Rapid Analysis for 567 Pesticides and Endocrine Disrupters by GC/MS using Deconvolution Reporting Software

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Introduction

More than 700 pesticides are approved for use around the world, many of which are suspected endocrine disrupters. Other pesticides, though no longer used, persist in the environment where they bioaccumulate in the flora and fauna. Analytical methods target only a subset of the possible compounds. The analysis of food and environmental samples for pesticides is usually 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. Even then, it can be difficult to detect trace levels of contaminants in the presence of the remaining matrix.

For efficiency, multi-residue 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. 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. Today, more and more pesticide laboratories are relying upon LC with mass spectral detection (LC/MS) and GC/MS as their primary analytical tools. 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 methods.

Using the techniques of retention time locking (RTL) and RTL database searching[1-3] together with spectral deconvolution, 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 co-elute with matrix compounds while RTL helps to eliminate false positives and gives greater confidence in the results.

Methods and Materials

Instrumentation: An Agilent Technologies (Wilmington, DE USA) 6890 N GC was equipped with an Agilent 5973 inert Mass Selective Detector (MSD) and an Agilent 7683 Automatic Sampler. Injections of five to 25 µL were made using a PTV inlet in the solvent vent mode. A 30-m X 0.25-mm X 0.25-µm HP-5MS (Agilent) column was operated with helium carrier gas in the constant pressure mode with the following 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). The PTV inlet parameters were as follows: 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; The following parameters were set for the MSD: scan range - 45-500 u; Tune – Autotune; Source, quad, and transfer line temperatures – 230, 150, 280°C, respectively; Solvent delay – 4 min. The following software packages for data acquisition and data processing were obtained from Agilent: G1701DA (Ver. D01.00 sp1) GC/MS ChemStation with the addition of Agilent G1716AA Deconvolution Reporting Software, the G1033A NIST MS Library with the NIST Automated Mass Spectral Deconvolution and Identification System (AMDIS)[4] software, and the G1049A RTL Pesticide Library.

Samples: Vegetable extracts were obtained from Dr. Mark Lee 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 Dr. Mark Lee and were analyzed in this laboratory using the Deconvolution Reporting Software.

Results and Discussion

Deconvolution Reporting Software (DRS): The 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 MS Library, and 3) the AMDIS software, also from NIST. Included in the DRS, are mass spectral and locked retention time 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 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. The DRS then sends the data file to AMDIS, which deconvolutes the spectra and searches the pesticide/endocrine disrupter database using the deconvoluted full spectra. A filter can be set in AMDIS, which requires the analyte's retention time to fall within a specified time window. Because RTL is used to reproduce the database retention times with high precision, this window can be quite small – typically 10-20 seconds. Finally, the deconvoluted spectra for all of the hits 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.

Pesticides in an herbal mix: Figure 1 shows a total ion chromatogram from the extract of an herbal mix. Figure 2 shows the MSD Deconvolution Report for that sample. 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 1: Total ion chromatogram of an herbal Mix. Figure 2 shows the deconvolution report for this sample, indicating the presence of several pesticides.
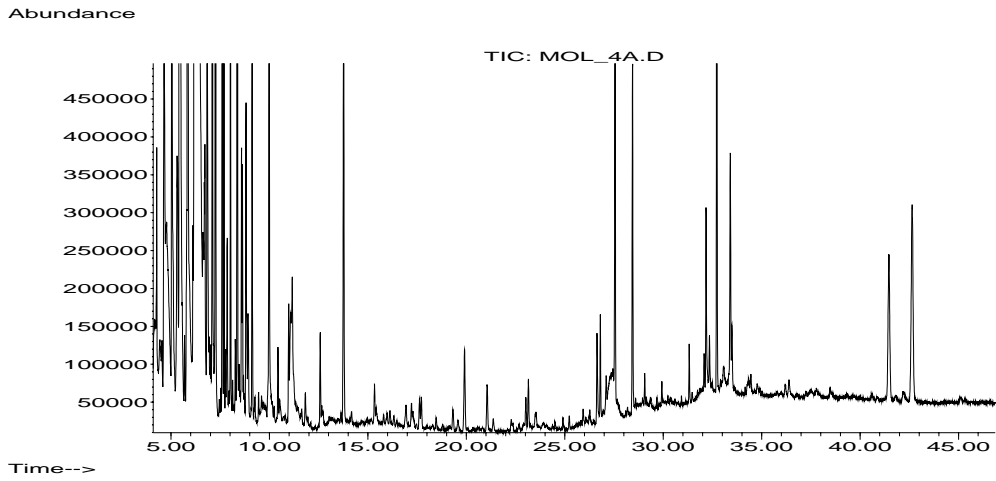
Figure 2: MSD Deconvolution Report generated for the extract of an herbal mix. The GC/MS total ion chromatogram for this extract is shown in Figure 1.

The DRS report in Figure 2 lists the retention time, CAS number, and compound name for each hit. Phenanthrene-d_{10} is listed at the bottom of the report as the internal standard 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*. True quantitation is possible when calibration curves have been determined for individual compounds, but this is not practical for all of the pesticides in the database. Experience has shown that most estimates reported using an average pesticide response factor fall within a factor of five of their true values.

Column 5 in the report shows the match factor obtained through AMDIS deconvolution and library searching of the deconvoluted full spectrum. In this case, several more targets were identified by AMDIS than were found by the ChemStation software, which is typical for dirty samples. When locked retention times are available for compounds in the spectral library, a requirement can be set which stipulates that a peak must fall within a narrow time window around the predicted retention time. Column 6 shows the retention time difference (in seconds) between the compound's library retention time and its actual value in the chromatogram.

Figure 2 shows that the software identified a number of 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 entire NIST mass spectral library, which contains about 147,000 entries. When the NIST library search finds a compound in the top 100 matches that agrees with the AMDIS results, its match factor is listed in column seven. The match 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 2), which eluted at 34.770 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.

Analysis of Surface Water Samples: 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.   In one study, the California Department of Food and Agriculture analyzed seventeen surface water extracts for pesticides. TICs for two typical samples are shown in Figure 3. The CDFA used RTL and RTL database searching but with no deconvolution. The same data files were then analyzed using the DRS for comparison.

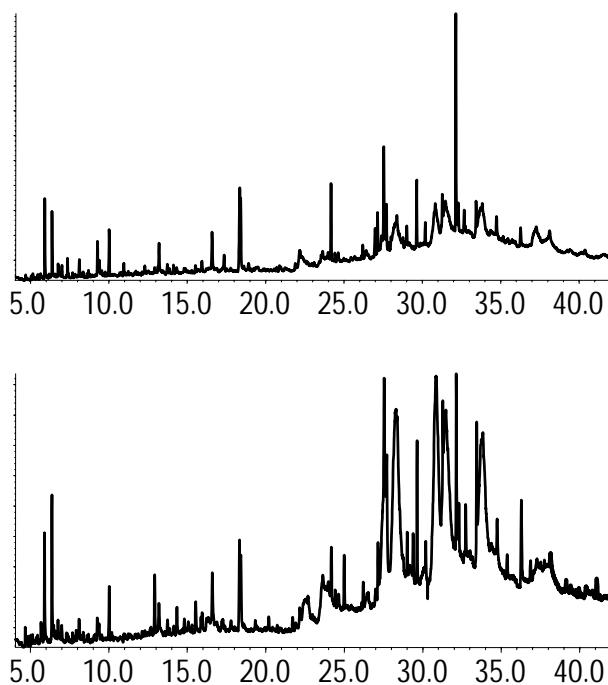**Figure 3:** TICs of typical surface water extracts provided by the CDFA.

Table 1 shows the results from the initial CDFA analysis of the seventeen 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 eight 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 pesticide hits 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 1: 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 devonvolution. 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

The AMDIS software from NIST is a powerful tool for finding trace contaminants in very complex mixtures. However, the software is complex and it can take a long time to understand all of its features, set points, and nuances. By incorporating AMDIS into the Deconvolution Reporting Software, it becomes almost transparent and runs in the background. The user needs to make only a few AMDIS set point choices in the beginning, which could be used for most or all subsequent samples. The inclusion of RTL and a retention time database in the analytical method has proven to be essential for complex samples to avoid numerous false positives. Experience so far has shown that the combination of ChemStation target compound analysis, AMDIS deconvolution with RTL Pesticide Library searching, and NIST library confirmation is able to find and identify pesticides and endocrine disrupters that would otherwise be missed. The amount of time required to review the data is greatly reduced.

Acknowledgements

References
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[3] Weiner K. and Prest H. (1999) Agilent Technologies Application Note No. 5968-8657E.
[4] 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