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].

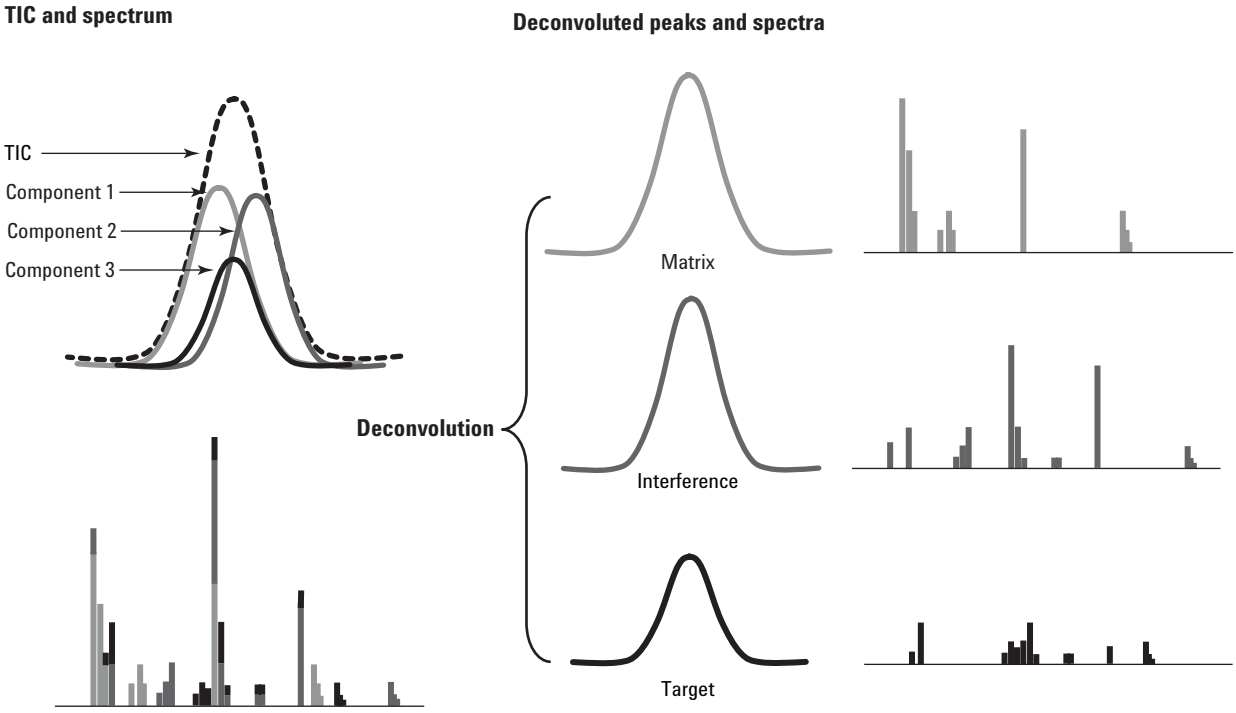

Figure 1. An illustration of mass spectral deconvolution process.

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.

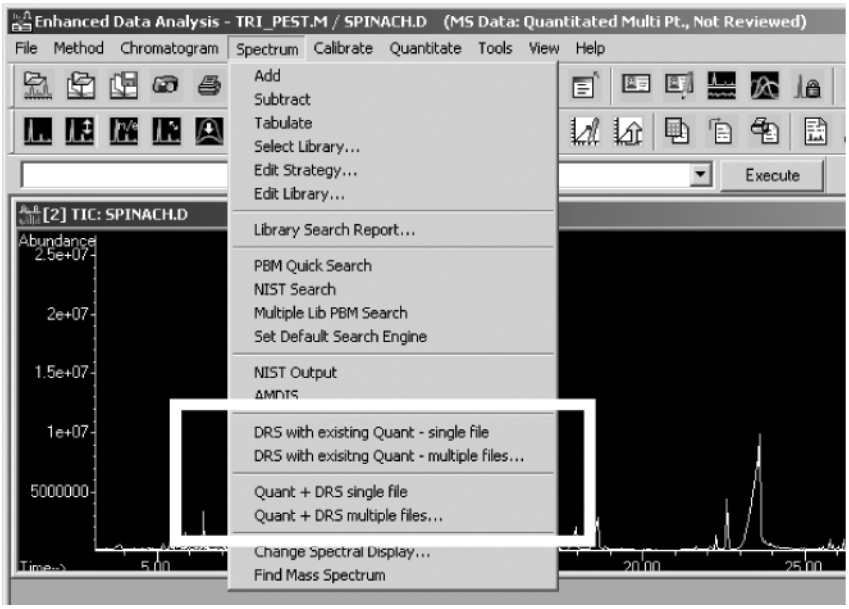

Figure 2. ChemStation pull down menu showing options for running the DRS on single or multiple files.

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.

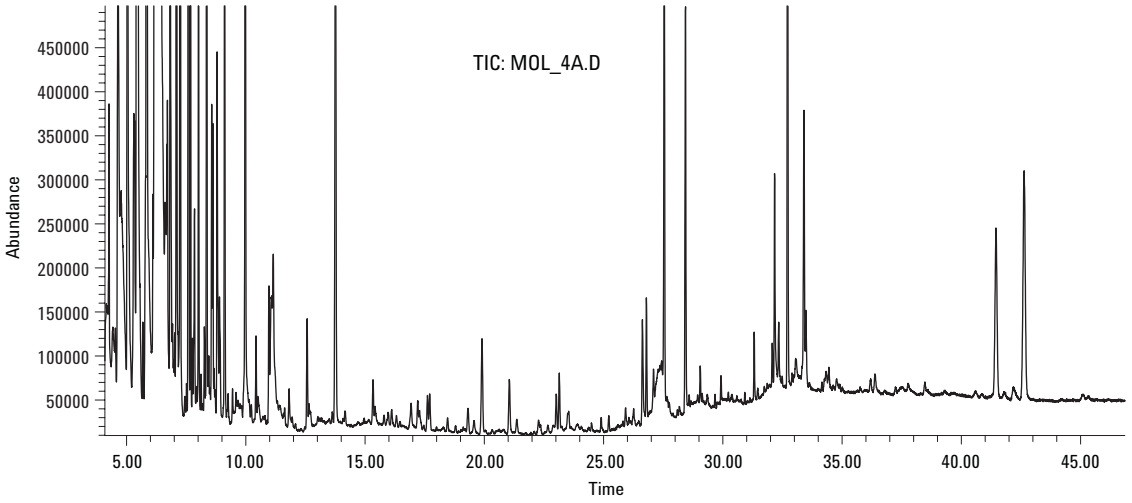

Figure 3. TIC of an herbal mix.

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Address C:\MSDChem\1\DATA\Hans Mol Data Feb 04 samples\Mar03_X4\MOL_4A.D\MOL_4A.htm Go Links

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

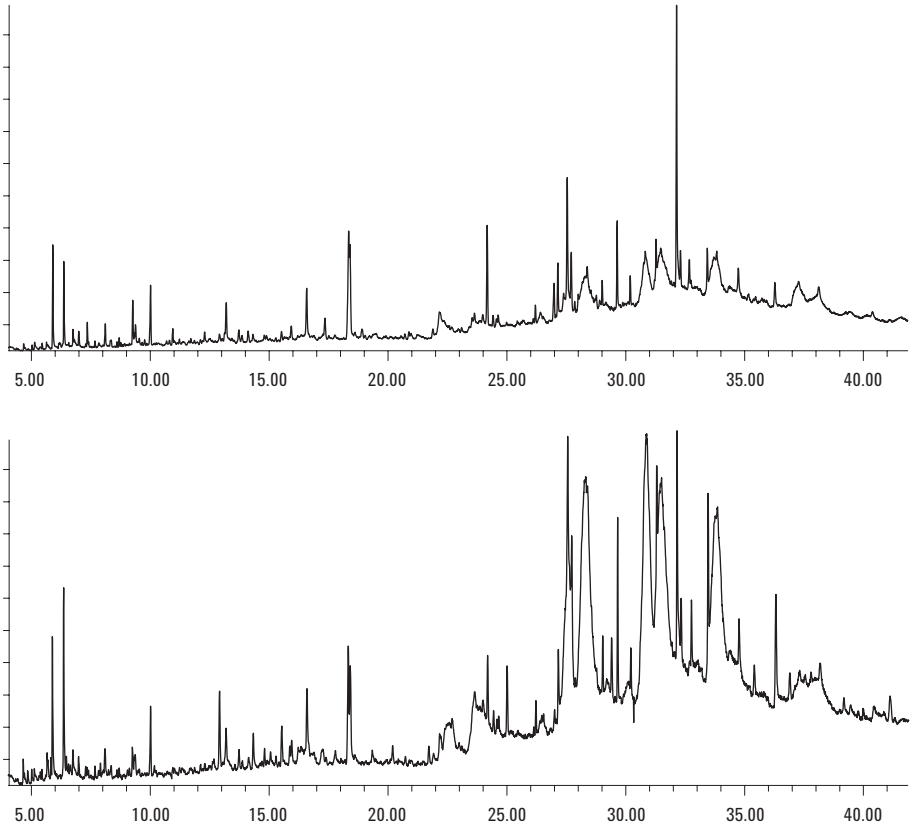

Figure 5. TICs of typical surface water extracts provided by the CDFA.

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.

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References

1. C.D.S Tomlin, editor. The Pesticide Manual, 13th edition, British Crop Protection Council, Surry, UK (2003).
2. http://www.who.int/pcs/docs/Classif_Pestic_2000-02.pdf
3. J. Cook, M.P. Beckett, B. Reliford, W. Hammock, and M. Engel (1999) *J. AOAC Int.*, **82** (6), 1419-1435.
4. M.A. Luke, J.E. Froberg, and H.T. Masumoto, (1975) *J. Assoc. Off. Anal. Chem.* **58**, 1020-1026.
5. M. Luke, J. Froberg, G. Doose, and H. Masumoto, (1981) *J. Assoc. Off. Anal. Chem.* **64**, 1187-1195.
6. B. McMahon and N. Hardin (1994) Pesticide Analytical Manual, Vol. 1, 3rd Ed., U.S. Food and Drug Administration, Washington, DC.
7. J. Fillion, R. Hindle, M. Lacroux, and J. Selwyn (1995) *J. AOAC Int.* **78**, 1252-1266.
8. J. Fillion, F. Sauvé, and J. Selwyn (2000) *J AOAC Int.,* **83**, 698-712.
9. P. Wylie and B. Quimby, "A Method Used to Screen for 567 Pesticides and Suspected Endocrine Disrupters," Agilent Technologies, publication 5967-5860E www.agilent.com/chem.
10. H. Prest, P. Wylie, K. Weiner, and D. Agnew, "Efficient Screening for Pesticides and Endocrine Disrupters Using the 6890/5973 GC/MSD System," Agilent Technologies, publication 5968-4884E www.agilent.com/chem.
11. K. Weiner and H. Prest, "Retention Time Locking: Creating Custom Retention Time Locked Screener Libraries," Agilent Technologies, publication 5968-8657E www.agilent.com/chem.
12. National Institute of Standards and Technology, AMDIS Literature and Downloads website:

    http://www.amdis.net/What_is_AMDIS/AMDIS_Literature_and_Downloads/amdis_literature_and_downloads.html.

13. V. Giarocco, B. Quimby and M. Klee, “Retention Time Locking: Concepts and Applications,” Agilent Technologies, publication 5966-2469E www.agilent.com/chem.

14. http://www.chem.agilent.com/cag/servsup/usersoft/main.html#RTL.

15. M. Szelewski and C.K. Meng, “Building and Editing RTL Screener Databases and Libraries,” Agilent Technologies, publication 5989-0916EN www.agilent.com/chem.

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