A complete automated tuning was provided with the TSQ 8000 GC-MS, ensuring reproducible tuning, even when different users were working with the instrument. The tune contained an automated leak check using the ratio between the native air/water background and a metered amount of air introduced into the source.

The tuning was saved to the instrument automatically and the last tune file was coupled to the instrument method by default, but it was also perfectly possible to attach another tune file to the method.
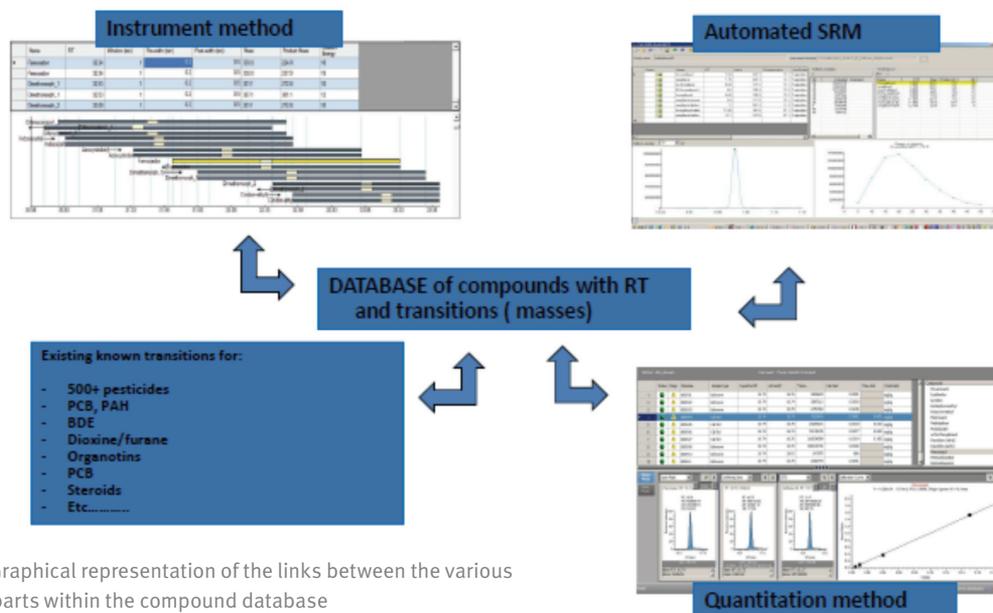
Figure 1. Graphical representation of the links between the various software parts within the compound database

Results and Discussion

Method Productivity & Performance

The goal of the developed method was to decrease the total work required by the laboratory for the analyses of so many compounds. For this entire list of compounds to be analyzed effectively, the TRACE 1310 GC and TG-XLBMS column were used to optimize the chromatographic separation of critical isomer pairs.
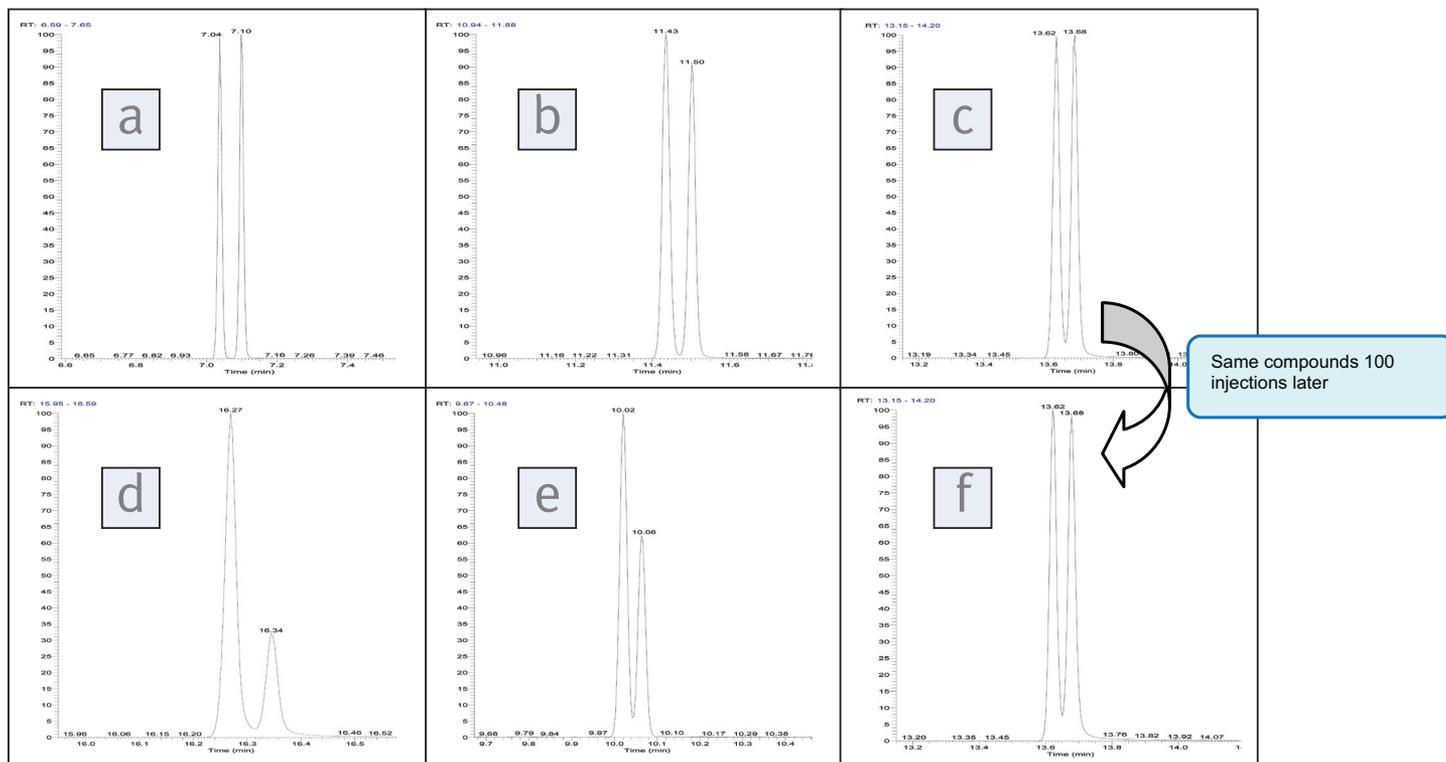
Figure 2: Chromatograms of several critical separations in a standard at 2000 pg absolute injection; except for benzo(b) and (k) fluoranthene that are depicted in building material with a concentration of 400 pg

> a: phenanthrene and anthracene
> b: chrysene and benzo(a)anthracene
> c: benzo(b) and benzo(k)fluroanthene
> d: indeno (1,2,3,c,d)pyrene and dibenzo(a,h) anthracene
> e: o,p DDD and p,p DDT
> f: benzo(b) and benzo(k)fluoranthene in building material **after 100 injections of samples**

The chromatographic performance was such that all compounds eluted within 17 minutes. The same separations were observed after more than 100 injections of water, soil, and building material extracts.

Calibration Curves

Calibration curves were produced in the range of 2 µg/L to 700 µg/L for the OCPs and PCBs. A higher range, 2 µg/L to 2,500 µg/L, was necessary for the PAHs. The curves were not corrected for internal standard calibration.

All curves had a regression coefficient higher than 0.995. Curves for a selection of target compounds are plotted in Figures 3 & 4.
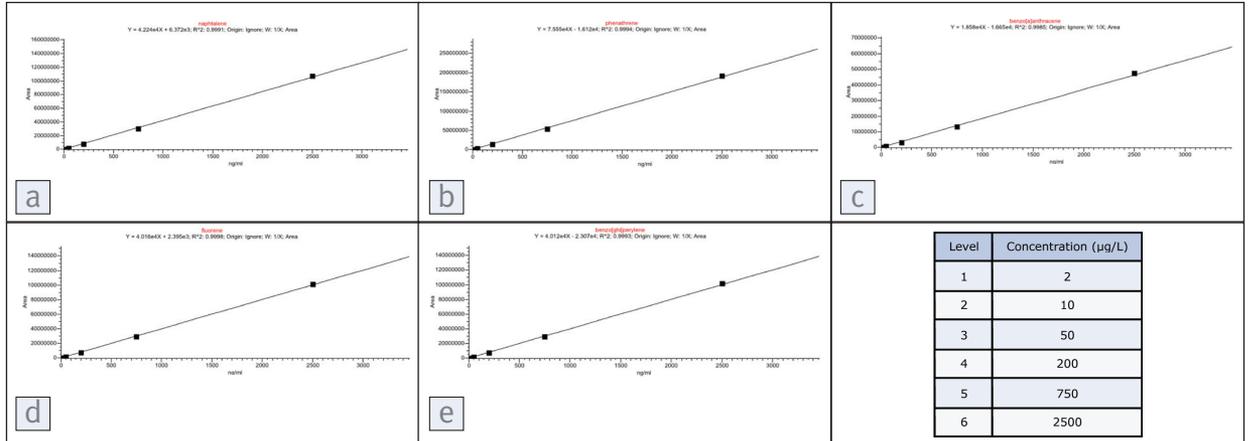
Figure 3: Calibration curves of various PAHs. The compounds and their respective regression coefficients were:

  a: naphthalene with R^2=0.9991
  b: phenanthrene with R^2= 0.9994
  c: benzo(a) anthracene with R^2= 0.9985
  d: fluorene with R^2= 0.9998
  e: benzoperylene with R^2= 0.9993
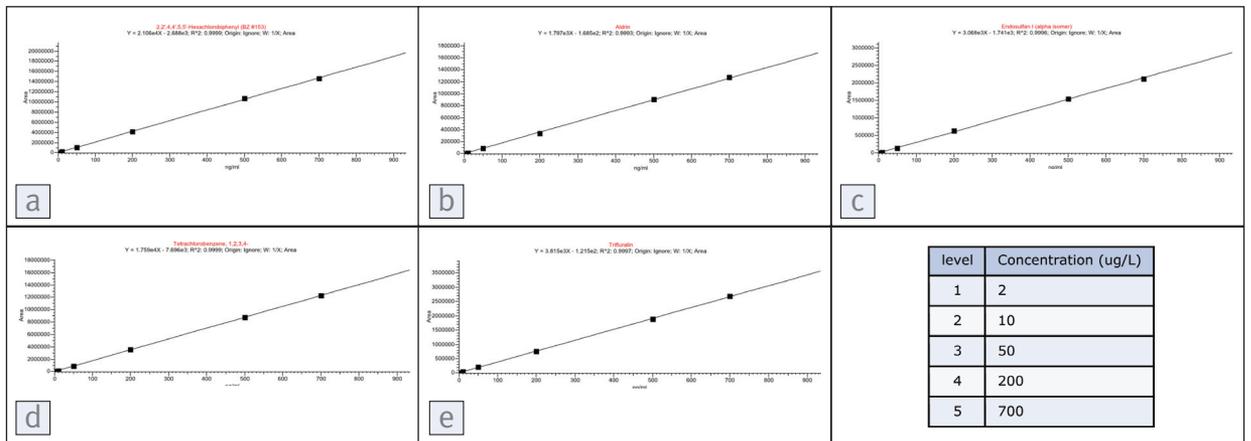
Figure 4: Calibration curves of various pesticides. The compounds and their respective regression coefficients were:

  a: PCB153 with R^2=0.9999
  b: aldrin with R^2= 0.9993
  c: alpha endosulfan with R^2= 0.9996
  d: tetrachlorobenzene with R^2= 0.9999
  e: trifluralin with R^2= 0.9999

Compounds at 2 µg/L level

At the lowest calibrated level (2 µg/L or 2 pg on column), all compounds gave excellent responses and high signal-to-noise values. A selection of extracted SRM chromatograms at this level are given in Figure 5.
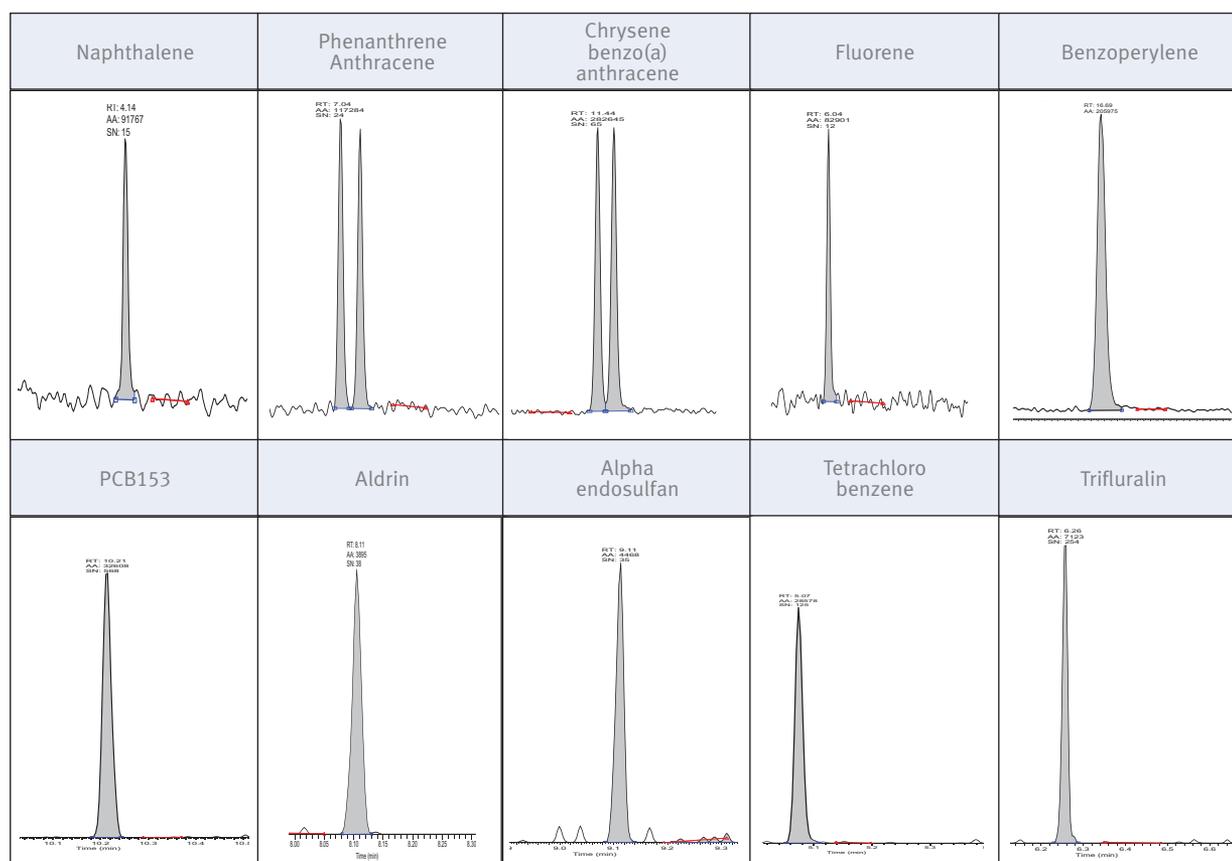
Figure 5: Peaks at 2 µg/L level; 2 pg absolute amount injected on column

Precision in Spiked Matrix Samples

In all three matrices, the repeatability was established by performing repeat injections of spiked soil extracts, spiked water extracts, and spiked building material extracts. All samples were analyzed seven times, and the RSD was calculated using an external calibration (Table 2).

Repeatability for all compounds in the matrix was below 10% RSD. TraceFinder software performed all integrations without manual intervention.

Table 2: Relative standard deviation of seven injected samples in various matrices

Compound	% RSD	% RSD	% RSD
	Building Material	Soil	Water
PCB180	2.5	6.4	5.3
PCB118	2.8	5.7	4.3
Benzo[a]anthracene	2.7	1.6	6.7
Benzo[a]pyrene	2.5	2.4	7.2
Benzo[b]fluoranthene	2.2	3.2	7.5
BHC-gamma (Lindane, gamma HCH)	2.9	7.3	7.8
Dieldrin	4.2	3.5	6.9
Endosulfan I (alpha isomer)	2.9	7.2	7.2
Endosulfan II (beta isomer)	3.4	7.7	7.3

Ion Ratio Stability

All compounds had at least two transitions in the method, and the two ions had been monitored throughout the samples, blanks, and standards.

Hexachloroethane	Ratio	Benzoperylene	Ratio
Average	0.479	Average	2.910
Standard deviation	0.025	Standard deviation	0.124
RSD	5.3%	RSD	4.3%
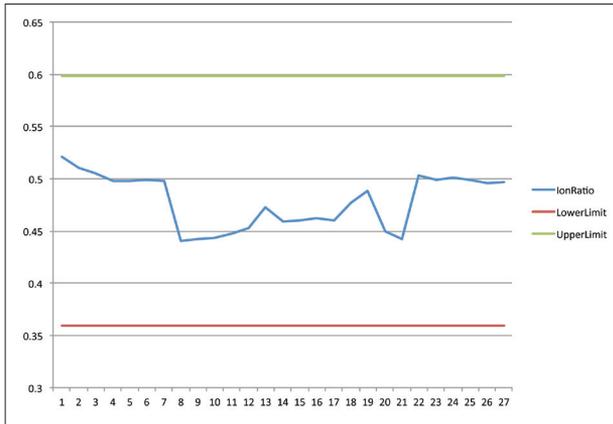
Figure 6: Ion ratio of hexachloroethane, plotted with the upper and lower allowed limit according to the EU guidelines for performance of analytical methods.[4] The average and the standard deviation are shown in the table above.

Throughout the complete series of calibration curves, water samples, soil samples, and building materials, the ion ratios were calculated. The ion ratio precision demonstrated good confirmation in both samples and standard injections across the concentration range.

Sample Results

A small selection of compound peaks at low levels in matrix is shown in Figure 7, demonstrating the sensitivity and selectivity of the measurements.

Below, a selection of matrix and compounds at low levels, and the concentrations, are given as the absolute amount on column.
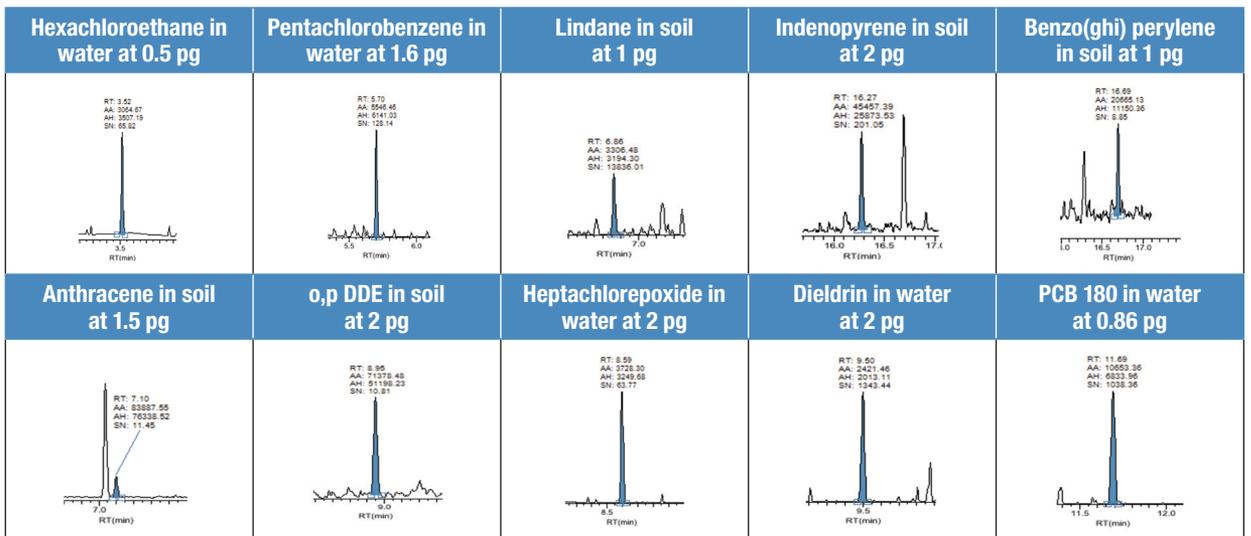
Figure 7: Compound peaks in various sample matrices at low levels

Conclusions

- The TSQ 8000 GC-MS/MS enabled simple method development, validation, and management using a combination of integrated software tools.

- The new method increased productivity in the laboratory by combining three separate methods into one, from three injections down to one injection.

- Quantitative performance of the system and methodology was excellent with a good level of linearity, excellent sensitivity, and high precision in a variety of environmental sample types.

References

1. Analysis of emerging persistent organic pollutants using GC-MS/MS; Kalachova *et al*. SETAC, Berlin 2012.

2. Ziegenhals, K.; Hubschmann, H.J. Fast-GC/HRMS to quantify the EU priority PAH. *J. Sep. Sci.* **2008**, *31*, 1779 – 1786.

3. Thermo Scientific Application Brief AB52998: Introducing AutoSRM: MRM Simplicity for High Performance Results; Cole J.

4. REGULATION (EC) No 2002/657 on analytical performance criteria.

5. Pesticides Method Reference, 2nd ed. 2011, Thermo Fisher Scientific, Austin, TX, USA, P/N 120390.

Addendum: SRM Transitions

Parent Mass (Da)	Product Mass (Da)	Collision Energy (V)	RT (min)	Start Time* (min)	Stop Time* (min)	Name
427.77	357.80	25	13.20	12.68	13.68	2,2',3,3',4,4',5,5'-Octachlorobiphenyl (BZ #194)
429.76	357.80	25	13.20	12.68	13.68	2,2',3,3',4,4',5,5'-Octachlorobiphenyl (BZ #194) Confirming 1
391.81	321.84	25	11.70	11.16	12.16	2,2',3,4,4',5,5'-Heptachlorobiphenyl (BZ #180)
393.81	323.84	25	11.70	11.16	12.16	2,2',3,4,4',5,5'-Heptachlorobiphenyl (BZ #180) Confirming 1
357.84	287.88	25	10.20	9.70	10.70	2,2',4,4',5,5'-Hexachlorobiphenyl (BZ #153)
359.84	289.87	25	10.20	9.70	10.70	2,2',4,4',5,5'-Hexachlorobiphenyl (BZ #153) Confirming 1
289.92	219.94	20	7.87	7.37	8.37	2,2',5,5'-Tetrachlorobiphenyl (BZ #52)
291.92	219.94	20	7.87	7.37	8.37	2,2',5,5'-Tetrachlorobiphenyl (BZ #52) Confirming 1
323.88	253.91	20	9.88	9.38	10.38	2,3',4,4',5-Pentachlorobiphenyl (BZ #118)
325.88	255.91	20	9.88	9.38	10.38	2,3',4,4',5-Pentachlorobiphenyl (BZ #118) Confirming 1
255.96	185.97	20	7.48	6.98	7.98	2,4,4'-Trichlorobiphenyl (BZ #28)
257.96	185.97	20	7.48	6.98	7.98	2,4,4'-Trichlorobiphenyl (BZ #28) Confirming 1
153.07	126.05	45	5.58	5.08	6.08	acenaphtene
153.07	151.07	40	5.58	5.08	6.08	acenaphtene Confirming 1
164.14	160.00	30	5.55	5.05	6.05	acenaphtene D10
164.14	162.00	20	5.55	5.05	6.05	acenaphtene D10 Confirming 1
152.06	102.03	30	5.43	4.93	5.93	acenaphtylene
152.06	126.05	20	5.43	4.93	5.93	acenaphtylene Confirming 1
276.08	272.08	60	16.70	16.20	17.20	benzo[ghi[perylene
276.08	274.08	40	16.70	16.20	17.20	benzo[ghi[perylene Confirming 1
216.89	180.91	8	6.48	5.98	6.98	BHC-alpha (benzene hexachloride)
218.89	182.91	8	6.48	5.98	6.98	BHC-alpha (benzene hexachloride) Confirming 1
240.17	212.00	30	11.40	10.94	11.94	chrysene D12
240.17	236.00	30	11.40	10.94	11.94	chrysene D12 Confirming 1
235.01	164.98	20	9.50	9.00	10.00	DDD-o,p'
237.01	164.98	20	9.50	9.00	10.00	DDD-o,p' Confirming 1
495.69	425.73	25	14.30	13.79	14.79	Decachlorobiphenyl (BZ #209)
497.69	427.73	25	14.30	13.79	14.79	Decachlorobiphenyl (BZ #209) Confirming 1
278.08	274.08	60	16.30	15.82	16.82	dibenzo[ah]anthracene

* Start and Stop Times are set automatically in timed-SRM mode of the TSQ 8000 by using a standard acquisition window of 60 s for all compounds

Addendum: SRM Transitions

Parent Mass (Da)	Product Mass (Da)	Collision Energy (V)	RT (min)	Start Time* (min)	Stop Time* (min)	Name
278.08	276.08	30	16.30	15.82	16.82	dibenzo[ah]anthracene Confirming 1
170.96	135.97	15	4.88	4.38	5.38	Dichlorobenzonitrile, 2,6- (Dichlobenil)
172.96	137.97	15	4.88	4.38	5.38	Dichlorobenzonitrile, 2,6- (Dichlobenil) Confirming 1
276.91	240.92	12	9.50	9.00	10.00	Dieldrin
278.91	242.92	12	9.50	9.00	10.00	Dieldrin Confirming 1
165.08	139.04	30	6.04	5.54	6.54	fluorene
165.08	163.08	30	6.04	5.54	6.54	fluorene Confirming 1
269.88	234.89	15	7.67	7.17	8.17	Heptachlor
271.88	236.89	15	7.67	7.17	8.17	Heptachlor Confirming 1
283.81	248.84	20	6.57	6.07	7.07	Hexachlorobenzene
285.81	250.83	20	6.57	6.07	7.07	Hexachlorobenzene Confirming 1
224.80	189.90	18	4.26	3.76	4.76	Hexachlorobutadiene
226.90	189.90	18	4.26	3.76	4.76	Hexachlorobutadiene Confirming 1
226.90	191.90	18	4.26	3.76	4.76	Hexachlorobutadiene Confirming 2
310.83	240.87	25	8.25	7.75	8.75	Isobenzan (Telodrin)
312.83	242.87	25	8.25	7.75	8.75	Isobenzan (Telodrin) Confirming 1
227.01	169.01	20	10.80	10.30	11.30	Methoxychlor, o,p'-
227.01	184.08	20	10.80	10.30	11.30	Methoxychlor, o,p'- Confirming 1
128.06	77.05	30	4.15	3.65	4.65	naphtalene
128.06	102.03	20	4.15	3.65	4.65	naphtalene Confirming 1
136.11	108.03	25	4.12	3.62	4.62	naphtalene D8
136.11	134.06	25	4.12	3.62	4.62	naphtalene D8 Confirming 1
247.85	141.92	25	5.68	5.18	6.18	Pentachlorobenzene
247.85	212.87	25	5.68	5.18	6.18	Pentachlorobenzene Confirming 1
264.00	230.00	30	14.40	13.85	14.85	perylene D12
264.00	260.00	30	14.40	13.85	14.85	perylene D12 Confirming 1
188.00	158.00	30	7.01	6.51	7.51	phenathrene D10
188.00	160.00	30	7.01	6.51	7.51	phenathrene D10 Confirming 1
207.00	136.00	16	6.14	5.64	6.64	TCMX
244.00	209.00	16	6.14	5.64	6.64	TCMX Confirming 1
242.00	207.00	16	6.14	5.64	6.64	TCMX Confirming 2
213.89	107.95	30	4.84	4.34	5.34	Tetrachlorobenzene, 1,2,4,5 +1,2,3,5
213.89	142.93	30	4.84	4.34	5.34	Tetrachlorobenzene, 1,2,4,5 +1,2,3,5 Confirming 1
264.09	160.05	15	6.26	5.76	6.76	Trifluralin
306.10	264.09	15	6.26	5.76	6.76	Trifluralin Confirming 1
213.89	107.95	30	5.07	4.57	5.57	Tetrachlorobenzene, 1,2,3,4-
213.89	142.93	30	5.07	4.57	5.57	Tetrachlorobenzene, 1,2,3,4- Confirming 1
218.89	182.91	8	6.75	6.25	7.25	BHC-beta Confirming 1
216.89	180.91	8	6.75	6.25	7.25	BHC-beta
218.89	182.91	8	6.85	6.35	7.35	BHC-gamma (Lindane, gamma HCH) Confirming 1
216.89	180.91	8	6.85	6.35	7.35	BHC-gamma (Lindane, gamma HCH)
178.08	152.07	25	7.04	6.54	7.54	phenathrene
178.08	176.08	20	7.04	6.54	7.54	phenathrene Confirming 1
178.08	152.07	25	7.11	6.61	7.61	anthracene
178.08	176.08	20	7.11	6.61	7.61	anthracene Confirming 1
216.89	180.91	8	7.14	6.64	7.64	BHC-delta

* Start and Stop Times are set automatically in timed-SRM mode of the TSQ 8000 by using a standard acquisition window of 60 s for all compounds

Parent Mass (Da)	Product Mass (Da)	Collision Energy (V)	RT (min)	Start Time* (min)	Stop Time* (min)	Name
218.89	182.91	8	7.14	6.64	7.64	BHC-delta Confirming 1
216.89	180.91	8	7.24	6.74	7.74	BHC-epsilon
218.89	182.91	8	7.24	6.74	7.74	BHC-epsilon Confirming 1
188.14	160.00	30	7.53	7.03	8.03	Alachlor Confirming 1
188.14	158.00	30	7.53	7.03	8.03	Alachlor
352.83	252.88	15	8.60	8.10	9.10	Heptachlor exo-epoxide (isomer B)
352.83	281.88	15	8.60	8.10	9.10	Heptachlor exo-epoxide (isomer B) Confirming 1
288.86	252.88	15	8.65	8.15	9.15	Heptachlor endo-epoxide (isomer A) Confirming 1
288.86	218.95	15	8.65	8.15	9.15	Heptachlor endo-epoxide (isomer A)
246.05	175.97	25	8.93	8.43	9.43	DDE-o,p'
317.94	245.95	20	8.93	8.43	9.43	DDE-o,p' Confirming 1
325.88	255.91	20	8.99	8.49	9.49	2,2',4,5,5'-Pentachlorobiphenyl (BZ #101) Confirming 1
323.88	253.91	20	8.99	8.49	9.49	2,2',4,5,5'-Pentachlorobiphenyl (BZ #101)
202.08	200.08	30	9.10	8.60	9.60	pyrene Confirming 1
202.08	176.08	35	9.10	8.60	9.60	pyrene
246.05	175.97	25	9.39	8.89	9.89	DDE-p,p'
317.94	245.95	20	9.39	8.89	9.89	DDE-p,p' Confirming 1
242.89	207.91	10	9.95	9.45	10.45	Endosulfan II (beta isomer) Confirming 1
240.89	205.91	10	9.95	9.45	10.45	Endosulfan II (beta isomer)
235.01	164.98	20	10.00	9.52	10.52	DDD-p,p'
237.01	164.98	20	10.00	9.52	10.52	DDD-p,p' Confirming 1
237.01	165.07	20	10.10	9.56	10.56	DDT-o,p' Confirming 1
235.01	165.07	20	10.10	9.56	10.56	DDT-o,p'
359.84	289.87	25	10.60	10.13	11.13	2,2',3,4,4',5'-Hexachlorobiphenyl (BZ #138) Confirming 1
357.84	287.88	25	10.60	10.13	11.13	2,2',3,4,4',5'-Hexachlorobiphenyl (BZ #138)
237.01	165.07	20	10.50	10.03	11.03	DDT-p,p' Confirming 1
235.01	165.07	20	10.50	10.03	11.03	DDT-p,p'
228.08	202.08	35	11.50	11.00	12.00	benzo[a]anthracene
228.08	226.08	30	11.50	11.00	12.00	benzo[a]anthracene Confirming 1
228.08	202.08	35	11.40	10.94	11.94	chrysene
228.08	226.08	30	11.40	10.94	11.94	chrysene Confirming 1
252.09	226.08	35	13.60	13.12	14.12	benzo[b]fluoranthene
252.09	250.09	30	13.60	13.12	14.12	benzo[b]fluoranthene Confirming 1
252.09	250.09	30	14.30	13.76	14.76	benzo[a]pyrene Confirming 1
252.09	226.08	35	14.30	13.76	14.76	benzo[a]pyrene
252.09	250.09	30	13.70	13.21	14.21	benzo[k]fluoranthene Confirming 1
252.09	226.08	35	13.70	13.21	14.21	benzo[k]fluoranthene
202.08	176.08	35	8.72	8.22	9.22	fluoranthene Confirming 1
202.08	200.08	30	8.72	8.22	9.22	fluoranthene
292.90	185.93	30	8.11	7.61	8.61	Aldrin
292.90	257.91	10	8.11	7.61	8.61	Aldrin Confirming 1
262.91	192.93	30	8.48	7.98	8.98	Isodrin
262.91	190.93	30	8.48	7.98	8.98	Isodrin Confirming 1
240.89	205.91	10	9.11	8.61	9.61	Endosulfan I (alpha isomer)
242.89	207.91	10	9.11	8.61	9.61	Endosulfan I (alpha isomer) Confirming 1

* Start and Stop Times are set automatically in timed-SRM mode of the TSQ 8000 by using a standard acquisition window of 60 s for all compounds

Addendum: SRM Transitions

Parent Mass (Da)	Product Mass (Da)	Collision Energy (V)	RT (min)	Start Time* (min)	Stop Time* (min)	Name
276.08	272.08	60	16.30	15.77	16.77	indeno[123cd]pyrene
276.08	274.08	40	16.30	15.77	16.77	indeno[123cd]pyrene Confirming 1
202.90	167.90	10	3.51	3.01	4.01	Hexachloroethane Confirming 1
117.00	82.00	25	3.51	3.01	4.01	Hexachloroethane Confirming 2
200.90	165.9	10	3.51	3.01	4.01	Hexachloroethane

* Start and Stop Times are set automatically in timed-SRM mode of the TSQ 8000 by using a standard acquisition window of 60 s for all compounds

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