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Organophosphorus Pesticides in Apple Matrix by GC/MS/FPD using an Agilent J&W DB-35ms Ultra Inert GC Column

Application Note

Environmental and Food Safety

Author

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Abstract

The Agilent J&W DB-35ms Ultra Inert (UI) 20 m × 0.18 mm × 0.18 µm column effectively resolved the analytes of interest producing excellent peak shape for even the more problematic organophosphorus (OP) pesticides. The detection limits for most of the pesticides were 15-25 ng/mL. Recovery studies were performed by spiking with a standard solution to achieve the desired concentrations in an apple matrix; 150, 300 and 750 ng/mL GC/MS/SIM and 50, 100, 250 ng/mL FPD in phosphorus mode. Recoveries were >77% for most of the pesticides by GC/MS/SIM and >75% by GC/FPD.

This application note details a quick and effective analytical method for the determination of low ppm and trace level organophosphorus pesticides residues in apple extract. A capillary flow technology (CFT) device was installed post-column to split the effluent between the MSD and FPD, implementing an automated backflush that diminished residual sample carryover and reduced instrument cycle times. This multi-signal configuration allowed for full scan, selective ion monitoring (SIM), and flame photometric detection from a single injection.

A simplified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method provided sufficient sample matrix cleanup while preserving low level analyte detection.

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Introduction

Organophosphorous (OP) pesticides are widely used in the agricultural industry for crop protection. Human toxicities for this class of molecules have shown acute as well as chronic effects from pesticide poisoning. OP pesticides affect the nervous system of insects and mammals by inhibiting an enzyme, acetylcholinesterase, important in helping regulate nerve impulses [1].

Children are considered more susceptible to organophosphate toxicity because their pesticide dose per body weight is larger compared to that of adults [2]. Children also have lower levels of detoxifying enzymes that deactivate OP pesticides, contributing to their vulnerability to pesticide exposure [3,4]. Recent studies have shown a correlation between OP pesticides exposure and an increased risk for attention deficit hyperactivity disorder (ADHD) and other neurodevelopmental deficits in children [3,5,6,7]. Because the main source of exposure for children is through consumption of food containing OP pesticide residues [2,8], analytical testing capable of determining residual pesticides in food samples is critical.

The multiresidue determination of pesticides in fruits and vegetables usually involves an organic extraction of the pesticides from the plant matrix, followed by a cleanup procedure to remove co-extractives and other interferences. Anastassiades et al [9] developed a QuEChERS method for the analysis of pesticide residues in produce. This approach simplifies the traditional, labor intensive extraction and cleanup procedure, while providing a fast, robust, and cost effective method suitable for extracting pesticide residues.

Chromatographically active compounds such as organophosphorus pesticides can adsorb onto active sites in the sample flow path, particularly at trace levels, compromising an analyte's response. These pesticides tend to show peak tailing through interaction with active sites in a chromatographic system. This makes analysis challenging, particularly in difficult sample matrices. Minimizing activity in the GC column is essential to ensure accurate quantitation. Agilent's J&W DB-35ms Ultra Inert (UI) column minimizes column activity so difficult and active analytes can be consistently analyzed at trace levels. The use of the midpolarity DB-35ms UI phase also offers additional selectivity over a nonpolar phase, which can assist in resolving potentially coeluting peaks, or shift a peak of interest away from matrix interferences.

A gas chromatographic system capable of multisignal detection can provide complementary data for identification, confirmation, and quantitation of target analytes from a single injection. This method provides simultaneous detection of organophosphorus pesticides by GC/MS/SIM and FPD in phosphorus mode by splitting the column effluent between the MSD and FPD. The approach chosen here uses a GC/MSD/FPD system to identify and confirm the order of elution for peaks of interest. Once the elution order is established, the chromatographic parameters can easily be transferred to a GC/FPD system. The use of FPD detection without flow splitting is expected to increase sensitivity threefold, further improving lower level detection.

The GC/MS system was also equipped with backflush capability, which shortens instrument cycle time by backflushing late-eluting matrix components through the inlet purge valve. Long bakeout times between injections are avoided by using this technique. Backflushing has the additional benefit of increasing the time intervals for source cleaning by effectively clearing deleterious matrix components from the system [10].

Experimental

An Agilent 7890 GC with an Agilent 5975C MSD equipped with a flame photometric detector and Agilent 7683B automatic liquid sampler were used for this series of experiments. A purged two-way capillary flow technology (CFT) device was used to split the effluent 3:1 to the MSD:FPD. The CFT device also allowed for post-column backflush. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists the flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions

GC/MSD:	Agilent 7890 GC/Agilent 5975C Series GC/MSD
Sampler:	Agilent 7683B automatic liquid sampler, 5.0 µL syringe (Agilent p/n 5181-1273)
CFT Device:	Purged 2-way splitter (Agilent p/n G3180B) Split Ratio 3:1 MSD:FPD
MSD Restrictor:	1.2 m × 0.15 mm id deactivated fused silica tubing
FPD Restrictor:	1.4 m × 0.15 mm id deactivated fused silica tubing
PCM 1:	3.8 psi constant pressure
Inlet:	1 µL splitless; 250 °C, purge flow 60 mL/min at 0.25 min, gas saver on at 2 min 20 mL/min
Column:	Agilent J&W DB-35ms UI 20 m × 0.18 mm × 0.18 µm (Agilent p/n121-3822UI)
Carrier:	Helium, constant pressure 43.5 psi at 95 °C
Oven:	95 °C (1.3 min), 15 °C/min to 125 °C, 5 °C/min to 165 °C, 2.5 °C/min to 195 °C, 20 °C/min to 280 °C (3.75 min)
Postrun Backflush:	5 min at 280 °C, PCM 1 pressure 70 psi during backflush, 2 psi inlet pressure during backflush
MSD:	310 °C transfer line, 310 °C source, 150 °C quad

Table 2. Flow Path Supplies

Vials:	Amber crimp top glass vials (Agilent p/n 5183-4496)
Vial Caps:	Crimp caps (Agilent p/n 5181-1210)
Vial inserts:	250 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 liner:	Deactivated dual taper Helix liner (Agilent p/n 5188-5398)
Ferrules:	0.4 mm id short; 85/15 vespel/graphite (Agilent p/n 5181-3323)
PCT fittings:	Internal nut (Agilent p/n G2855-20530)
PCT ferrules:	SilTite ferrules, 0.25 mm id (Agilent p/n 5188-5361)
20x magnifier:	20x Magnifier loop (Agilent p/n 430-1020)

Reagents and Chemicals

All reagents and solvents were HPLC or Ultra Resi grade. Acetonitrile (ACN) from Honeywell (Muskegon, MI, USA), toluene from Burdick & Jackson, and acetone from JT Baker was purchased through VWR International (West Chester, PA, USA). The 12-component custom pesticide standard was prepared by Ultra Scientific (N. Kingstown, RI, USA).

Solutions and Standards

The OP pesticide stock standard solution (100 µg/mL of 12 organophoshorus pesticides) was diluted in acetone to yield spiking solutions 1 and 10 µg/mL. A surrogate standard, triphenyl phosphate (TPP), was prepared at concentrations of 1, 15 and 100 µg/mL in toluene. The spiking solutions were used to prepare the calibration curves in the matrix blank extract by appropriate dilution.

Sample Preparation

An organic apple sample was purchased from a local grocery store. The apple was chopped into small cubes and frozen at –80 °C overnight. The samples were then comminuted thoroughly to achieve sample homogeneity. The sample extraction method used the QuEChERS method. Figure 1 illustrates the sample preparation procedure graphically in a flow chart.

Samples containing 15 (± 0.1) grams of apple were weighed into centrifuge tubes. QC samples were spiked with appropriate amount of spiking solutions to yield QC samples with quantitative concentrations relative to the 3:1 split ratio of 150, 300, and 750 ng/mL levels for GC/MS-SIM determination, and 50, 100, and 250 ng/mL by flame photometric detection. Each sample received a 15-mL aliquot of ACN. Two ceramic bars (Agilent p/n 5982-9313) were added to each sample to aid in sample extraction. The samples were vortexed for 1 minute. An Agilent original QuEChERS extraction salt packet (Agilent p/n 5982-5555) containing 6 grams of $MgSO_4$ and 1.5 grams sodium chloride was added to each centrifuge tube. The capped tubes were shaken on a Geno/Grinder @1500 rpm for 1 minute. The samples were centrifuged at 4000 rpm for 5 min.

An 8 mL aliquot of the upper layer was transferred to an Agilent QuEChERS General Fruits and Vegetables dispersive SPE 15 mL tube (Agilent p/n 5982-5058). The dSPE tube was vortexed for 1 minute and then centrifuged at 4000 rpm for 3 minutes to complete the sample extraction. The extract from the dSPE tube was transferred to a GC vial and analyzed by SIM GC/MS and GC/FPD using the chromatographic conditions listed in Table 1.

Extractions of water and acetonitrile aliquots were prepared in the same manner as the samples and served as reagent blanks.

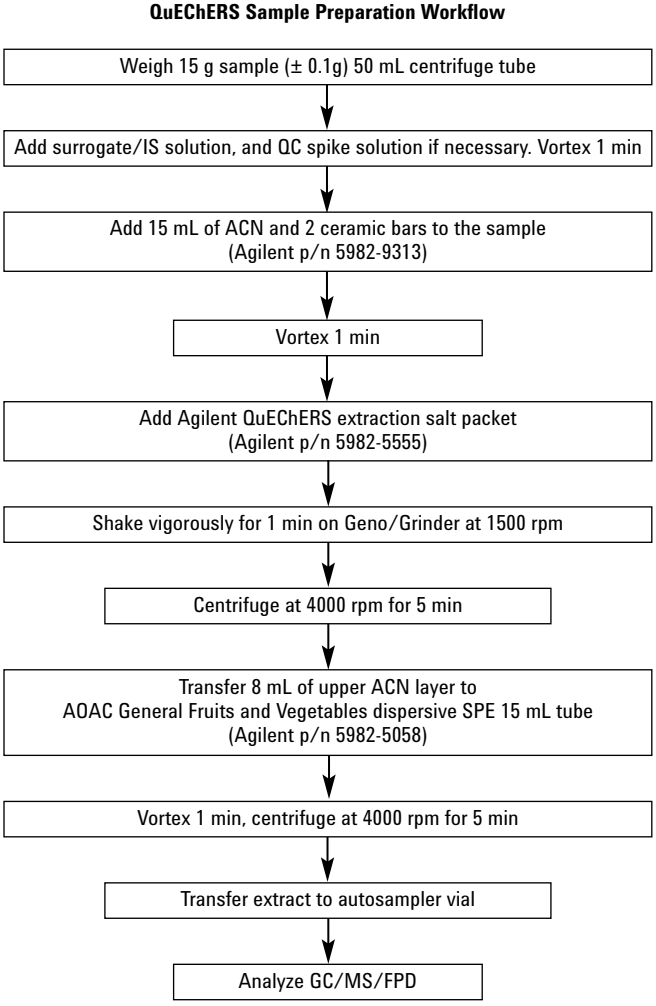

Figure 1. Flow chart of the Agilent QuEChERS extraction procedure for apple samples.

Discussion of Results

The organophosphorus pesticides were resolved on an Agilent J&W DB-35ms UI 20 m × 0.18 mm × 0.18 µm analysis column in about 30 minutes. The 12-component pesticide matrix-matched standard shown in Figure 2 shows good peak shapes for the pesticides in both the GC/MS/SIM and FPD chromatograms. Organophosphorus pesticides, particularly the more polar pesticides can be problematic, often yielding broad peak shapes or excessive tailing making reliable quantitation at low levels difficult. The high level of inertness of the DB-35ms UI column results in better peak shape and decreased sample adsorption allowing lower detection limits. Figure 3 depicts the excellent peak shape seen for four of the more polar OP pesticides (oxydemeton-methyl, methamidophos, mevinphos, acephate) with the DB-35ms UI column.

The performance of the DB-35ms UI high efficiency column yielded excellent linearity and recovery over the calibration range of this study. The linearity of the column as defined by the r^2 values of the pesticide standard curve was ≥0.992 for all the pesticides using both detectors. The individual OP pesticide analyte values are shown in Table 3.

The GC/MS/SIM analysis was able to detect down to the 15–20 ng/mL range for most of the pesticides. A higher SIM signal is necessary to quantify the more volatile pesticides below the 30 ng/mL range due mainly to matrix interferences. Because flame photometric detection in phosphorus mode is selective only for analytes containing phosphorus, it is able to detect low levels of OP pesticides in complex matrices without the matrix interferences. The FPD was able to detect the OP pesticides down to 15 ng/mL with the exception of naled, which could only be detected at higher levels (>25 ng/mL). Naled can undergo debromination, which can have an impact on detection, especially at trace levels. The detection levels for the targeted OP pesticides were well below the US maximum residue levels (MRLs) in an apple matrix, except in the case of chlorpyrifos, which has an MRL of 10 ppb for apples and grapes [11]. Analysis by GC/FPD without flow splitting offers increased sensitivity to monitor the lower levels of detection needed for chlorpyrifos.

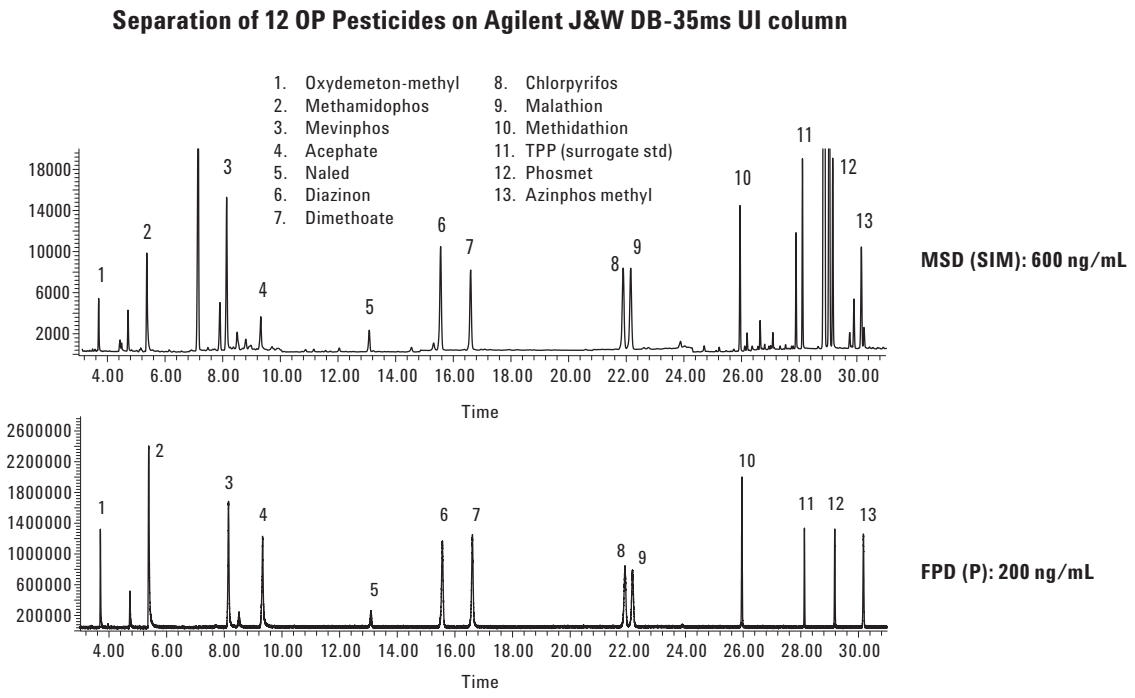

Figure 2. GC/MS-SIM and FPD chromatograms of a matrix matched organophosphorus pesticides standard analyzed on an Agilent J&W DB-35ms UI 20 m × 0.18 mm × 0.18 µm capillary GC column (Agilent p/n 121-3822UI). Chromatographic conditions are listed in Table 1. The effluent split ratio is MSD:FPD = 3:1.

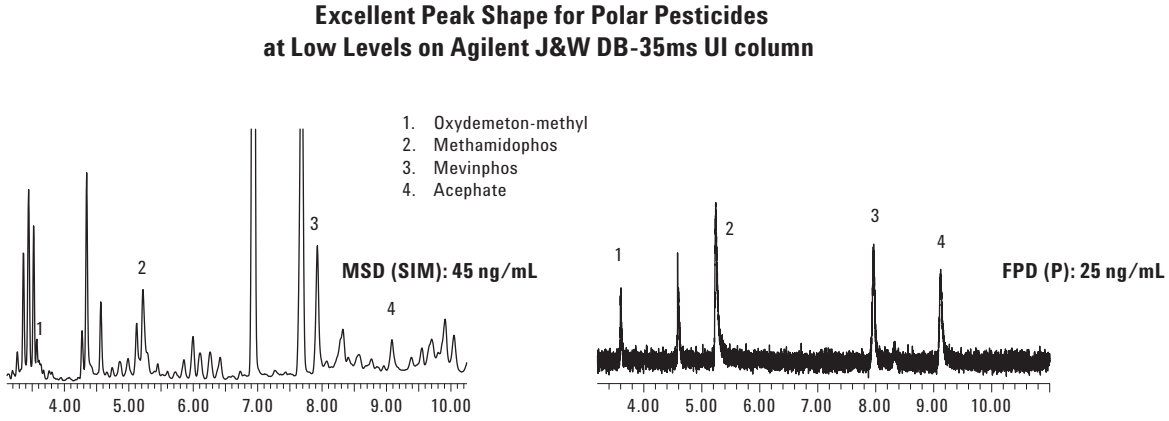

Figure 3. Enlarged section of GC/MS/SIM and FPD chromatograms of the more problematic polar pesticides analyzed on an Agilent J&W DB-35ms UI capillary column (Agilent p/n 121-3822UI). Chromatographic conditions are listed in Table 1. The effluent split ratio is MSD:FPD = 3:1.

Table 3. Correlation Coefficients for the OP Pesticides Calibration Standards Analyzed by GC/MS-SIM and FPD in Phosphorus Mode with a Split Ratio for MSD:FPD = 3:1

Excellent Linearity of OP Pesticides on Agilent J&W DB-35ms UI Column

	MSD	***FPD***
Oxydemeton-methyl	0.994	0.997
Methamidophos	0.997	0.997
Mevinphos	0.997	0.999
Acephate	0.997	0.999
Naled	0.992	0.996
Diazinon	0.996	0.997
Dimethoate	0.997	0.999
Chlorpyrifos	0.997	0.998
Malathion	0.995	0.999
Methidathion	0.996	0.999
TPP	0.999	0.997
Phosmet	0.997	0.999
Azinphos methyl	0.995	0.999

r^2 values for 30, 75, 150, 300, 525, 750, 1500 ppb MSD Calibration Levels
25, 50, 100, 175, 250, 500 ppb FPD Calibration Levels

The extraction process using the QuEChERS method was effective in retaining the OP pesticides in the spiked apple sample and providing sufficient cleanup of the sample matrix for GC/MS analysis. Figure 4 shows the organophosphorus pesticide mix spiked into an apple matrix sample. The matrix was prepared using a QuEChERS sample preparation approach that included extraction/partitioning and dispersive-SPE. A GC/MS/SIM blank matrix trace is shown below the analyte trace to indicate the level of potential matrix interference with the analytes of interest. Peak shapes for the organophosphorus pesticides are still quite sharp and well resolved indicating excellent performance on the DB-35ms UI column in fruit matrix.

Recoveries were determined by GC/MS/SIM at the 150, 300, and 750 ng/mL levels, and 50, 100, and 250 ng/mL using the FPD in phosphorus mode. The recoveries for most of the pesticides were greater than 75% with average RSDs below 10%. Recoveries for the individual OP pesticides are listed in Table 4. Lower recoveries were noted for the more polar pesticides: oxydemeton-methyl, methamidophos, and acephate. One possible explanation is that these polar, highly water soluble pesticides may have been partially lost through incomplete partitioning into the aqueous layer during the extraction step [12].

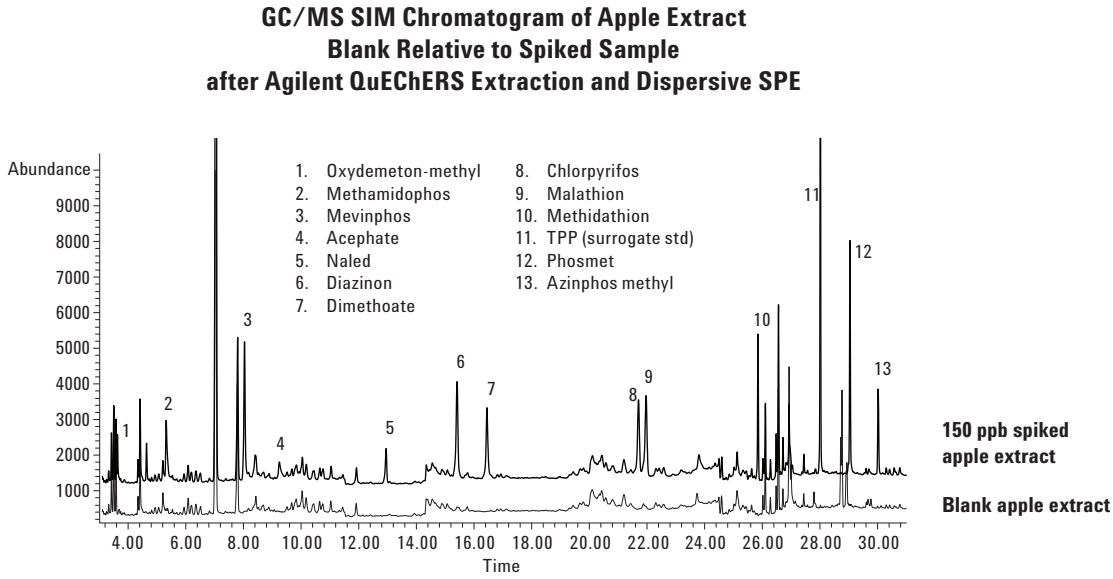

Figure 4. GC/MS/SIM chromatogram of the apple extract blank and a 150 ng/mL spiked apple extract analyzed on an Agilent J&W DB-35ms UI capillary column (Agilent p/n 121-3822UI). Chromatographic conditions are listed in Table 1.

Table 4. Recovery and Repeatability of OP Pesticides in Spiked Apple Matrix with an Agilent J&W DB-35ms UI Column (Agilent p/n 121-3822UI) (continued)

Recovery and Repeatability of OP Pesticides in Spiked Apple Matrix by SIM GC/MS with Agilent J&W DB-35ms UI Column

	150 ng/mL fortified QC		300 ng/mL fortified QC		750 ng/mL fortified QC	
Analysis	**% Recovery**	**RSD (n=6)**	**% Recovery**	**RSD (n=6)**	**% Recovery**	**RSD (n=6)**
Oxydemeton methyl	64.6	5.5	64.0	5.0	61.9	8.2
Methamidophos	68.9	8.5	78.6	3.9	83.8	3.9
Mevinphos	88.7	4.3	93.4	2.6	97.0	3.2
Acephate	77.5	6.4	80.3	5.2	84.3	2.6
Naled	92.9	6.2	87.6	2.4	80.3	5.7
Diazinon	84.5	3.0	89.0	2.7	90.9	3.0
Dimethoate	90.6	2.9	92.9	3.3	96.6	3.6
Chlorpyrifos	87.0	3.7	91.7	3.1	95.6	3.6
Malathion	92.5	3.8	91.9	3.4	97.3	3.6
Methidathion	89.6	4.4	92.1	3.4	99.2	3.7
TPP (surrogate std)	100.6	3.8	101.5	3.1	100.1	3.0
Phosmet	85.9	5.1	86.8	3.4	95.1	3.8
Azinphos methyl	88.6	4.2	84.3	3.5	95.4	3.9

Table 4. Recovery and Repeatability of OP Pesticides in Spiked Apple Matrix with an Agilent J&W DB-35ms UI Column (Agilent p/n121-3822UI)

Recovery and Repeatability of OP Pesticides in Spiked Apple Matrix by GC/FPD with Agilent J&W DB-35ms UI Column

	150 ng/mL fortified QC		300 ng/mL fortified QC		750 ng/mL fortified QC	
Analysis	**% Recovery**	**RSD (n=6)**	**% Recovery**	**RSD (n=6)**	**% Recovery**	**RSD (n=6)**
Oxydemeton methyl	45.8	8.5	60.6	9.4	62.4	7.7
Methamidophos	63.4	9.2	75.5	5.9	83.7	4.3
Mevinphos	80.5	5.0	90.1	3.5	93.5	4.1
Acephate	64.1	11.1	78.5	7.5	81.3	7.9
Naled	97.2	12.0	87.6	8.0	78.6	8.7
Diazinon	80.1	2.3	86.7	2.9	90.3	3.5
Dimethoate	80.6	7.2	91.0	3.6	93.6	4.6
Chlorpyrifos	80.8	4.7	91.5	5.5	96.6	4.4
Malathion	84.7	4.4	92.9	4.5	96.9	4.8
Methidathion	84.7	7.9	93.9	2.6	96.1	4.1
TPP (surrogate std)	99.6	2.3	99.7	6.0	95.9	3.2
Phosmet	76.6	5.5	89.3	2.6	92.4	4.5
Azinphos methyl	79.4	7.6	88.5	4.5	93.8	3.2

Conclusions

This application note successfully shows a quick and efficient analytical method to monitor low and trace level organophosphorus pesticides residue in apple samples. Splitting the column effluent to both an MSD and FPD facilitated selectivity, identification, and confirmation of OP pesticides from a single injection, thereby increasing laboratory productivity. Using GC/MS in Full Scan mode enabled identification of specific pesticides, while SIM mode offered selectivity and sensitivity for quantitation of the pesticides at trace levels. Confirmation and further specificity was achieved by FPD in phosphorus mode. FPD detection was effective at minimizing matrix interferences enabling lower detection.

The Agilent QuEChERS method for general fruits and vegetables was successful at providing enough sample cleanup to minimize matrix interferences while still maintaining low level analyte detection. The simple QuEChERS extraction method allows for faster sample prep facilitating higher sample throughput. Residual sample matrix carryover is removed through use of backflush, which eliminates the need for a bakeout cycle, significantly reducing analytical run times.

The Agilent J&W DB-35ms UI capillary column resolves the targeted OP pesticides and provides excellent peak shapes for the polar pesticides allowing for more reliable quantitation at low levels. Detection levels for the OP pesticides were at or below the US maximum residue levels (MRLs) for various fruits. Matrix-matched calibration standards yielded regression coefficients $r^2 \geq 0.992$ and recoveries from fortification studies were greater than 75% with an average RSD <10% for both GC/MS/SIM and FPD, further demonstrating the effectiveness of using an Agilent J&W DB-35ms UI column for residual pesticide determination.

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Printed in the USA
January 20, 2011
5990-7165EN

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Sensitive Femtogram Determination of Aflatoxins B_1, B_2, G_1 and G_2 in Food Matrices using Triple Quadrupole LC/MS

Application Note

Food Safety

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Abstract

A simple and inexpensive sample cleanup procedure based on a dispersive solid phase adsorption approach (C18) is effective in removing background matrix contaminants for reliable determination of aflatoxins in food at the femtogram level by triple quadrupole LC/MS. This application demonstrates fast analysis time (< 6 min) with good chromatographic resolution and separation for all four aflatoxins. Standard curves for each aflatoxin analyte show good linearity (> 0.998) across a wide concentration range (0.1–100 µg/L). Recoveries using the dispersive solid phase adsorption approach were between 85–110% for each aflatoxin for all four spiked food matrices and were comparable to other widely used SPE routines. The limit of detection was determined to be < 0.15 µg/kg and the limit of quantitation < 0.5 µg/kg for all four sample matrices. Precision data was typically below 5% RSD for all analytes.

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Introduction

Aflatoxins are a group of mycotoxins produced as metabolites by the fungi aspergillus flavus and aspergillus parasiticus [1]. They can be found in various foods including grains, nuts, and spices [2]. There are four major naturally occurring aflatoxins: B_1, B_2, G_1 and G_2 (Figure 1). Exposure to them can cause cancer in humans and live stock, therefore reliable and sensitive analytical methods for the determination of aflatoxins are required to safeguard our food supply.

Experimental

These analyses were performed using an Agilent G6460A Triple Quadrupole LC/MS/MS System equipped with Agilent Jet Stream Technology [3] using an Agilent 1200 Series SL LC. The LC system consisted of a binary pump (G1312B), vacuum degasser (G1379B), a low carryover automatic liquid sampler (G1367D), thermostatted column compartment (G1316B) and MassHunter data system.

Aflatoxin standards and foods

Purified aflatoxin standards (B_1, B_2, G_1 and G_2) were obtained from Sigma-Aldrich. Aflatoxin-free corn flour, wheat, peanut and walnut samples obtained from a local grocery store were used for recovery studies.

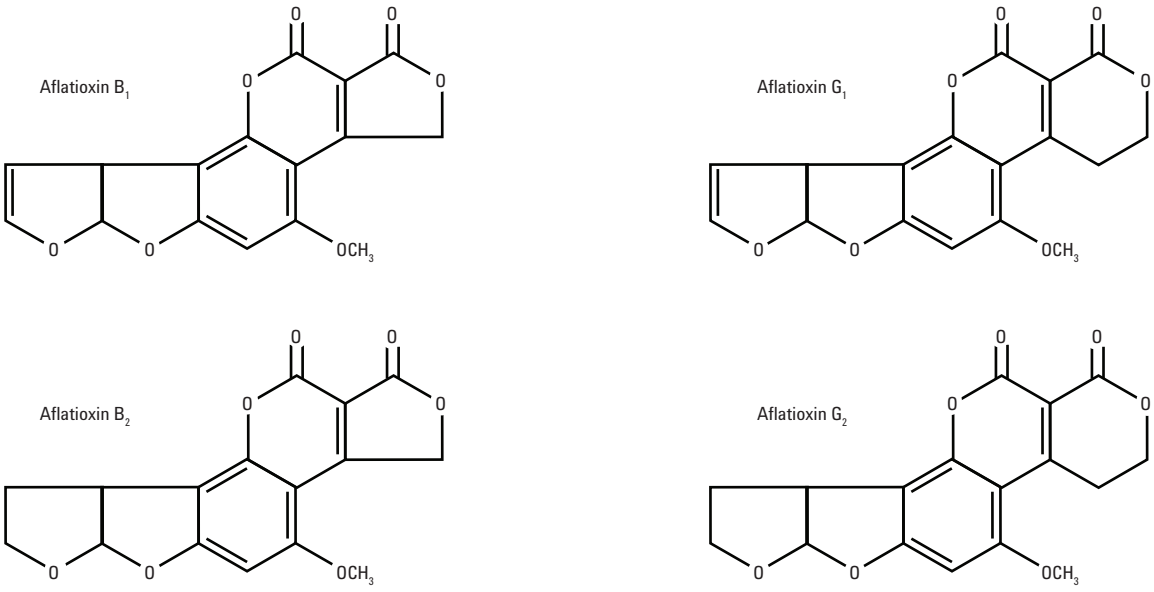


Figure 1. Structures of aflatoxins B_1, B_2, G_1 and G_2.

Instrumentation

Rapid Resolution HPLC Conditions and Configuration:

- Agilent 1200 Series Binary Pump SL (G1312B)
- High Performance WP Sampler SL Plus (G1367D)

  Sampler Thermostat (G1330B)
- Thermostatted Column Compartment SL, including 10P/Two-Position switching valve (G1316B with option #057)

Method Conditions:

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm	
Column temperature:	40 °C	
Injection volume:	5 µL	
Autosampler temp:	4 °C	
Needle wash:	Flushport (100% methanol), 5 seconds	
Mobile phase:	A = 10 mM NH_4 acetate in water B = 100% methanol	
Gradient flow rate:	0.6 mL/min (no split)	
Gradient:	Time (min)	%B
	0	5
	5	100
	6	100
Analysis time:	6 min	
Equilibration time:	1.5 min	
Total run time:	7.5 min	

Mass Spectrometer Source Conditions and Configuration:

Agilent 6460 Triple Quadrupole LC/MS equipped with Agilent Jet Stream Technology.

Ion Source Conditions:

Ion Mode:	ESI/Agilent Jet Stream, Positive ionization
Capillary Voltage:	4000 V
Drying gas (nitrogen):	10 L/min
Drying gas temperature:	325 °C
Nebulizer gas (nitrogen):	50 psi
Sheath Gas temperature:	350 °C
Sheath Gas flow:	11 L/min
Nozzle Voltage:	0 V
Q1 and Q2 Resolution:	0.7 amu [autotune]
Delta EMV:	400V

The Triple Quadrupole MS MRM parameters are listed in Table 1. All fragmentor voltage (frag) settings and respective collision energies (CE) and the most abundant MS/MS product ions per analyte were determined automatically using the Agilent MassHunter Optimizer Software.

Table 1. MRM Transitions for Aflotoxins and Respective Internal Standards

Name	Retention time (min)	Fragmentor voltage (V)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
Aflatoxin B_1	4.68	130	313.1	241.1	35
				285.1	20
				269.1	25
Aflatoxin B_2	4.57	130	315.1	287.1	25
				259.1	25
				243.1	40
Aflatoxin G_1	4.40	130	329.1	243.1	25
				311.1	20
				283.1	20
Aflatoxin G_2	4.26	130	331.1	245.1	30
				285.1	25
				313.1	25
Isotope B_1	4.68	130	330.1	301.1	20
				255.1	40
Isotope B_2	4.57	130	332.1	303	25
				273.0	30
Isotope G_1	4.40	130	346.1	257.1	25
				299.1	25
Isotope G_2	4.26	130	348.1	330.1	25
				259.1	30

Sample Preparation and Recovery Studies

Corn flour, ground wheat, peanut and walnut samples (10 g each) were spiked with a mixture of four aflatoxin standards, each at 5 and 25 ng/g. This was then extracted using 40 mL of acetonitrile-water (84:16, v/v) for 30 min with shaking at room temperature. The extract was cleaned up using both C18 powdered adsorbent material (ODS SPE bulk sorbent, Agilent p/n 5982-1182) and MycoSep 226 multifunctional SPE (Romer). Aliquots (0.4 mL) of the cleaned up extracts were diluted with 0.6 mL 10 mM ammonium acetate in water.

The sample was then centrifuged at 14,000 rpm for 3 min prior to LC/MS/MS analysis.

Each food matrix and spike level was conducted in seven replicates to represent and maintain statistical integrity. A schematic of this sample preparation is illustrated in Figure 2.

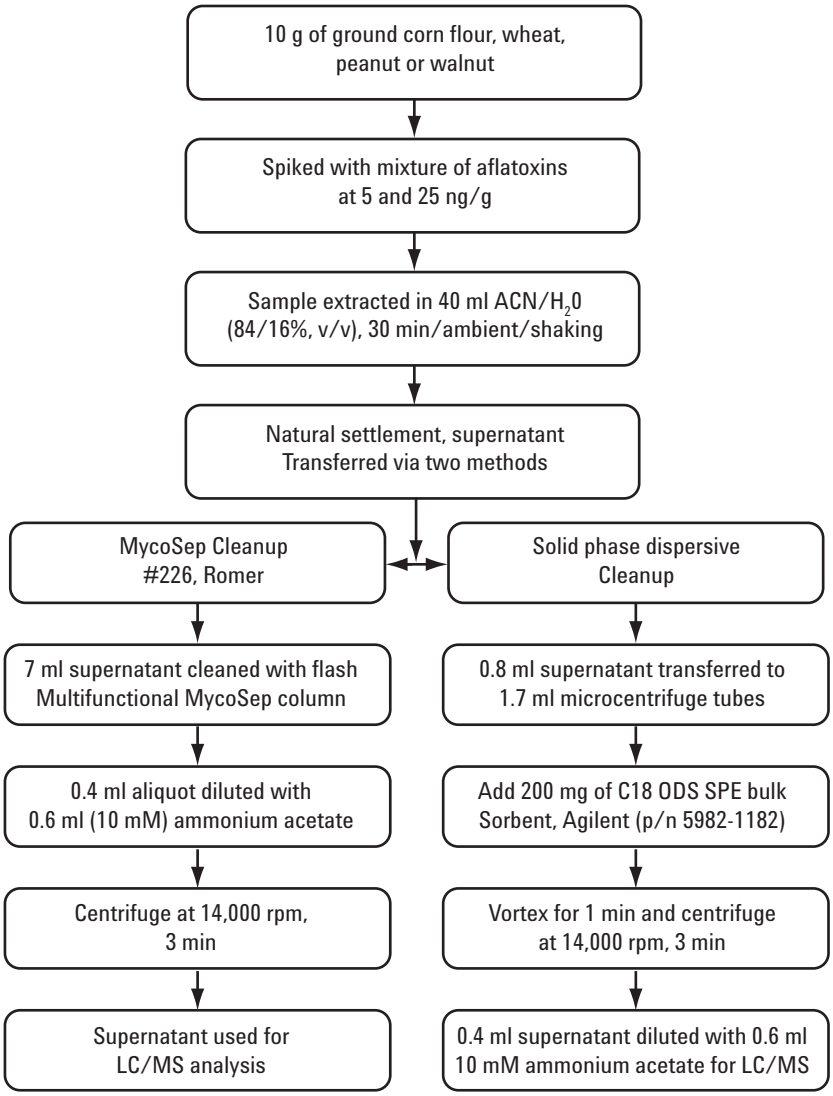


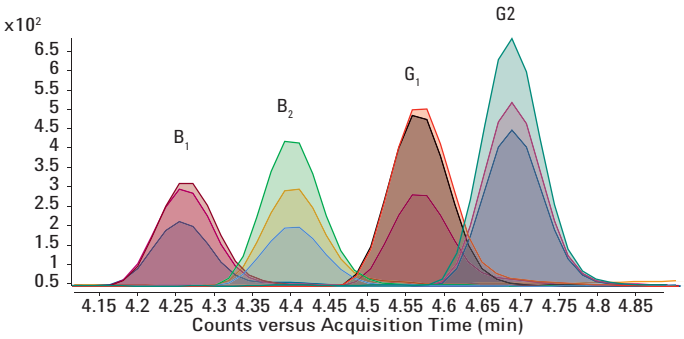
Figure 2. Schematic matrix sample preparation workflow showing the dispersive solid phase adsorption approach versus a widely used SPE approach.

Results and Discussion

The rapid chromatography conditions as outlined in the experimental section yielded good chromatographic resolution for each aflatoxin analyte and each analysis was completed in six minutes. A typical chromatogram is shown in Figure 3(a), which illustrates 1 ppb concentration level of each aflatoxin together with the corresponding isotopically labeled internal standards at a concentration level of 2.5 ppb (Figure 3(b)). These chromatograms show overlaid extracted ion chromatograms (EICs.)

Standard curves for aflatoxins B1, B2, G1 and G2 all showed a good linearity through the concentration range 0.1 to 100 ppb each with a linear correlation (R^2) of greater than 0.999. Figure 4 illustrates an overlay of each standard curve on the same scale, but without internal standard correction. The use of internal standards effectively adjusted for matrix differences, as shown in Figure 5.

(a) Overlaid aflatoxin EICs Quant ion and two Qualifiers (1 ppb)



(b) Overlaid Isotopically labelled aflatoxin EICs Quant ion and Qualifier ion (2.5 ppb)

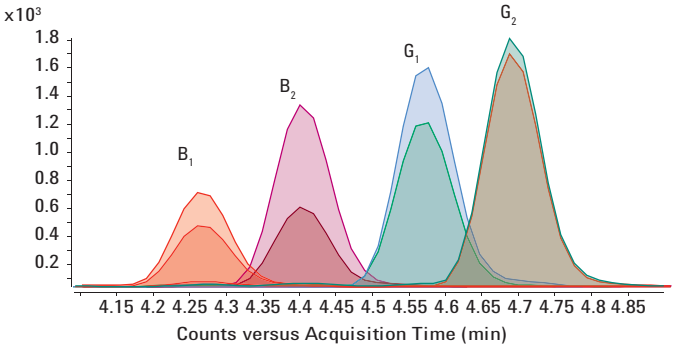


Figure 3. LC/MS/MS chromatogram of aflatoxin B_1, B_2, G_1 and G_2 standards at 1 ppb with corresponding isotopically labelled internal standards at 2.5 ppb.

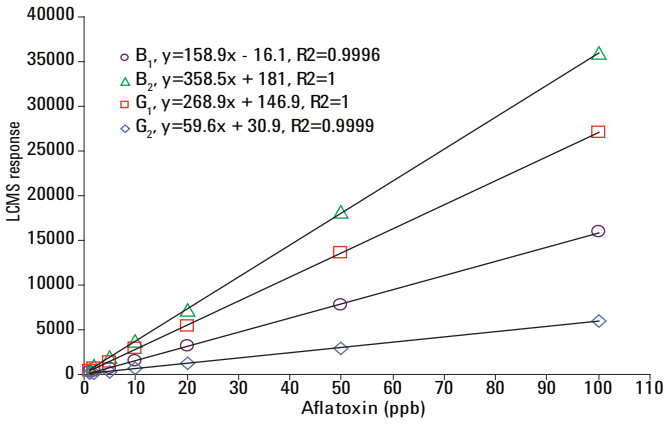


Figure 4 Overlaid standard curves for aflatoxins B_1, B_2, G_1 and G_2.

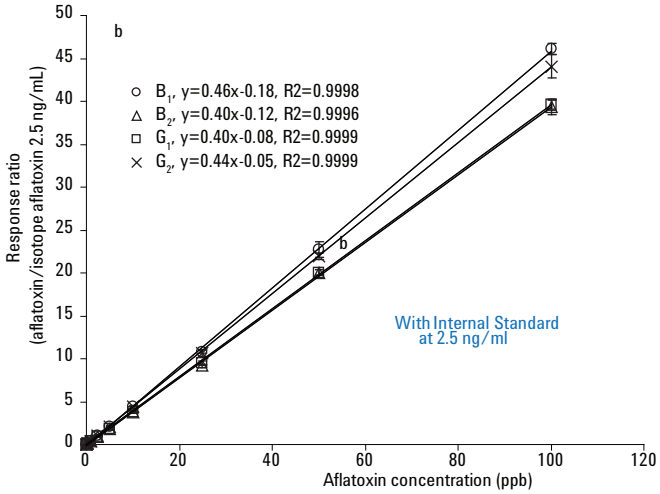


Figure 5. Overlaid standard curves for aflatoxins B_1, B_2, G_1 and G_2 with internal standard correction.

In order to determine the limits of detection (LOD) and reporting (LOR), seven separate and mutually exclusive batches of each food matrix were tested via the two sample preparation protocols and analytical methodology outlined previously. The results outlined in this document are derived from the average values across the seven batches of each matrix (N=7.)

Limits of detection were determined using the protocol of chromatographic signal-to-noise ratio of above 3/1 (peak to peak.) Limits of reporting were determined using the protocol of chromatographic signal-to-noise ratio above 10/1 (peak to peak.)

Table 2 details the observed LODs and LORs for each aflatoxin across the series of four food matrices. The limit of detection overall was determined to be < 0.15 µg/kg and the limit of quantitation < 0.5 µg/kg for all four sample matrices and both sample preparation routines.

Tables 3 and 4 summarize the LOD data obtained across the seven batches. Table 3 data is presented with respect to dispersive solid phase adsorption only, and Table 4 data using the Mycosep SPE sample preparation only.

Table 2. Limits of Detection and Reporting Observed for Aflatoxins B_1, B_2, G_1 and G_2 Across Four Food Matrices via Dispersive SPA and Mycosep SPE Sample Preparation Approaches

	Aflatoxin	Mycosep (#226, Romer)		Dispersive C18 ODS SPE Bulk Sorbent, Agilent (p/n 5982-1182)	
Food Matrix		**LOD ng/g (S/N>3)**	**LOR ng/g (S/N>10)**	**LOD ng/g (S/N>3)**	**LOR ng/g (S/N>10)**
Corn	B_1	0.047	0.16	0.060	0.20
(Ave 7-batches)	B_2	0.036	0.12	0.085	0.28
	G_1	0.08	0.28	0.10	0.35
	G_2	0.046	0.15	0.033	0.11
Wheat	B_1	0.068	0.23	0.012	0.042
(Ave 7-batches)	B_2	0.11	0.36	0.037	0.12
	G_1	0.14	0.47	0.15	0.50
	G_2	0.038	0.13	0.11	0.36
Peanut	B_1	0.051	0.17	0.056	0.19
(Ave 7-batches)	B_2	0.045	0.15	0.069	0.23
	G_1	0.07	0.23	0.05	0.15
	G_2	0.052	0.17	0.14	0.45
Walnut	B_1	0.12	0.41	0.093	0.31
(Ave 7-batches)	B_2	0.035	0.12	0.098	0.33
	G_1	0.03	0.10	0.12	0.40
	G_2	0.047	0.16	0.04	0.13

Table 3. LOD Results Observed for Aflatoxins B_1, B_2, G_1 and G_2 via the Dispersive SPA

Food Matrix	B_1 LOD (ng/g)	B_2 LOD (ng/g)	G_1 LOD (ng/g)	G_2 LOD (ng/g)
Corn	0.060	0.085	0.100	0.033
Wheat	0.012	0.037	0.150	0.110
Peanut	0.056	0.069	0.050	0.140
Walnut	0.093	0.098	0.120	0.040
Average	0.055	0.072	0.105	0.080
Mass On-Column (fg)	275	360	525	400

Table 4. LOD Results Observed for Aflatoxins B_1, B_2, G_1 and G_2 via the Mycosep, #226 SPE Sample Preparation Approach

Food Matrix	B_1 LOD (ng/g)	B_2 LOD (ng/g)	G_1 LOD (ng/g)	G_2 LOD (ng/g)
Corn	0.047	0.036	0.080	0.046
Wheat	0.068	0.110	0.140	0.038
Peanut	0.051	0.045	0.070	0.052
Walnut	0.120	0.035	0.030	0.047
Average	0.072	0.057	0.080	0.046
Mass On-Column (fg)	360	283	400	229

Sample Preparation Approach

Recovery studies were extensively undertaken for both sample cleanup techniques in parallel across the four matrices and across the seven batches for each aflatoxin analyte. Figures 6 (a) and (b) graphically depict the recovery trends across the four food matrices for the dispersive C18 cleanup approach and the Mycosep SPE cleanup, respectively. As illustrated, the recoveries for both sample cleanup procedures were between 85-110 % for each of the aflatoxins for all four spiked food matrices, with the MycoSep cleanup method only marginally better than the C18 one for walnut samples .

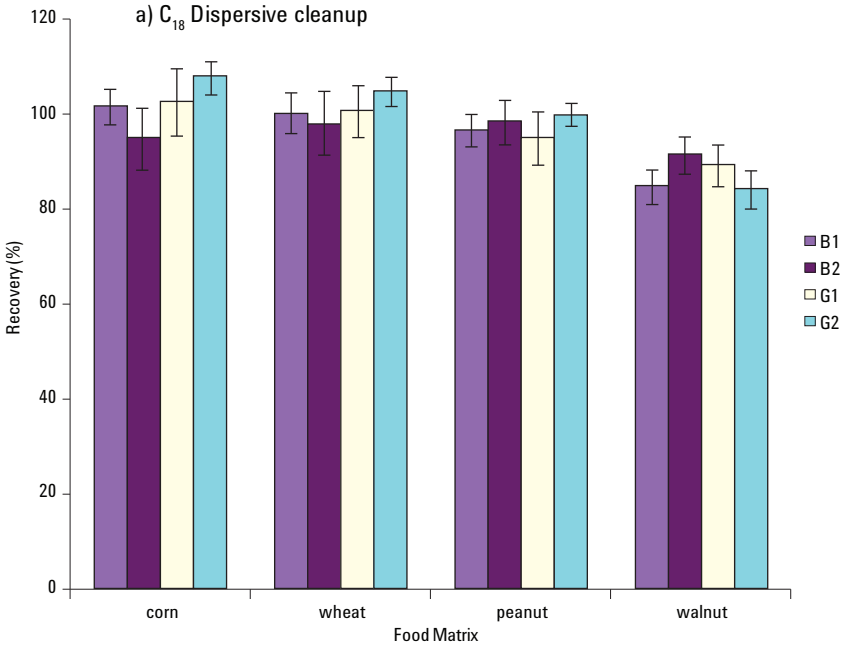


Figure 6(a). Recovery of aflatoxin B_1, B_2, G_1 and G_2 from food matrices using C_{18} dispersive cleanup.

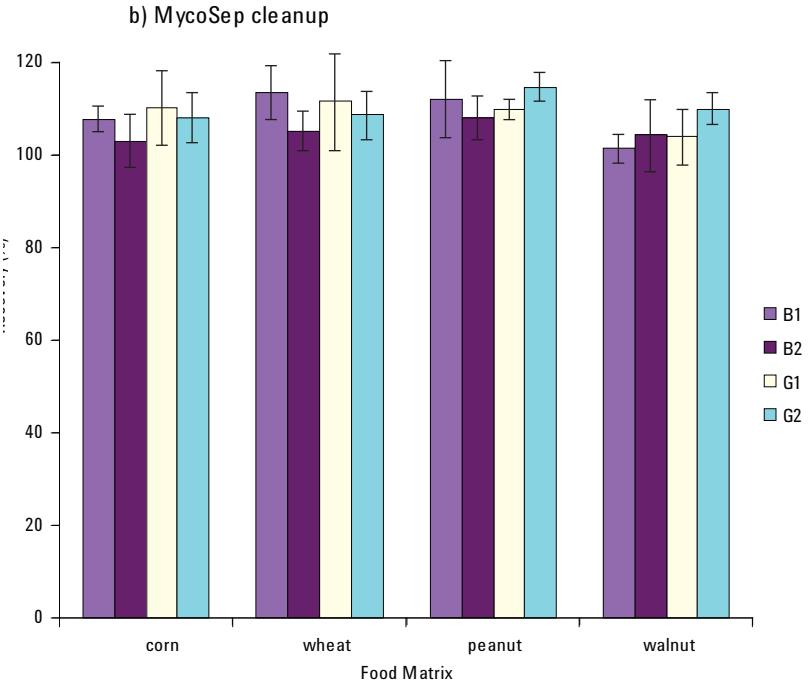


Figure 6(b). Recovery of aflatoxin B_1, B_2, G_1 and G_2 from food matrices using Mycosep, #226 SPE cleanup.

Aflatoxin analyte recovery data for each separate food matrix is detailed in Tables 5 through 8 and was undertaken at two concentration spiked levels of 5 ng/g and 25 ng/g. Each sample batch tested was split and divided between the two cleanup procedures outlined in this document following the natural settlement and supernatant transfer step outlined in Figure 2(a).

Table 5. Spiked Corn Samples – Recovery Studies (% Recovery, ± RSD, N=7)

	Aflatoxin	Corn spiked at 5 ng/g C18 cleanup	Corn spiked at 25 ng/g C18 cleanup	Corn spiked at 5 ng/g Mycosep#226	Corn spiked at 25 ng/g Mycosep#226
No Internal Standard	B_1	101.7 ± 3.7	95.7 ± 3.0	107.8 ± 2.8	105.4 ± 2.8
	B_2	95 ± 6.3	95.4 ± 1.4	103.0 ± 5.7	105.3 ± 2.0
	G_1	102.7 ± 7.1	96.8 ± 1.75	110.2 ± 7.9	103.7 ± 3.0
	G_2	107.9 ± 3.5	97.8 ± 0.88	108.1 ± 5.4	104.3 ± 2.4
Internal Standard	B_1	102.3 ± 2.9	100.1 ± 2.4	108.2 ± 5.1	97.8 ± 3.3
	B_2	100.0 ± 7.9	94.0 ± 3.1	101.7 ± 4.7	92.8 ± 3.5
	G_1	107.3 ± 3.5	97.0 ± 6.0	110.3 ± 3.6	102.5 ± 1.8
	G_2	101.3 ± 5.6	100.4 ± 3.8	104.9 ± 5.4	97.2 ± 6.3

Table 6. Spiked Wheat Samples – Recovery Studies (% Recovery, ± RSD, N=7)

	Aflatoxin	Wheat spiked at 5 ng/g C18 cleanup	Wheat spiked at 25 ng/g C18 cleanup	Wheat spiked at 5 ng/g Mycosep#226	Wheat spiked at 25 ng/g Mycosep#226
No Internal Standard	B_1	100.1 ± 4.4	96.6 ± 2.9	113.5 ± 5.9	100.6 ± 1.8
	B_2	98.2 ± 6.9	96.4 ± 2.6	105.1 ± 4.5	102.1 ± 4.4
	G_1	100.5 ± 5.5	105.4 ± 3.8	111.5 ± 10.4	106.1 ± 4.0
	G_2	104.9 ± 3.2	106.7 ± 1.3	108.6 ± 5.2	103.7 ± 2.9
Internal Standard	B_1	100.9 ± 3.6	109.3 ± 4.7	107.5 ± 4.8	111.7 ± 4.9
	B_2	85.2 ± 7.7	99.8 ± 2.8	92.4 ± 6.3	101.0 ± 4.0
	G_1	110.6 ± 7.8	112.8 ± 1.8	117.6 ± 7.7	109.3 ± 5.7
	G_2	108.4 ± 6.2	108.3 ± 3.9	115.6 ± 7.1	109.8 ± 3.6

Table 7. Spiked Peanut Samples – Recovery Studies (% Recovery, ± RSD, N=7)

	Aflatoxin	Peanut spiked at 5 ng/g C18 cleanup	Peanut spiked at 25 ng/g C18 cleanup	Peanut spiked at 5 ng/g Mycosep#226	Peanut spiked at 25 ng/g Mycosep#226
No Internal Standard	B_1	96.7 ± 3.4	97.0 ± 4.6	112.0 ± 8.4	104.9 ± 1.7
	B_2	98.3 ± 4.7	97.4 ± 2.9	108.0 ± 4.6	104.5 ± 2.0
	G_1	95.0 ± 5.6	95.0 ± 4.9	109.9 ± 2.1	105.7 ± 3.4
	G_2	100.0 ± 2.3	100.0 ± 2.0	114.7 ± 3.2	106.3 ± 1.1
Internal Standard	B_1	101.8 ± 3.6	96.1 ± 2.0	100.0 ± 6.8	103.0 ± 3.5
	B_2	102.5 ± 5.5	100.2 ± 5.0	99.4 ± 4.1	102.9 ± 2.7
	G_1	105.7 ± 7.3	99.2 ± 2.2	105.2 ± 4.3	101.7 ± 5.2
	G_2	107.5 ± 10.9	104.9 ± 6.7	109.3 ± 8.7	102.4 ± 3.1

Table 8. Spiked Walnut Samples – Recovery Studies (% Recovery, ± RSD, N=7)

	Aflatoxin	Walnut spiked at 5 ng/g C18 cleanup	Walnut spiked at 25 ng/g C18 cleanup	Walnut spiked at 5 ng/g Mycosep#226	Walnut spiked at 25 ng/g Mycosep#226
No Internal Standard	B_1	84.9 ± 3.7	85.2 ± 2.2	101.4 ± 3.2	101.0 ± 2.3
	B_2	91.5 ± 3.9	89.8 ± 2.8	104.2 ± 7.9	106.3 ± 2.9
	G_1	89.4 ± 4.4	86.7 ± 1.5	103.9 ± 5.9	101.7 ± 4.2
	G_2	84.0 ± 4.0	83.1 ± 1.3	109.9 ± 3.4	106.3 ± 1.5
Internal Standard	B_1	106.5 ± 4.9	98.9 ± 4.1	93.8± 1.4	100.2 ± 2.9
	B_2	99 ± 5.4	96.5 ± 3.5	92.4 ± 2.7	98.7 ± 4.4
	G_1	103.2 ± 5.9	94.9 ± 2.5	102.8 ± 9.0	102.1 ± 3.9
	G_2	100.2 ± 6.2	97.5 ± 4.6	99.5 ± 6.8	101.2 ± 3.8

Conclusions

An inexpensive and rapid LC/MS/MS method has been developed for the analysis and confirmation of aflatoxins B_1, B_2, G_1 and G_2 in cereals and nuts, with a detection limit of less than 1 ppb. This method is inclusive of sample preparation using a dispersive C18 solid phase adsorption approach (Agilent bulk sorbent p/n 5982-1182.) The performance of this simple sample cleanup procedure was comparable to that of a widely used and generally accepted SPE approach in terms of matrix cleanup and aflatoxin recoveries.

Aflatoxin limits of detection were determined to be less than 0.15 µg/kg and aflatoxin limits of reporting were all less than 0.5 µg/kg for all four sample matrices (corn, wheat, peanut and walnut.)

Standard curves for aflatoxins B_1, B_2, G_1 and G_2 showed a good linearity through the concentration range of 0.1 to 100 ppb with a linear correlation (R^2) of greater than 0.999 for all analytes.

Aflatoxin recoveries were between 85-110% for each of the aflatoxins for all four spiked food matrices using the dispersive C18 solid phase adsorption approach.

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1. Microhim Acts 153, 2006, 101-108
2. Rapid Commun. Mass Spectrum, 2009; 23: 3-11
3. "Agilent Jet Stream Thermal Gradient Focussing Technology" Agilent Technologies Publication 5990-3494EN.

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Printed in the USA
March 9, 2011
5990-6894EN

Agilent Technologies

Improved Isolation and Analysis of Mycotoxins from Cereals, Beer and Wine

Application Note

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Introduction

Trichothecenes are important mycotoxins produced primarily by the genus fusarium. The toxic effects of fusarium toxins on human and animals are well documented and reliable and sensitive analysis methods are therefore required to protect the population and its food sources[1]. This application shows the optimized extraction and clean-up of 12 type A- and B-trichothecenes (Neosolaniol [NEO], HT-2 toxin [HT-2], T-2 toxin [T-2], T-2 triol, T-2 tetraol, Monoacetoxyscirpenol [MAS], Diacetoxyscirpenol [DAS], Deoxynivalenol [DON], 3-Acetyl-deoxynivalenol [3ADON], 15-Acetyl-deoxynivalenol [15ADON], Nivalenol [NIV], Fusarenon [FUS]) and Zearalenone (ZEA) in cereals and cereal-based food, as well as the four polar mycotoxins DON, T-2, HT-2 and ZEA in wheat beer and sake wine matrices. The clean-up was optimized on Bond Elut Mycotoxin, a newly developed solid phase extraction (SPE) sorbent. The LC-MS-MS method presented here gives quantitative and qualitative information and has the accuracy and resolution required for reporting results to European and US governmental agencies[2].

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Sample Preparation

Cereals and cereal-based food samples

Typical clean-up methods of trichothecenes and ZEA from cereals and cereal-based foods use commercially available polar clean-up columns[3]. Substances that interfere with the detection of the mycotoxins are retained while trichothecenes are not. This purification method, however, gives low recoveries for the polar toxins: NIV, T-2 tetraol, and DON.

To address these problems, the extraction step was optimized by marginally increasing the polarity of the extraction solvent to ACN/H_2O (80/20; v/v) as previously described in Varian Application Note 295[4]. Trials with the polar DON reference material from Food Analysis Performance Assessment Scheme (FAPAS) confirm that the best recovery data is achieved with the Bond Elut Mycotoxin method (Table 1).

Table 1. Recovery comparison of Food Analysis Performance Assessment Scheme (FAPAS) certified reference material for DON applying LC-MS-MS

Method 1: Clean up on Bond Elut Mycotoxin
Method 2: Clean up on polar charcoal-alumina sorbent (PS)

Reference Material	Certified Value (µg/kg)	Method 1 (µg/kg)	Method 2 (µg/kg)
FAPAS T2210	463 ± 167	495 ± 5	395 ± 15

Wheat beer and sake wine samples

Prior to clean-up by the Bond Elut cartridge, the wheat beer and sake wine samples were degassed by sonication for 30 min at room temperature. The degassed samples were filtered through Whatman no.1 filter paper (Florham Park, NJ), and the solution of multiple mycotoxins was added to a final concentration of either 35 or 350 ng/g with an internal standard (ZAN) concentration of 50 ng/g. This mixture was applied to a Bond Elut Mycotoxin column. Fully automated clean-up was performed on a Zymark SPE workstation (Hopkinton, MA) according to the Bond Elut Mycotoxin method (Table 2).

Table 2. Bond Elut Mycotoxin Method

1. Pass 4 mL of the filtrated sample extract through a Bond Elut Mycotoxin column (part number 12165001B).
2. Evaporate 2 mL of the eluate to dryness at 50 °C under a gentle stream of nitrogen.
3. Reconstitute in 0.5 mL acetonitrile/water (20/80; v/v).
4. Inject into LC-MS-MS.

LC-MS-MS

The LC-MS-MS system utilized in these experiments is the 320-MS triple quadrupole mass spectrometer fitted with an electrospray ionization (ESI) source, two ProStar 210 liquid chromatography pumps, and an Agilent ProStar 430 autosampler. Separation was according to the gradient program shown in Table 3.

Table 3. Gradient program and LC/API conditions

Time (min)	A (%)	B (%)	Flow (mL/min)
0:00	100	0	0.3
16:00	40	60	0.3
16:01	40	60	0.3
40	30	70	0.3
40:01	85	15	0.4
50:00	85	15	0.4

LC Conditions

Column:	Polaris C18-A 5 µm, 150 mm × 3.0 mm id (p/n A2000150X030)
Buffer A:	5 mM ammonium acetate, 1% acetic acid in 10% methanol
Buffer B:	5 mM ammonium acetate, 1% acetic acid in 100% methanol
Injection Solvent:	Buffer A
Injection Volume:	30 µL

API Conditions

Ionization Mode:	ESI (positive and negative)
Collision Gas:	1.8 mTorr argon
API Drying Gas:	30 psi at 250 °C
API Nebulizing Gas:	50 psi
Needle:	4500 V
Capillary:	Scanning
Detection:	1900 V

Results and Discussion

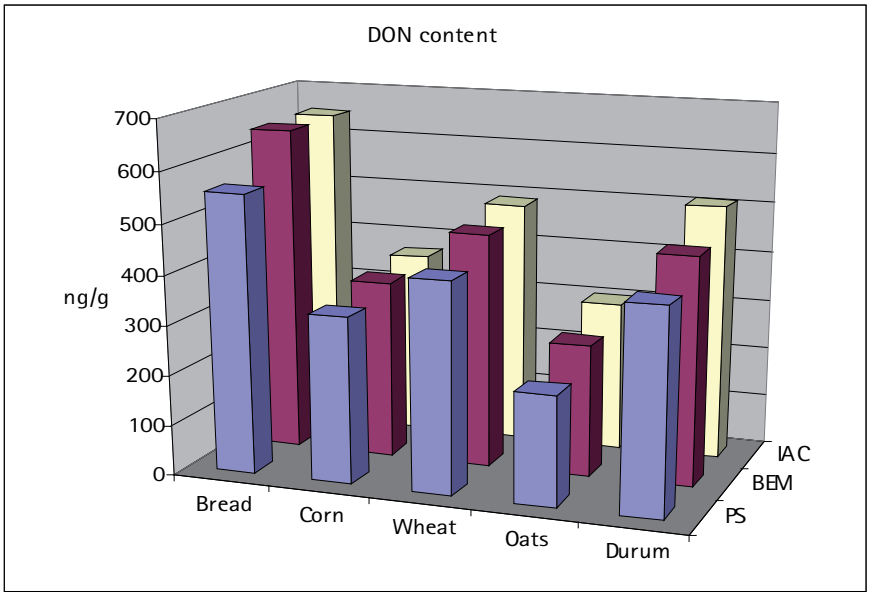
Cereals and cereal-based food samples

With the low increase of 6% water in the extraction solvent and with the clean-up step on Bond Elut Mycotoxin, recoveries (especially for the polar toxins DON, NIV, 3ADON and T-2 tetraol) were increased up to 31%.

If the determination of DON alone is of interest, then the highest content can be achieved with an extraction of 100% water and clean up with the more expensive IAC; however, for the determination of 12 trichothecenes with different polarities, the Bond Elut Mycotoxin cartridge provides good results.

Figure 1 shows the trichothecene content of 5 naturally contaminated samples after 3 different clean-up methods.

Up to 43% higher values were achieved using Bond Elut Mycotoxin for the clean-up of naturally contaminated samples containing the polar toxins DON, NIV, 3ADON, 15ADON and T-2 tetraol in comparison to the charcoal-alumina based polar method.



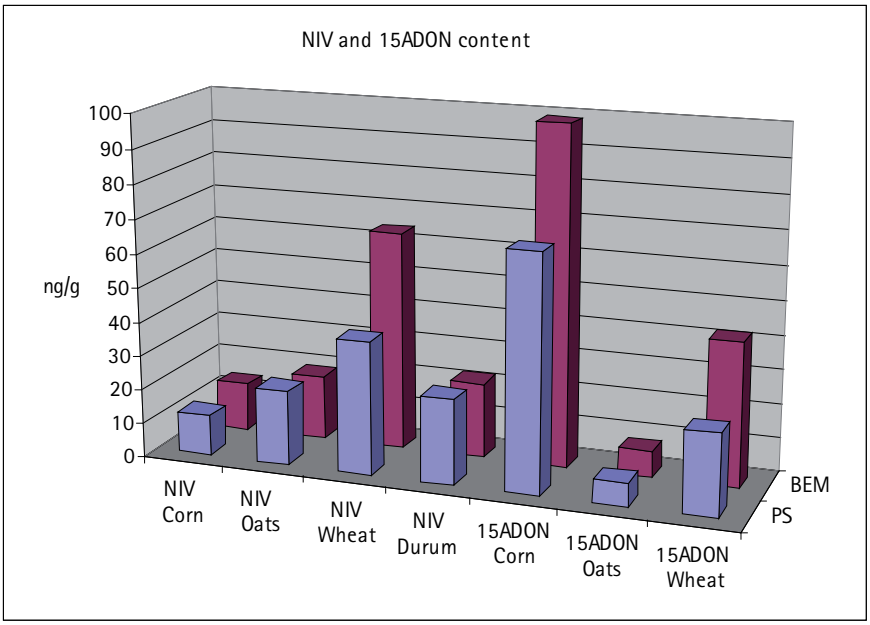


Figure 1. Trichothecene contents of 5 naturally contaminated samples analyzed with DONPrep (IAC), Polar charcoal-alumina sorbent (PS) and Bond Elut Mycotoxin cartridges (BEM) (n=3)

Wheat beer and sake wine samples

A mixture of HT-2, T-2, ZEA and ZAN (internal standard) at a low-level concentration of 35 ng/g was spiked into a complex matrix of wheat beer and/or sake wine.

Figure 2 shows the chromatograms with excellent peak shape and high signal response of the mycotoxins in a wheat beer matrix.

Table 4 shows the recovery rates calculated for high concentration of 350 ng/g and low concentration of DON, ZEA, T-2 and HT-2 from wheat beer and sake after clean-up on Bond Elut Mycotoxin. Very good recoveries (> 90%) and excellent reproducibility (n=3) are displayed for DON and ZEA. The recoveries of T-2 and HT-2 are >60% and they satisfy the European requirements. The EU guideline 2005/38/EG sets recovery limits for DON and ZEA in the range of 60–120% and 60–130% for T-2 and HT-2.

Summary

This application note describes a new, reliable, and cost-efficient clean-up method for the determination by triple quadrupole LC-MS-MS, of 12 type A- and B-trichothecenes in cereals and cereal-based foods. In addition, it shows the determination of four polar mycotoxins DON, T-2, HT-2 and ZEAN in wheat beer and sake wine matrices. The extracted mycotoxins are purified by means of the newly developed Bond Elut Mycotoxin SPE cartridge. The method exceeds the required limits of detection as well as ion ratio requirements for European Directives (ion ratios of <30%), such as SANCO D.1(06)D/412820.

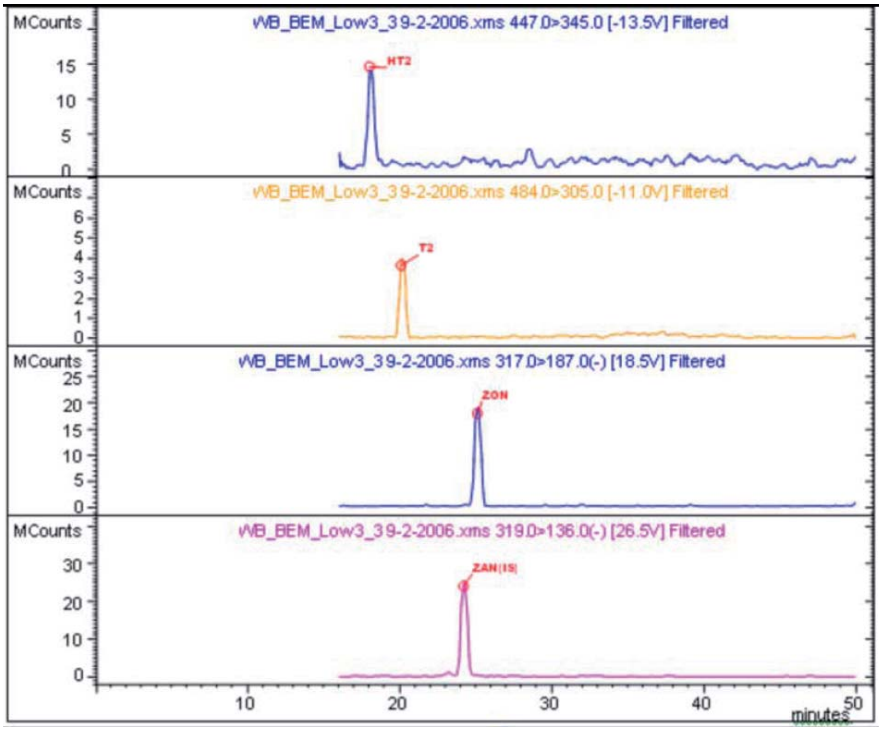


Figure 2. Example chromatograms of low-level spikes for various mycotoxins in a wheat beer matrix. Shown (top to bottom) are: HT-2, T-2, ZEA and ZAN (internal standard)

Table 4. Rates of recovery for selected mycotoxins after a clean-up step on Bond Elut Mycotoxin from wheat beer and sake at two concentrations; relative standard deviation is calculated from three replicates

	Wheat beer matrix			
	% Recovery	**% RSD**	**% Recovery**	**% RSD**
Mycotoxin	35 ng/g		350 ng/g	
DON	92	2.6	95.5	1.5
ZEA	116	6.1	101.9	1.3
T-2	61.3	12.6	60.1	1.1
HT-2	81.8	5.6	76.1	1.4

	Sake wine matrix			
	% Recovery	**% RSD**	**% Recovery**	**% RSD**
Mycotoxin	35 ng/g		350 ng/g	
DON	94.3	7.4	96.8	0.5
ZEA	99.3	1.3	99.8	0.8
T-2	101.3	1.3	66.0	0.9
HT-2	113.9	8.3	111	1

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[2] World Wide Regulations for Mycotoxins in Food and Feed in 2003. FAO Food and Nutrit. 2003, 81, 3.1–3.5.

[3] R. Krska, S. Baumgartner, R. Josephs (2001) The state-of-the-art in the analysis of Type A and B trichothecene mycotoxins in cereals; Fresenius J. Anal. Chem. 371, 285-299

[4] Agilent Application Note SI-00295, Bond Elut Mycotoxin; New SPE Sorbent for Clean-up of Fusarium toxin-contaminated Cereals and Cereal-based Foods, M.Klötzel, Uwe Lauber, Chemisches u. Veterinäruntersuchungsamt Stuttgart, Germany

www.agilent.com/chem

Published in UK, October 19, 2010

SI-01075

Agilent Technologies

Fast separation of EU and EPA Regulated PAHs on Agilent J&W FactorFour VF-17ms for PAH

Application Note

Author

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Introduction

When analyzing polycyclic aromatic hydrocarbons (PAHs) the bottleneck is the number of PAHs with the same mass. With MS detection different compounds with the same mass cannot be resolved. To resolve these PAHs the column selectivity and the oven program are very important. The Agilent FactorFour VF-17ms for PAH has perfect selectivity and optimized film thickness to analyze the PAHs, addressing the increasing need of many laboratories for improved productivity.

PAHs enclose a large group of organic compounds which contain two or more aromatic rings. Hundreds of individual PAHs can be formed during incomplete combustion or pyrolysis of organic matter, industrial processes and cooking and food processing. PAHs are therefore analyzed in food as well as environmental samples. Because of the difference in European (EU) and American (EPA) legislation, different sets of PAHs are prescribed (Table 1). The EU PAH list is used for food samples while the EPA list concerns environmental samples. This application note describes the analysis of EU and EPA PAHs in a single run, which can therefore be used for environmental as well as for food samples.

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Conditions

Technique:	GC/MS
Column:	VF-17ms for PAHs, 20 m x 0.15 mm, df = 0.05 µm (part number CP9009)
Temperature:	70 °C (0.5 min), 70 °C/min, 180 °C (1 min), 5 °C/min, 245 °C (1 min), 4 °C/min, 270 °C (1.5 min), 15 °C/min, 350 °C
Carrier Gas:	Helium, constant flow 1 mL/min
Injector:	300 °C, Splitless mode
Detector:	Quadrupole MS, EI in SIM, ion source 275 °C, transfer line 300 °C
Sample:	Concentration approx. 1 µg/mL
Injection Volume:	1 µL

Table 1. Peak Identification for Figure 1

Peak	Compound	MW	EU PAH	EPA PAH
1	Naphthalene	128		X
2	Acenaphthylene	152		X
3	Acenaphthene	154		X
4	Fluorene	166		X
5	Phenanthrene	178		X
6	Anthracene	178		X
7	Fluoranthene	202		X
8	Pyrene	202		X
9	Benzo(c)fluorene	216	X	
10	Benz(a)anthracene	228	X	X
11	Cyclopenta(c,d)pyrene	226	X	
12	Triphenylene	228		
13	Chrysene	228	X	X
14	6-Methylchrysene	242		
15	5-Methylchrysene	242	X	
16	Benzo(b)fluoranthene	252	X	X
17	Benzo(k)fluoranthene	252	X	X
18	Benzo(j)fluoranthene	252	X	
19	Benzo(a)pyrene	252	X	X
20	Indeno(1,2,3-cd)pyrene	276	X	X
21	Benzo(b)triphenylene	278		
22	Dibenz(a,h)anthracene	278	X	X
23	Benzo(g,h,i)perylene	276	X	X
24	Dibenzo(a,l)pyrene	302	X	
25	Dibenzo(a,e)pyrene	302	X	
26	Dibenzo(a,i)pyrene	302	X	
27	Dibenzo(a,h)pyrene	302	X	

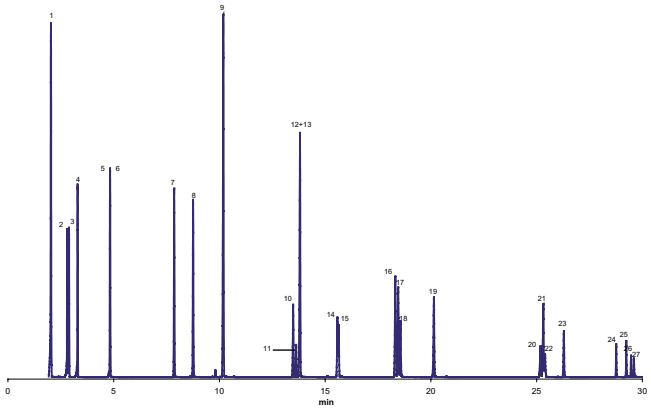

Figure 1. GC/MS analysis of EU and EPA PAHs on VF-17ms 20 m x 0.15 mm x 0.05 µm

Results and Discussion

When analyzing PAHs some peak groups are difficult to resolve on the MS because of their identical mass. One of the groups, Benz(a)anthracene, Cyclopenta(c,d)pyrene, Chrysene and Triphenylene, has compounds with different masses, but the difference between these masses, m/z 226 and 228, is difficult to distinguish with MS. Therefore these compounds should be resolved chromatographically (Figure 2). The same problem occurs when resolving Indendo(1,2,3-cd)pyrene, Benzo(b)triphenylene and Dibenz(a,h)anthracene with m/z 276 and 278 (Figure 4).

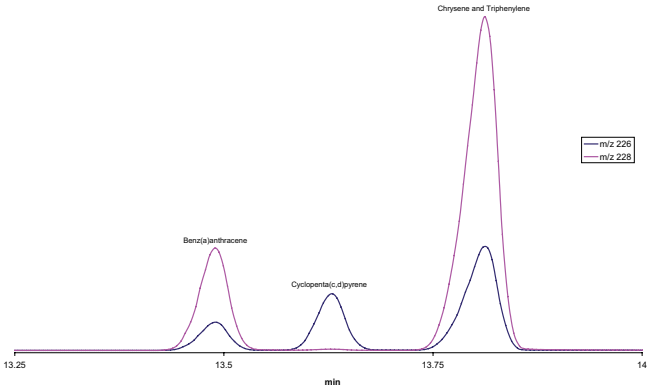

Figure 2. Separation of m/z 226 and 228

In the other difficult group, the Benzofluoranthene isomers, all three compounds have the same mass (m/z 252). With most analyses, Benzo(b)fluoranthene and Benzo(k)fluoranthene are not resolved very well. However, in this analysis, not only are Benzo(b)fluoranthene and Benzo(k)fluoranthene fully resolved, but also Benzo(j)fluoranthene (Figure 3).

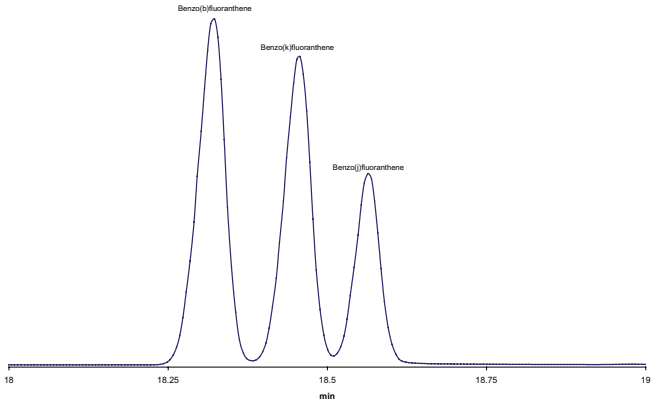

Figure 3. Separation of Benzofluoranthene isomers (m/z 252)

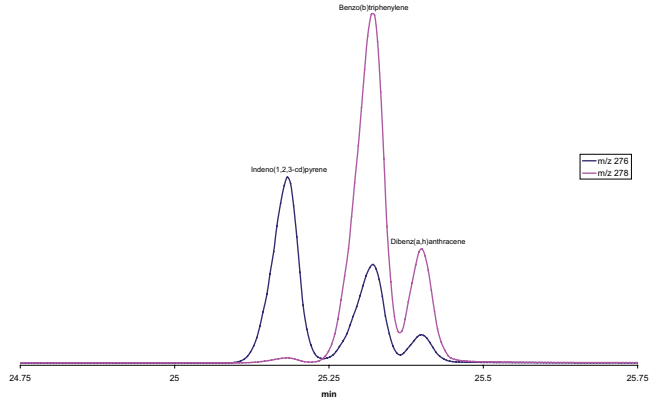

Figure 4. Separation of m/z 276 and 278

Conclusion

In this application note the EU and EPA regulated PAHs are perfectly separated using the VF-17ms for PAH in less than 30 minutes. With the optimized oven program the difficult groups are nearly baseline resolved. However, it should be noted that Triphenylene, which is not listed in either EPA or EU lists, can cause a false positive because of its co-elution with Chrysene.

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www.agilent.com/chem

Published in UK, October 08, 2010
SI-02125

Agilent Technologies

Multi-residue Analysis of PAHs, PCBs and OCPs using an Agilent J&W FactorFour VF-35ms Column

Application Note

Author

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Agilent Technologies, Inc.

Introduction

Multi-residue analysis involves the separation of different groups of compounds in a single operation. One of the most important advantages of multi-residue analysis is the opportunity to screen and quantify a multitude of components in a short time span, reducing analysis costs. A multi-residue method for the separation of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) is described here.

PAHs, PCBs and OCPs have different sources and belong to different chemical classes. PAHs contain two or more aromatic rings and are formed during incomplete combustion or pyrolysis of organic matter. OCPs are pesticides containing mainly carbon, hydrogen and chlorine atoms. They break down slowly and can remain in the environment long after application, and bioaccumulate in organisms during prolonged exposure. PCBs are characterized by two phenyl groups, with varying numbers of chlorine atoms. PCBs are used in many industrial and commercial applications, for example, as plasticizers in paints, plastics and rubber products, and in pigments and dyes. All of these compounds are typically very persistent in sediments, plants and animals.

The 16 EPA PAHs,17 PCBs (including the 6 EU marker PCBs) and 24 common OCPs were analyzed in a single run at different concentration levels. The concentration of the PAHs was ten times higher than the concentration of PCBs and OCPs, a concentration difference that is common also in real environmental samples.

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Conditions

Technique:	GC/MS
Column:	VF-35ms, 30 m x 0.25 mm, df=0.25 µm (part number CP8877)
Sample Conc:	OCPs and PCBs 0.1 µg/mL, PAHs 1 µg/mL
Temperature:	75 °C (1 min), 20 °C/min, 220 °C, 5 °C/min, 260 °C (2 min), 7 °C/min, 305 °C, 2 °C/min, 330 °C
Injection Volume:	1.0 µL
Carrier Gas:	Helium, constant flow, 1.0 mL/min
Injection:	100 °C (0.4 min), 600 °C/min, 300 °C (15 min), 50 °C/min, 120 °C, splitless
Detection:	Quadrupole MS, EI in SIM, Source 230 °C, Transfer line 280 °C

Results and Discussion

The VF-35ms column delivered a multi-residue analysis of 57 PAHs, PCBs and OCPs in 32.5 minutes (Figure 1). For PAHs, two pairs are difficult to resolve, namely benzo[b] fluoranthene/benzo[k]fluoranthene and indeno[1,2,3-c,d] pyrene/dibenz[a,h]anthracene. The first pair has the same mass and therefore cannot be separated by MS alone. The second pair have different masses (276 and 278 respectively) and again are difficult to resolve using only MS.

For PCB and OCP groups, PCB 138/PCB 163 and p,p'-DDD/o,p'-DDT have the same mass spectra and cannot be separated by MS. The OCP group also has another pair difficult to resolve, namely cis-heptachlor epoxide and trans-heptachlor epoxide. These compounds both have the main m/z 353 in their mass spectra. Confirmation of both compounds therefore requires an additional m/z.

Figures 2 to 6 show the peak pairs that are difficult to resolve.

Table 1. Peak Identification and SIM ions

Peak	Compound	Ions
1	Naphthalene	128
2	Acenaphthylene	152
3	Acenaphthene	154
4	Fluorene	166
5	Hexachlorobenzene	284, 249
6	α-HCH	181, 219
7	PCB 18	256, 186
8	γ-HCH	181, 219
9	β-HCH	181, 219
10	Phenanthrene	178
11	Anthracene	178
12	PCB 28	256, 186
13	PCB 31	256, 186
14	Heptachlor	272, 100
15	δ-HCH	181, 219
16	PCB 20	256, 186
17	PCB 52	292, 220
18	Aldrin	66, 263
19	PCB 44	292, 220
20	trans-Heptachlor epoxide	353, 81
21	cis-Heptachlor epoxide	81, 183
22	PCB 155	360, 290
23	trans-Chlordane	373, 326
24	PCB 101	326, 254
25	o,p'-DDE	246, 318
26	cis-Chlordane	373, 237
27	Fluoranthene	202
28	Endosulfan I	195, 241
29	p,p'-DDE	246, 318
30	Dieldrin	79, 263
31	Pyrene	202
32	o,p'-DDD	235, 165
33	PCB 118	326, 254
34	PCB 149	360, 290
35	Endrin	263, 81
36	PCB 153	360, 290
37	o,p'-DDT	235, 165
38	p,p'-DDD	235, 165
39	Endosulfan II	241, 195
40	PCB 105	326, 254
41	PCB 163	360, 290
42	PCB 138	360, 290
43	p,p'-DDT	235, 165
44	Endrin-aldehyde	345, 67
45	Endosulfan sulfate	272, 387
46	PCB 180	396, 324
47	Methoxychlor	227
48	Benz[a]anthracene	228
49	Chrysene	228
50	PCB 170	396, 324
51	PCB 194	430, 358
52	Benzo[b]fluoranthene	252
53	Benzo[k]fluoranthene	252
54	Benzo[a]pyrene	252
55	Indeno[1,2,3-c,d]pyrene	276
56	Dibenz[a,h]anthracene	278
57	Benzo[g,h,i]perylene	276

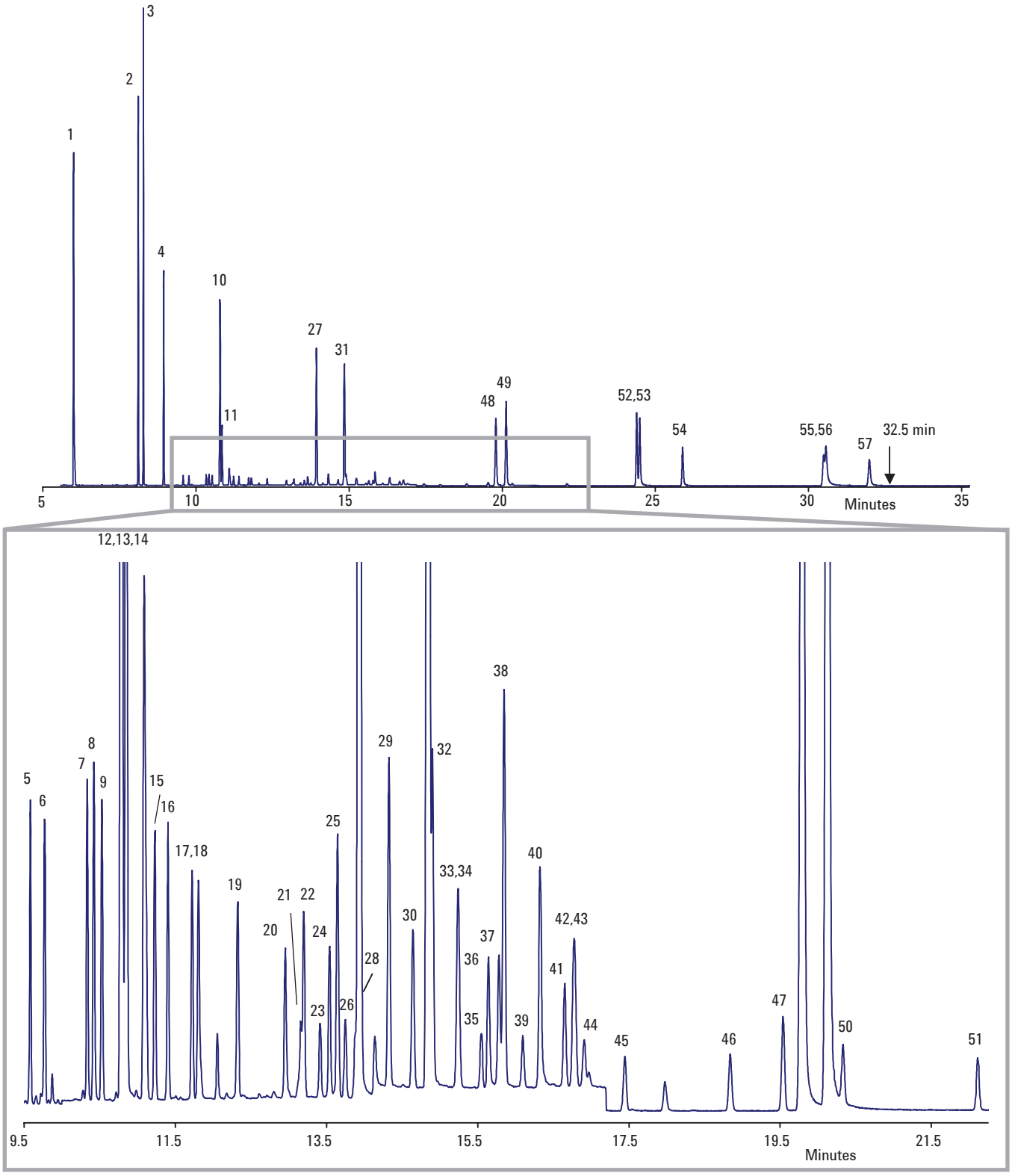

Figure 1. Total ion chromatogram multi-residue analysis on a VF-35ms column.

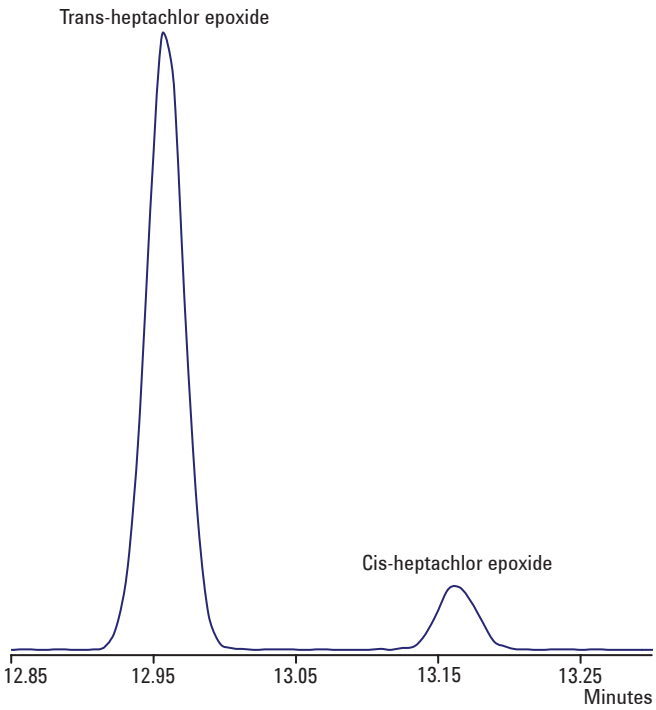

Figure 2. Zoomed total ion chromatogram of cis-heptachlor epoxide / trans-heptachlor epoxide

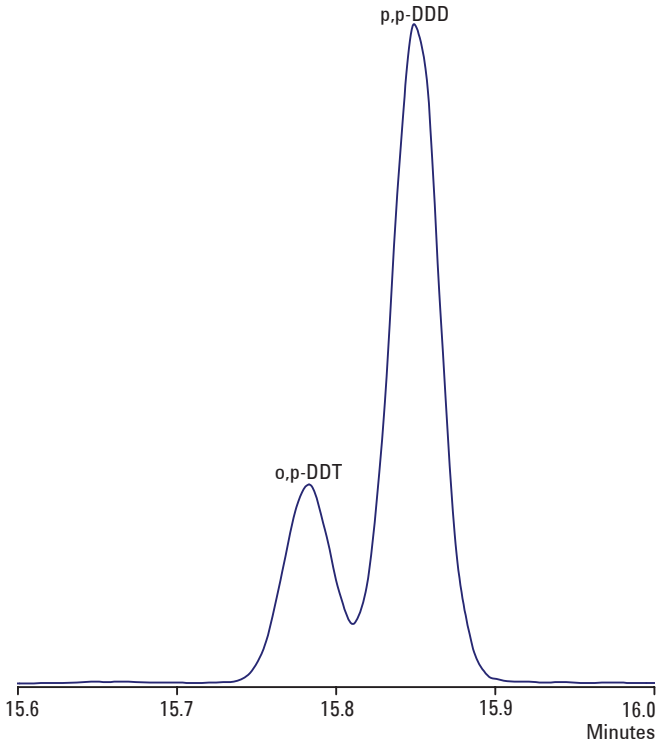

Figure 3. Selected ion chromatogram (m/z 235) of p,p'-DDD / o,p'-DDT

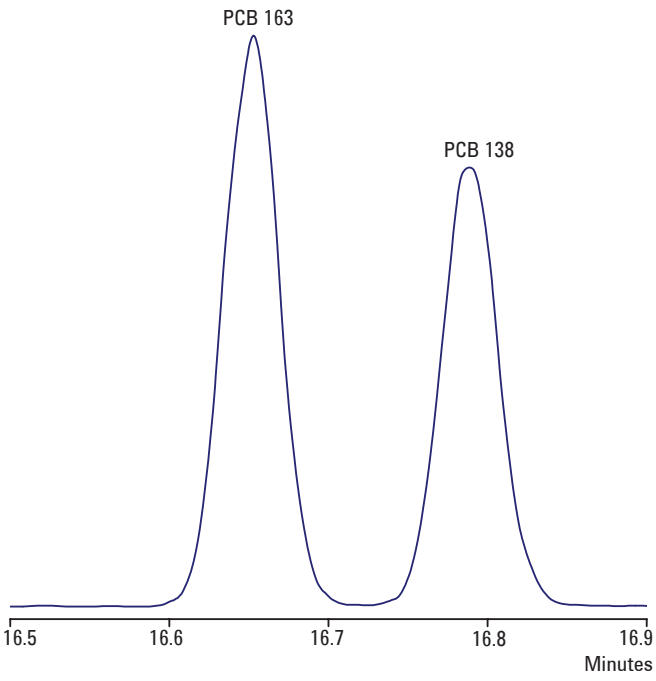

Figure 3. Selected ion chromatogram (m/z 360) of PCB 138/PCB 163

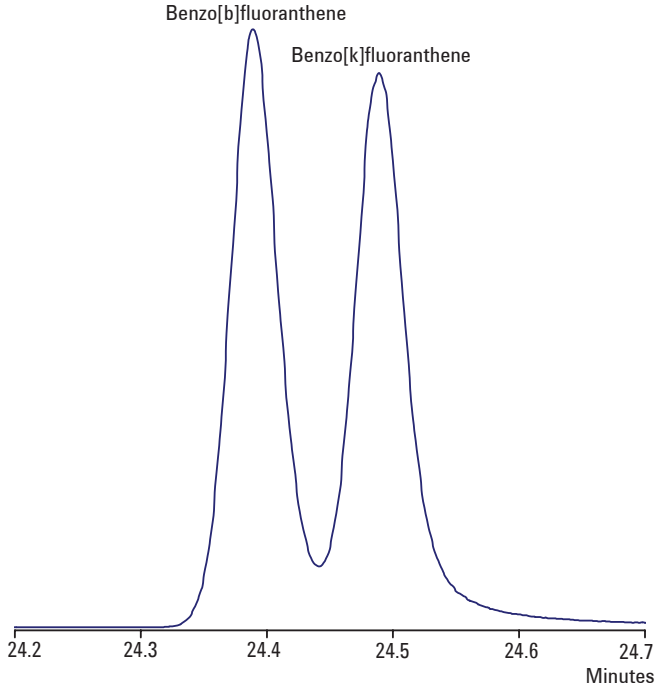

Figure 4. Zoomed total ion chromatogram of benzo[b]fluoranthene / benzo[k]fluoranthene

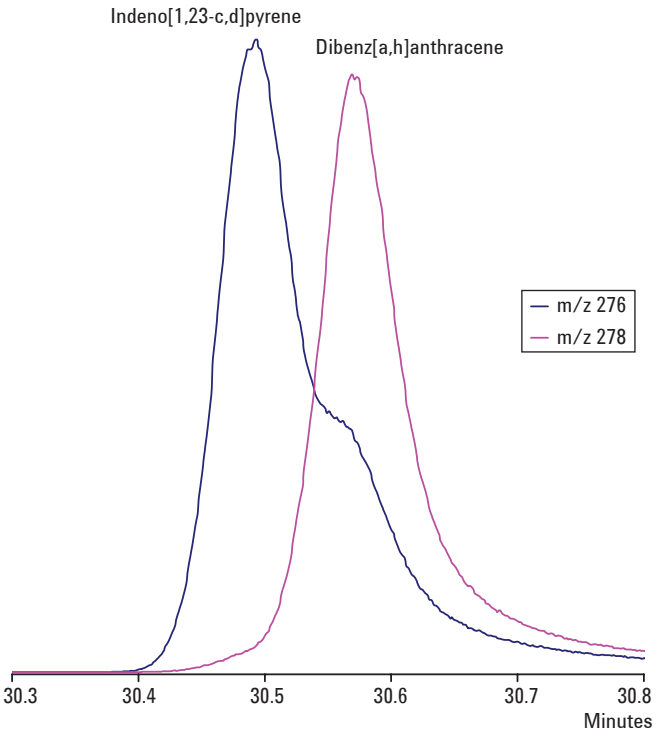

Figure 6. Selected ion chromatogram (m/z 276 and 278) of indeno[1,2,3-c,d]pyrene and dibenz[a,h]anthracene

Conclusion

Analyzing all of these compounds in a single run is normally problematic because every group presents its own difficulties in separation. However, with the VF-35ms column and optimized oven program, multi-residue separation is achieved in about 32 minutes. The VF-35ms column is based on a medium polarity, highly robust and inert phase, making it the ideal choice for demanding trace environmental and chemical analysis.

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http://www.akaction.org/fact_sheets/Organochlorine_Pesticide_Fact_Sheet.pdf

www.agilent.com/chem

Published in UK, October 11, 2010
SI-02396

Agilent Technologies

Extra Fast Separation of 16 US EPA 610 Regulated PAHs on Agilent J&W Select PAH

Application Note

Author

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Introduction

The difficulty in analyzing polycyclic aromatic hydrocarbons (PAHs) is that a number of them have the same mass. This makes their separation with GC/MS problematic, so that column selectivity and an optimized oven program are necessary for their resolution.

PAHs contain two or more aromatic rings and are formed during incomplete combustion or pyrolysis of organic matter, industrial processes, and cooking and food processing. Due to their carcinogenic activities, it is necessary to analyze PAHs in environmental and food samples. However, in PAH analysis, there is a difference between the European (EU) and American (EPA 610) legislation, with the EPA list used for environmental samples and the EU PAHs analyzed in food samples (Table 1). We describe here an optimized oven program for the Select PAH column that separates PAH in less than 7 minutes (Figure 1).

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Conditions

Technique:	GC/MS, Triple Quad
Column:	Select PAH, 15 m x 015 mm, df=0.10 µm (part number CP7461)
Sample:	SRM 1647c, concentration approximately 0.8-21 µg/mL (www.nist.com)
Injection Volume:	1 µL
Temperature:	70 °C (0.40 min), 120 °C/min, 180 °C, 40 °C/min, 350 °C (5 min)
Carrier Gas:	Helium, constant flow 1.2 mL/min
Injector:	300 °C, Splitless mode, 0.25 min @ 100 mL/min
Detector:	Triple Quad, EI in FS or SIM mode, ion source 275 °C, transfer line 300 °C

Results and Discussion

Conditions were optimized to give a fast separation of the 16 EPA PAHs. The most important isomers to resolve were phenanthrene and anthracene (m/z 178), benzo[b and k] fluoranthene (m/z 252), and indeno[1,2,3-c,d]pyrene and dibenzo[a,h]anthracene with m/z 276 and 278.

Table 1. Peak identification for Figure 1

Peak	MW	Compound	EPA 610	SFC1 and EFSA2 PAHs (15+1)	CAS
1	128	Naphthalene	x		91-20-3
2	152	Acenaphthylene	x		208-96-8
3	154	Acenaphthene	x		83-32-9
4	166	Fluorene	x		86-73-7
5	178	Phenanthrene	x		85-01-8
6	178	Anthracene	x		120-12-7
7	202	Fluoranthene	x		206-44-0
8	202	Pyrene	x		129-00-0
9	228	Benzo[a]anthracene	x	x	56-55-3
10	228	Chrysene	x	x	218-01-9
11	252	Benzo[b]fluoranthene	x	x	205-99-2
12	252	Benzo[k]fluoranthene	x	x	207-08-9
13	252	Benzo[a]pyrene	x	x	50-32-8
14	278	Dibenzo[a,h]anthracene	x	x	53-70-3
15	276	Indeno[1,2,3-c,d]pyrene	x	x	193-39-5
16	276	Benzo[g,h,i]perylene	x	x	191-24-2

[1] Scientific Committee on Food, one of the committees providing the European Commission with scientific advice on food safety. [2] European Food Safety Authority.

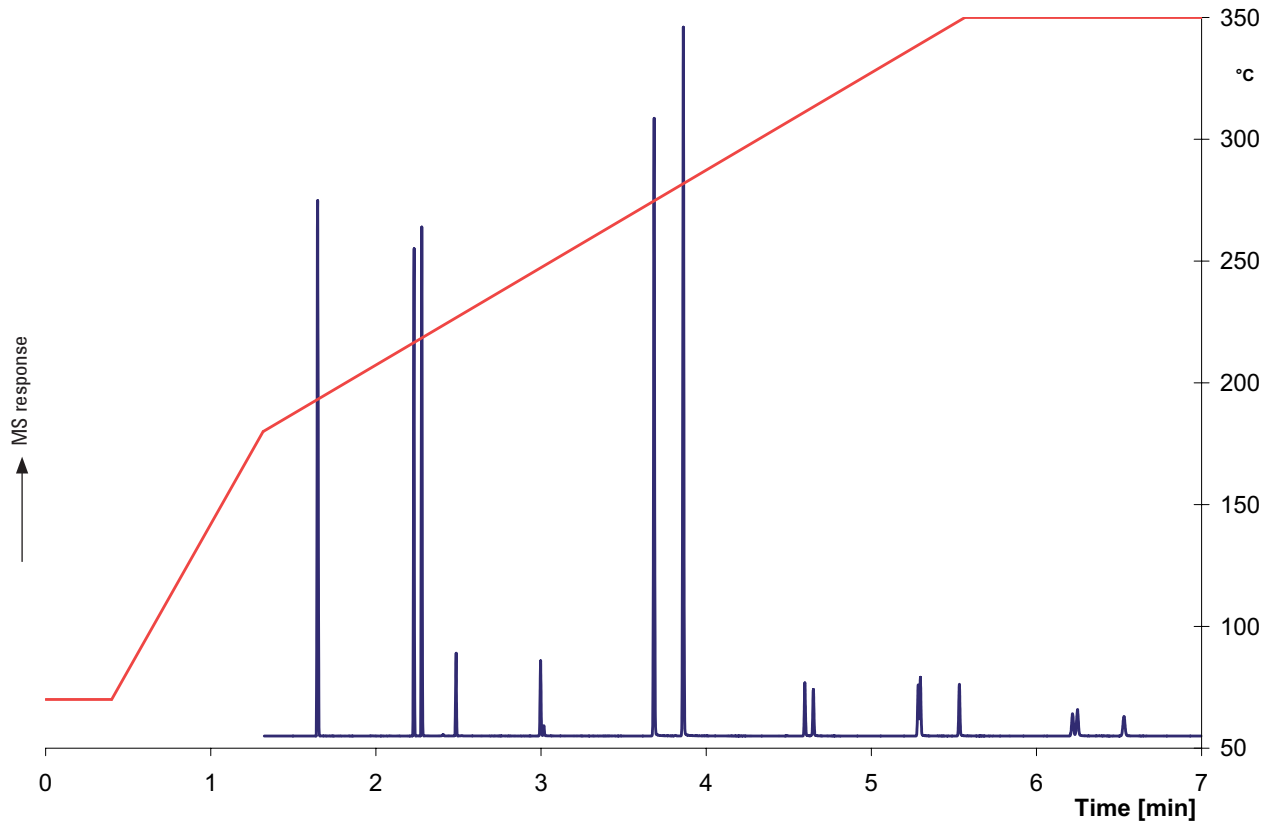

Figure 1. Fast GC/MS analysis of 16 US EPA PAHs on Select PAH

Table 2. Peak Identification for Figure 2

Peak	MW	Compound	EPA 610	CAS
1	128	Naphthalene	x	91-20-3
2	152	Acenaphthylene	x	208-96-8
3	154	Acenaphthene	x	83-32-9
4	166	Fluorene	x	86-73-7
5	178	Phenanthrene	x	85-01-8
6	178	Anthracene	x	120-12-7
7	202	Fluoranthene	x	206-44-0
8	202	Pyrene	x	129-00-0
9	228	Benzo[a]anthracene	x	56-55-3
10	228	Chrysene	x	218-01-9

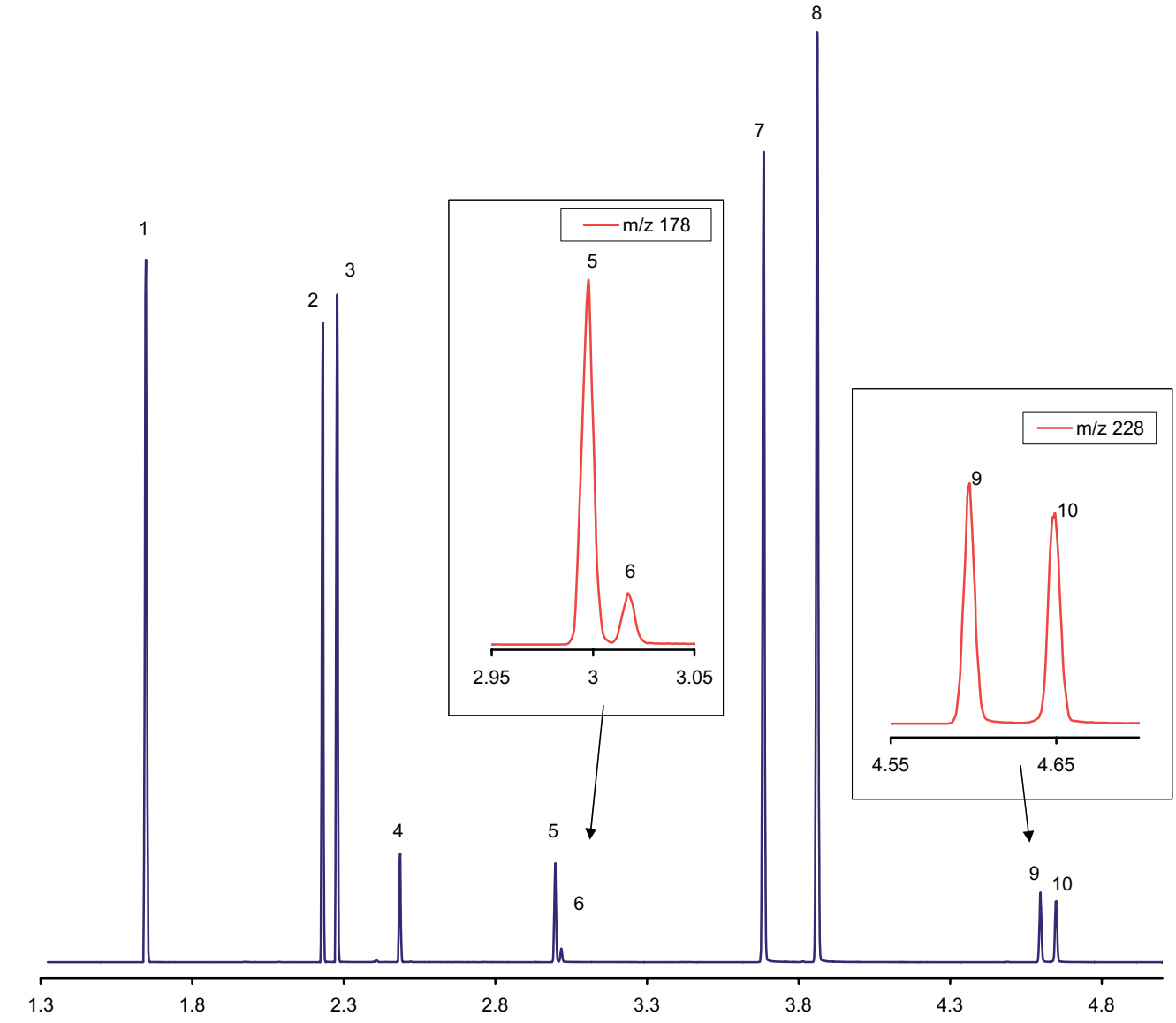

Figure 2. Details and peak identification, peak numbers 1 to 10

Table 3. Peak Identification for Figure 3

Peak	MW	Compound	EPA 610	CAS
11	252	Benzo[b]fluoranthene	x	205-99-2
12	252	Benzo[k]fluoranthene	x	207-08-9
13	252	Benzo[a]pyrene	x	50-32-8
14	278	Dibenz[a,h]anthracene	x	53-70-3
15	276	Indeno[1,2,3-c,d]pyrene	x	193-39-5
16	276	Benzo[g,h,i]perylene	x	191-24-2

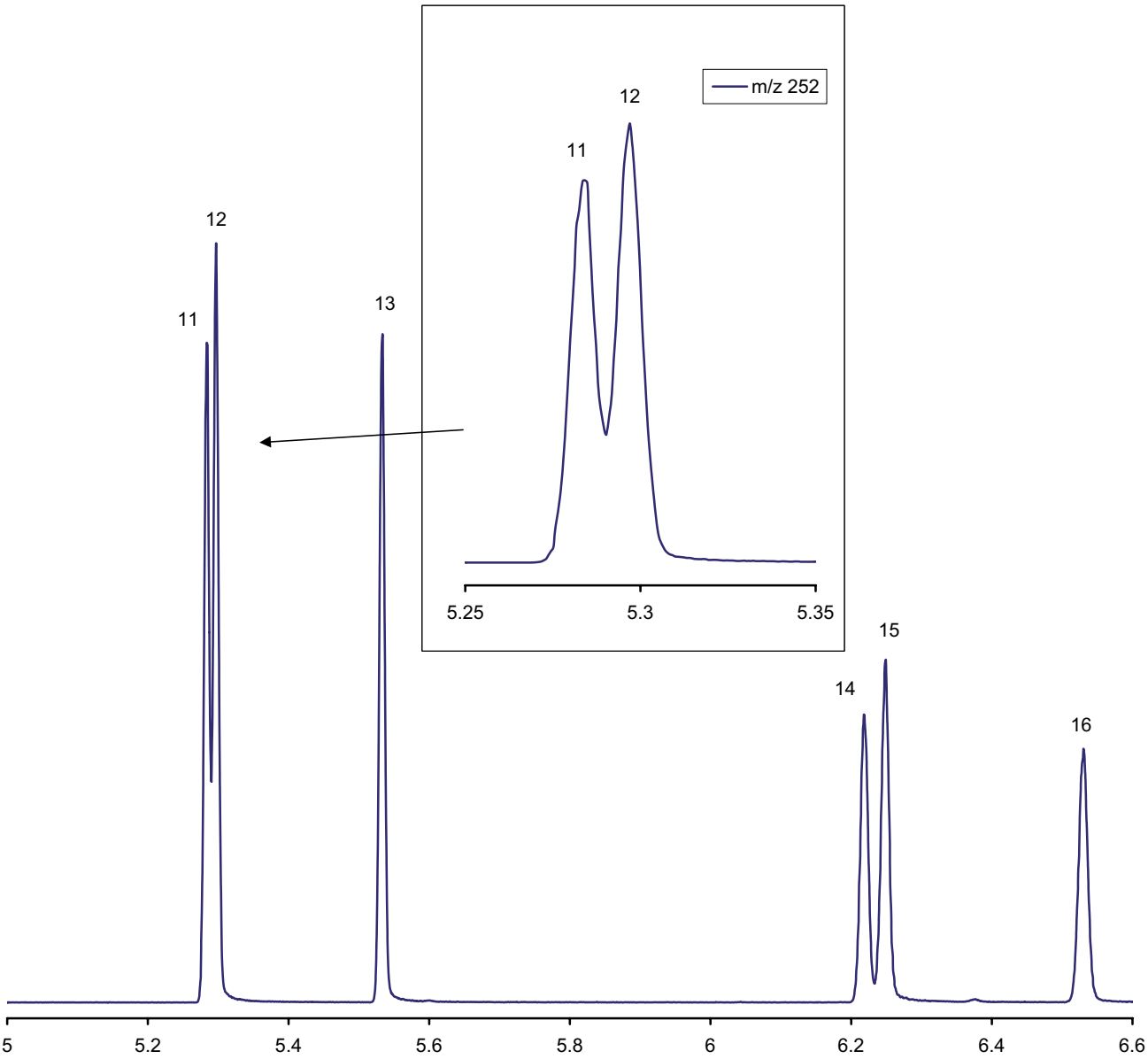

Figure 3. Details and peak identification, peak numbers 11 to 16

Conclusion

With the Select PAH GC column and the optimized oven program described here, all 16 EPA PAHs were resolved in a single run with a runtime of less than 7 minutes.

The Select PAH column also offers enhanced resolution of PAHs, preventing co-elution of interfering PAHs that can cause false positives and inaccurate results. Typical interferences are triphenylene on chrysene, and benzo[j] fluoranthene on benzo[k]fluoranthene. Application note SI-02259 describes the separation of 54 PAHs, including the 16 EPA PAHs and their interferences.

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www.agilent.com/chem

Published in UK, October 11, 2010

SI-02282

Agilent Technologies

Separation of EU and EPA Regulated PAHs on Agilent J&W FactorFour VF-17ms Column

Application Note

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Introduction

The difficulty in analyzing polycyclic aromatic hydrocarbons (PAHs) is the number of PAHs with the same mass. This makes the separation of these PAHs with GC/MS rather difficult, and column selectivity and an optimized oven program are necessary to resolve these PAHs. In this application note an optimized oven program for the FactorFour VF-17ms column is described.

PAHs are compounds which contain two or more aromatic rings. They are formed during incomplete combustion or pyrolysis of organic matter, industrial processes and cooking and food processing. PAHs are therefore analyzed in both environmental and food related matrices.

In PAH analysis there is a difference between the European (EU) and American (EPA) legislation. These regulatory bodies both describe a different set of PAHs (Table 1). The EPA list is especially used for environmental samples while the EU PAHs are analyzed in food samples. This application note shows a GC/MS method which resolves all EU and EPA PAHs with the VF-17ms column (Figure 1).

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Conditions

Technique:	GC/MS
Column:	VF-17ms, 30 m x 0.25 mm, df = 0.15 µm (part number CP8981)
Temperature:	70 °C (1 min), 40 °C/min, 180 °C, 2.5 °C/ min, 245 °C, 2.0 °C/min, 270 °C (10 min), 8.0 °C/min, 350 °C (5 min)
Carrier Gas:	Helium, constant flow 1 mL/ min
Injector:	300 °C, Splitless mode
Detector:	Quadrupole MS, EI in SIM, ion source 275 °C, transfer line 300 °C
Sample:	Concentration approx. 1 µg/ mL
Injection Volume:	1 µL

Table 1. Peak Identification for Figure 1

Peak	Compound	MW	EU PAH	EPA PAH
1	Naphthalene	128		X
2	Acenaphthylene	152		X
3	Acenaphthene	154		X
4	Fluorene	166		X
5	Phenanthrene	178		X
6	Anthracene	178		X
7	Fluoranthene	202		X
8	Pyrene	202		X
9	Benzo(c)fluorene	216	X	
10	Benz(a)anthracene	228	X	X
11	Cyclopenta(c,d) pyrene	226	X	
12	Triphenylene	228		
13	Chrysene	228	X	X
14	6-Methylchrysene	242		
15	5-Methylchrysene	242	X	
16	Benzo(b) fluoranthene	252	X	X
17	Benzo(k) fluoranthene	252	X	X
18	Benzo(j)fluoranthene	252	X	
19	Benzo(a)pyrene	252	X	X
20	Indeno(1,2,3-cd) pyrene	276	X	X
21	Benzo(b) triphenylene	278		
22	Dibenz(a,h) anthracene	278	X	X
23	Benzo(g,h,i)perylene	276	X	X
24	Dibenzo(a,l)pyrene	302	X	
25	Dibenzo(a,e)pyrene	302	X	
26	Dibenzo(a,i)pyrene	302	X	
27	Dibenzo(a,h)pyrene	302	X	

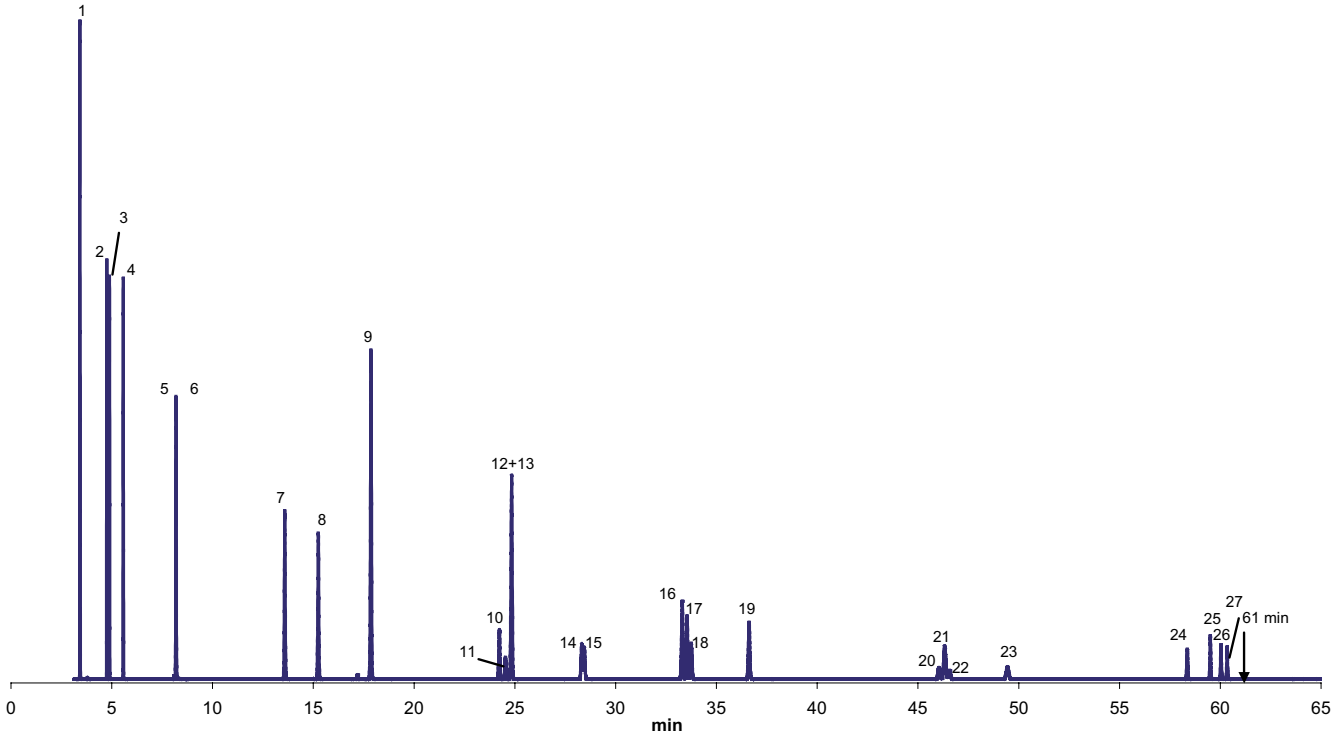

Figure 1. GC/MS analysis of EU and EPA PAHs on VF-17ms 30 m x 0.25 mm x 0.15 µm

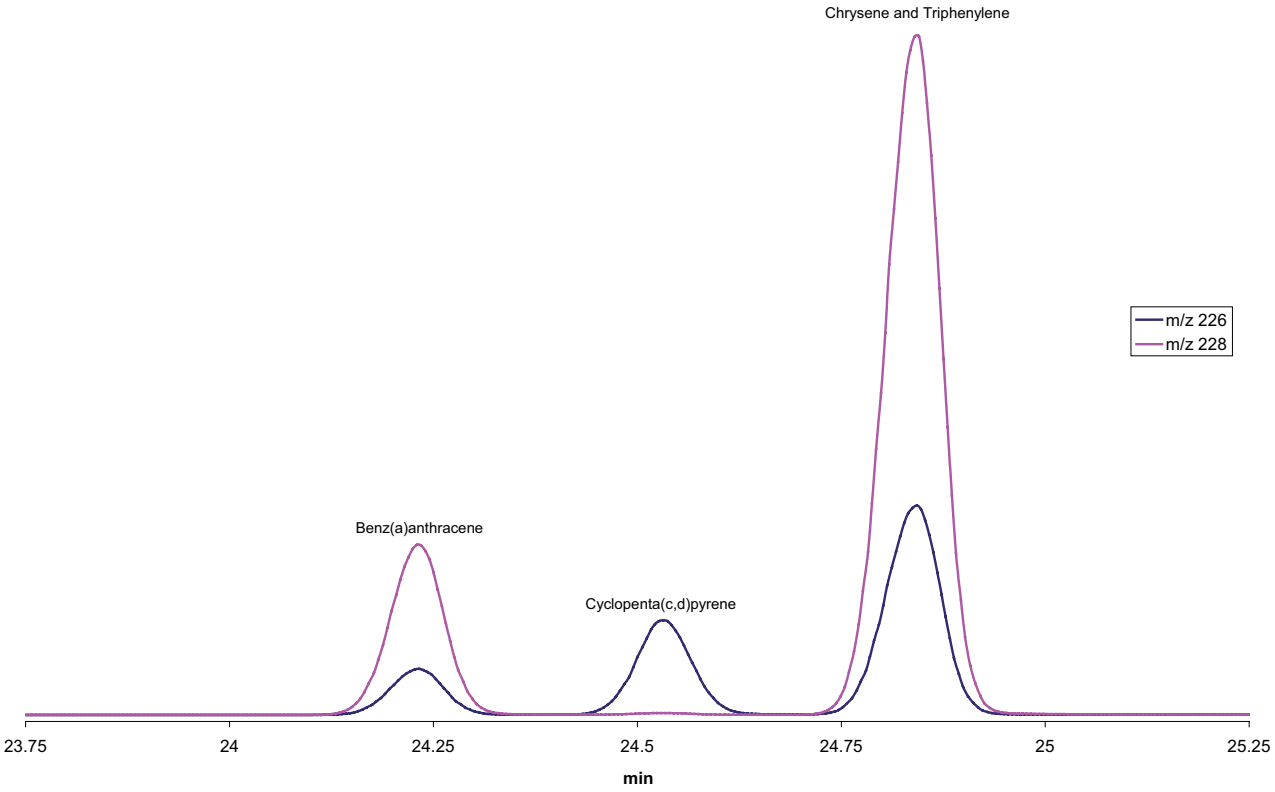

Figure 2. Separation of m/z 226 and 228

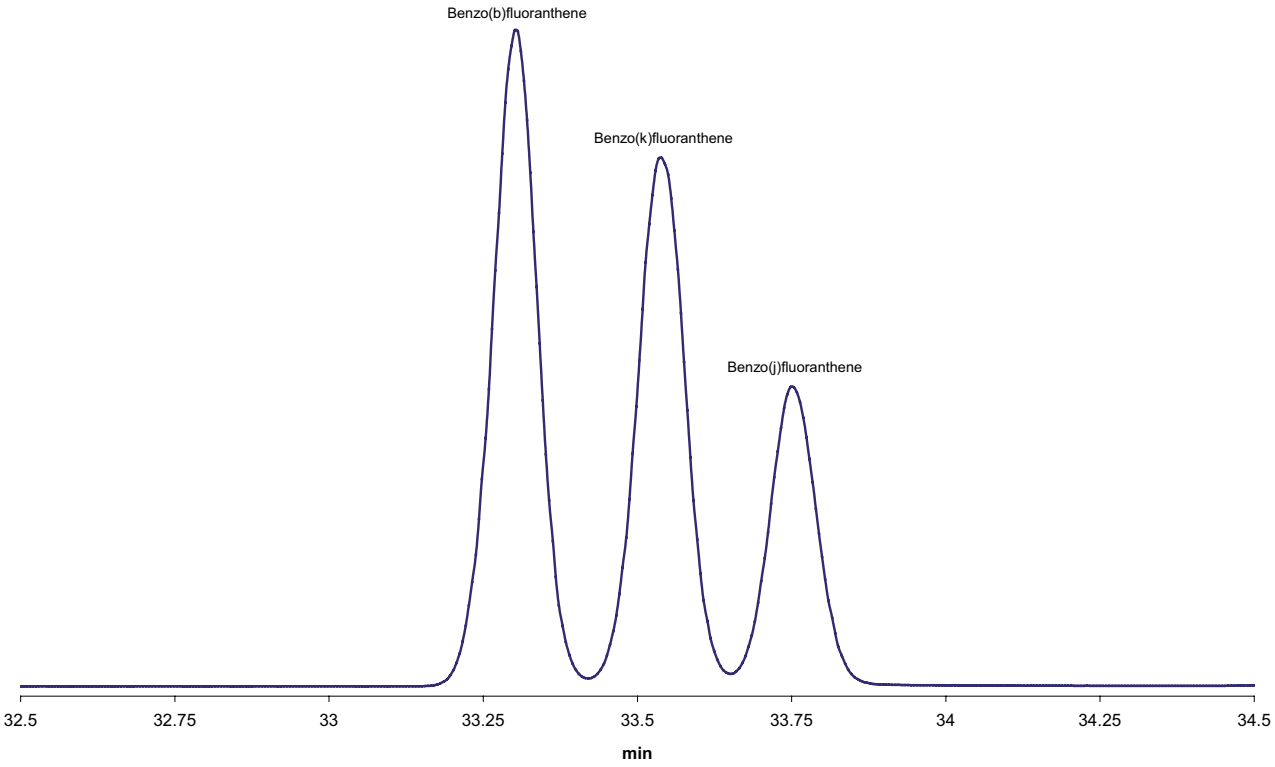

Figure 3. Separation of Benzofluoranthenes (m/z 252)

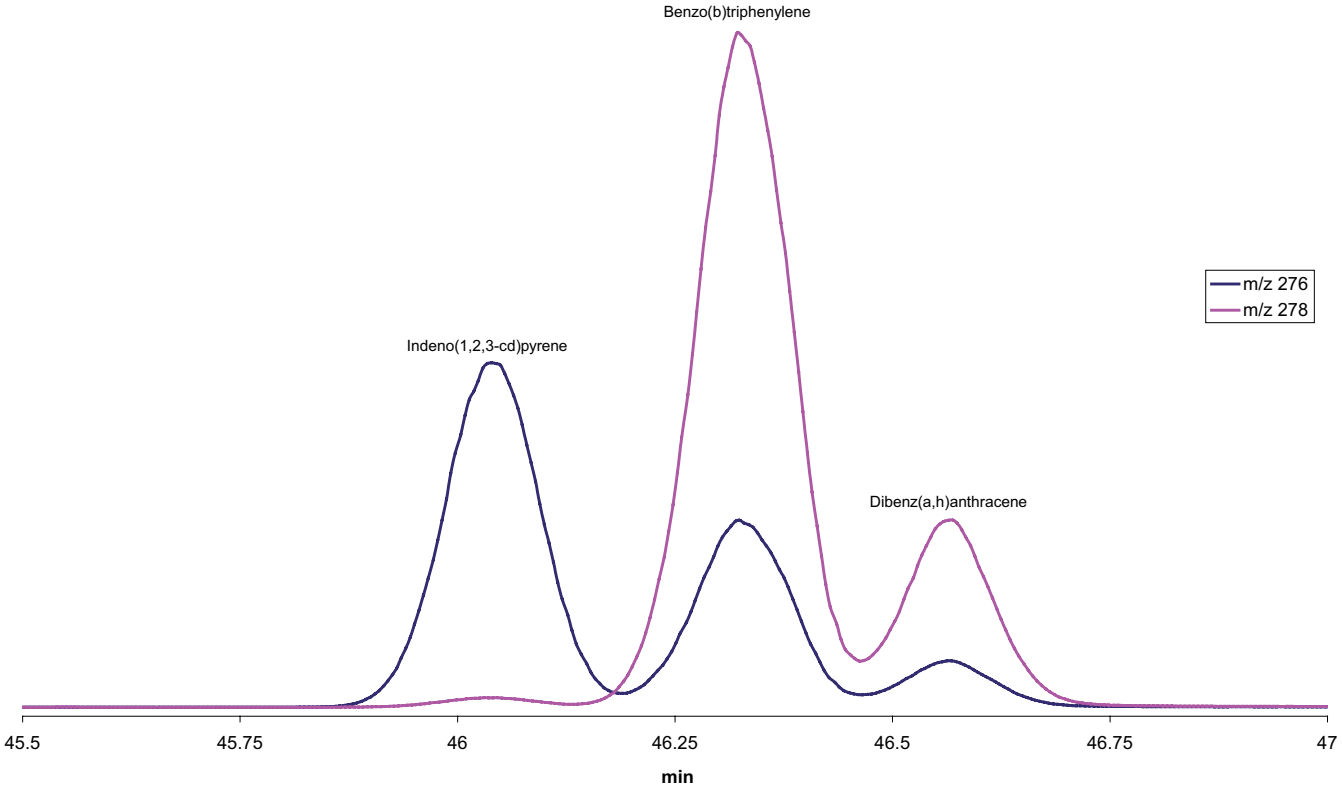

Figure 4. Separation of m/z 276 and 278

Results and Discussion

When performing this analysis there are 3 sets of peaks which are difficult to resolve. The first set, Benz(a) anthracene, Cyclopenta(c,d)pyrene, Chrysene and Triphenylene, has different masses, m/z 226 and 228. The compounds with m/z 228 also contain some m/z 226 and this makes it difficult to resolve this set only with MS (Figure 2). The same problem occurs when separating Indendo(1,2,3-cd)pyrene, Benzo(b)triphenylene and Dibenz(a,h)anthracene with m/z 276 and 278 (Figure 4).

The third set of PAHs which are difficult to resolve are the Benzofluoranthene isomers. These three isomers, Benzo(b) fluoranthene, Benzo(j)fluoranthene and Benzo(k)fluoranthene, have the same mass and can't be resolved with MS only (Figure 3).

For the above reasons the column has to resolve these components chromatographically which is showed in the figures.

Conclusion

The VF-17ms column phase as well as the GC oven program affect the separation of the difficult to resolve PAH sets. With the optimized oven program described in this application note all EU and EPA PAHs are resolved. It should be taken in mind that Triphenylene can cause a false positive because of the co-elution with Chrysene.

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www.agilent.com/chem

Published in UK, October 08, 2010
SI-02124

Agilent Technologies

Benefit of Low Temperature ELSD for the Analysis of Herbicides by HPLC

Stephen Bullock
Polymer Laboratories, now a part of Varian, Inc.

Introduction

Phenylurea compounds are used extensively in agriculture as selective herbicides, for pre- and post-emergence, by inhibiting photosynthesis. They are easily taken up from soil solution by the root systems of plants and rapidly translocated into stems and leaves by the transpiration system. Common phenylurea herbicides, such as diuron, have a relatively low tendency to adsorb to soils and sediments, while its degradation products have a relatively long lifetime. Consequently, the mobility and relative persistence of these herbicides increases the chance of migration to ground water via leaching and surface run-off. Developing analytical methods for the analysis of phenylurea herbicides and their metabolites in surface and ground water is vital to the understanding of herbicide fate and degradation pathways in the environment.

Unlike the majority of pesticides, the application of gas chromatography (GC) to the analysis of phenylurea herbicides is difficult because they are thermally unstable and degrade rapidly to isocyanates and amines[1]. In addition, thermal reactions in the GC detector and on column result in a lack of reproducibility preventing the quantitative screening of the degradation products. A better approach for thermally sensitive compounds is to use HPLC, coupled with evaporative light scattering detection.

Evaporative light scattering detectors respond to all compounds that are less volatile than the mobile phase. In the past, high ELSD operating temperatures were required to fully evaporate aqueous eluents, making the detection of semi-volatile analytes difficult or even impossible.

The Varian ELS detector is designed to evaporate highly aqueous mobile phases at ambient temperature. This is achieved by introducing a stream of dry nitrogen during the evaporation stage, which increases the vapor loading capacity of the surrounding gas and enables complete evaporation of the eluent to take place without raising the temperature. The advantage of characterizing samples at ambient temperature is demonstrated by the analysis of a mixture of ten phenylurea herbicides.

Instrumentation

Column: C18 5 µm, 250 x 4.6 mm
Detection: Varian ELSD (neb=25 °C, evap as shown, gas=1.6 SLM)

Materials and Reagents

Eluent A: Water
Eluent B: ACN

Sample Preparation

Sample: Phenylurea herbicide mixture

Conditions

Flow Rate: 0.7 mL/min
Injection Volume: 20 µL
Gradient: 10-80 % B in 40 min

Results and Discussion

The herbicide mixture contains compounds of different volatilities and the highly volatile herbicides are not detected as the ELSD temperature is increased. Only seven of the ten herbicides are detected at 70 °C, compared to all ten at 25 °C. Therefore, by operating the Varian ELS detector at ambient temperature the loss of semi-volatile components is minimized, the sample integrity is preserved and maximum sensitivity is achieved, as shown in Figure 1.

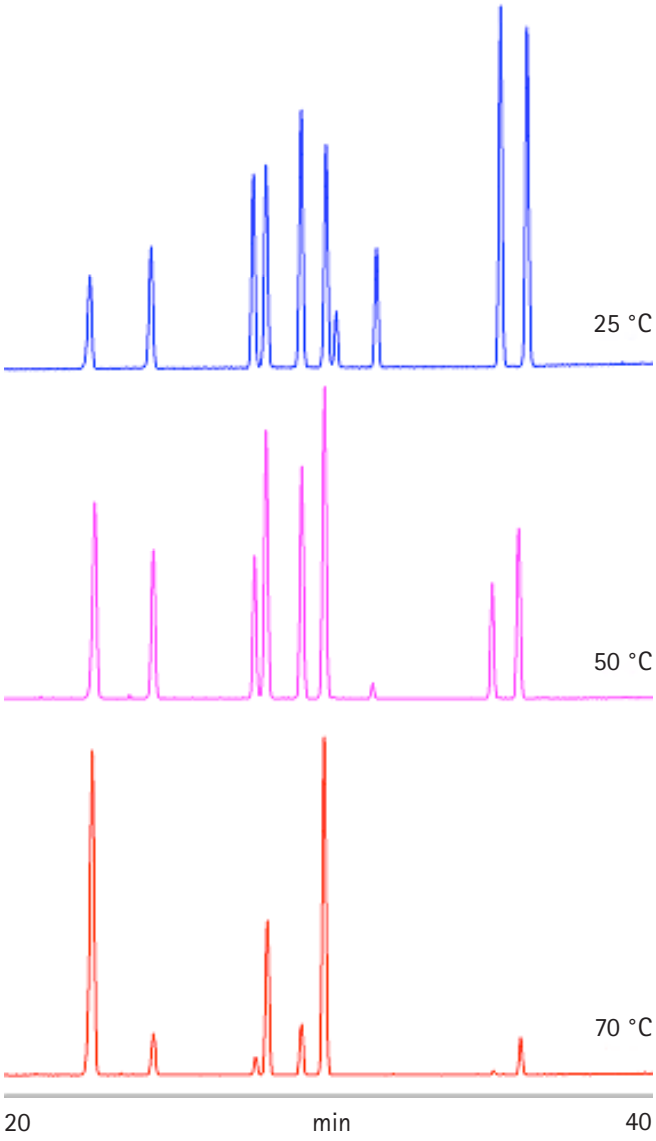

Figure 1. Varian ELSD reveals the presence of semi-volatile herbicides when operated at ambient temperature.

Conclusion

Separation and identification of semi-volatile compounds is straightforward using evaporative light scattering detection at the ambient temperatures available with the Varian ELS detector. The Varian ELS detector surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELS detector's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian ELS detector provides superior performance compared to detectors from other vendors for the analysis of semi-volatile compounds.

References

[1]Garland, S.M. et al. 2004. Practical approaches to the analyses for pesticide residues in essential oils. Rural Industries Research and Development Corporation, Barton, ACT, Australia.

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Application Note SI-01229 / TB1064

Fast analysis of coal tar polycyclic aromatic hydrocarbons on Agilent J&W Select PAH

Application Note

Author

John Oostdijk
Agilent Technologies, Inc.

Introduction

The difficulty in analyzing polycyclic aromatic hydrocarbons (PAHs) is the number of PAHs with the same mass. This makes their separation by GC/MS rather difficult, and so column selectivity and an optimized oven program are necessary for the resolution of PAHs. We describe here the fast analysis of a coal tar sample using an optimized oven program and a 15 m x 0.15 mm x 0.10 µm Select PAH column.

Coal tar is a brown or black liquid of high viscosity that smells of naphthalene and aromatic hydrocarbons. It is obtained from the destructive distillation of coal. In the past, coal tar was sourced as a by-product from the manufacture of coal gas but is now produced during the production of coke for steel making. The crude tar contains many organic compounds, such as benzene, naphthalene, methylbenzene and phenols, which can be obtained by distillation, leaving a residue of pitch. At one time coal tar was the major source of organic chemicals, most of which are now derived from petroleum and natural gas. Coal tar pitch is mainly used as binding agent in the production of carbon electrodes, anodes and Søderberg electrodes, for instance, by the aluminium industry. It is also used as a binding agent for refractories, clay pigeons, active carbon, coal briquetting, road construction and roofing. In addition, small quantities are used for heavy-duty corrosion protection.

The standard reference material for coal tar analysis (SRM 1597a, NIST) is a natural, combustion-related mixture of PAHs from a medium crude coke-oven tar that is dissolved in toluene. It is intended for use in the evaluation and validation of analytical methods for the determination of PAHs. A sample of SRM 1597a was therefore analyzed using an optimized oven program for the Select PAH column, demonstrating the excellent performance of the column with this reference material.

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Conditions

Technique:	GC/MS, Triple Quad
Column:	Select PAH, 15 m x 0.15 mm, df = 0.10 µm (part number CP7461)
Sample:	SRM 1597a, concentration approximately 0.2-1030 mg/kg (www.nist.gov)
Injection Volume:	1.0 µL SIM mode, 0.1 µL FS mode
Temperature:	70 °C (0.40 min), 70 °C/min, 180 °C
Program:	7 °C/min, 230 °C (7 min), 50 °C/min, 280 °C (7 min), 30 °C/min, 350 °C (4 min)
Carrier Gas:	Helium, constant flow 1.2 mL/min
Injection:	300 °C, Splitless mode, 0.5 min @ 100 mL/min
Detection:	Triple Quad, EI in FS or SIM mode, ion source 275 °C, transfer line 300 °C

Results and Discussion

The sample was analyzed directly in full scan and SIM mode using the same conditions as for a standard with regulated and interfering PAHs. Chromatograms with peak identifications are shown in Figures 1 to 3. Figure 1 shows chrysene (66 mg/kg) separated from triphenylene (12 mg/kg). Benzo[b,k,j]fluoranthene (66, 37 and 41 mg/kg) was separated from benzo[a]pyrene (94 mg/kg). The MS spectrum from chrysene is also shown. As well as m/z 228, other ions, such as m/z 226 and m/z 229, were formed. PAHs were mostly very stable and only low fragment ions were observed, for example m/z 113 and 114 in the mass spectrum.

Table 1. Peak identification for Figure 1

Peak	Name
BaA	Benzo[a]anthracene
BaP	Benzo[a]pyrene
BbFA	Benzo[b]fluoranthene
BkFa	Benzo[k]fluoranthene
BjFA	Benzo[j]fluoranthene
CHR	Chrysene
CPP	Cyclopenta[c,d]pyrene
TP	Triphenylene

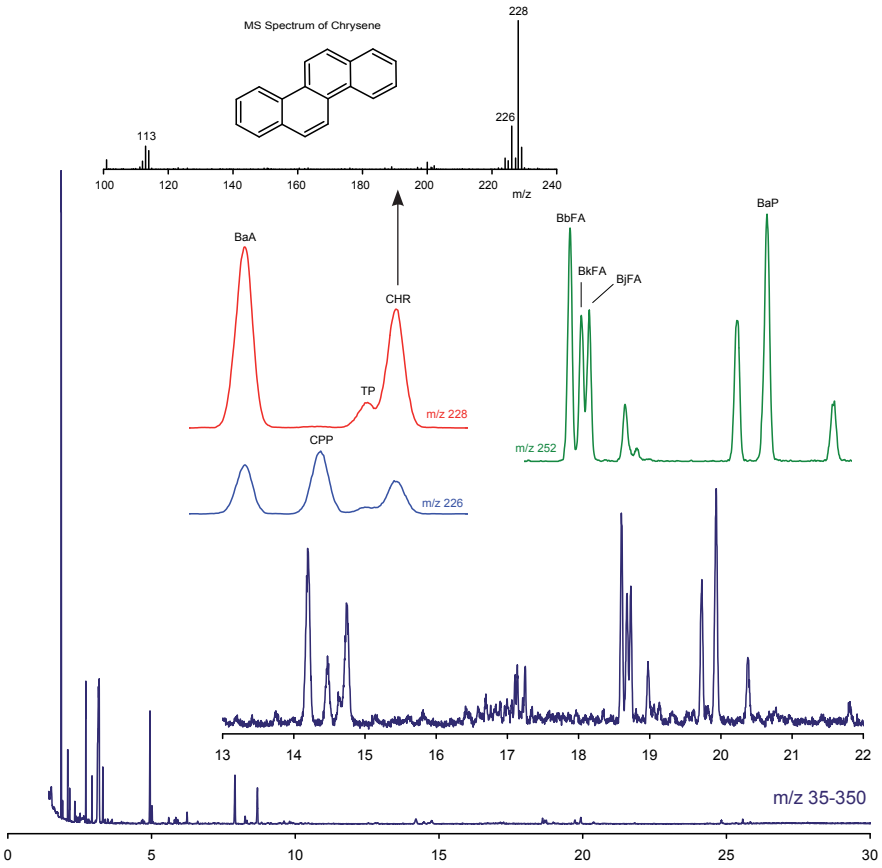

Figure 1. GC/MS analysis of 0.1 µL of SRM 1597a in full scan mode (m/z 35-350) on Select PAH

Table 2. Peak identification for Figure 2

Peak	Name
ACL	Acenaphthylene (263 mg/kg)
BaA	Benzo[a]anthracene (98 mg/kg)
BaP	Benzo[a]pyrene (94 mg/kg)
BghiP	Benzo[g,h,i]perylene (51 mg/kg)
DBaeP	Dibenzo[a,e]pyrene (9 mg/kg)
FA	Fluoranthene (327 mg/kg)
PHE	Phenanthrene (454 mg/kg)
PY	Pyrene (240 mg/kg)
NA	Naphthalene (1030 mg/kg)

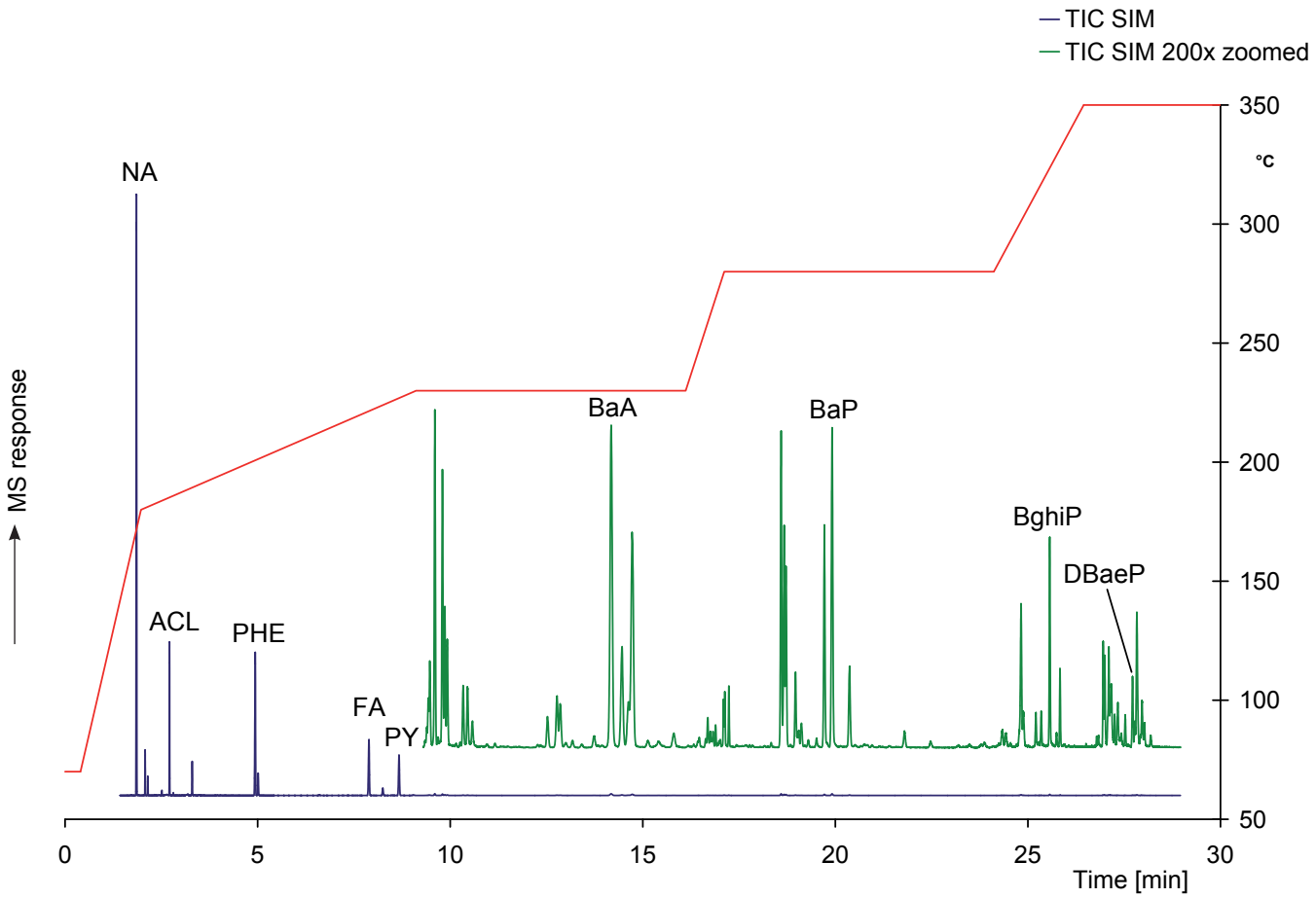

Figure 2. GC/MS analysis of 1 µL of SRM 1597a in SIM mode on Select PAH. The final part of the chromatogram was enlarged because of the large concentration difference in the sample

Table 3. Peak identification for Figure 3

Peak	Name
ATR	Anthanthrene (Dibenzo[d,e,f,m,n,o] chrysene)
BaFA	Benzo[a]fluoranthene
BaFL	Benzo[a]fluorene
BaP	Benzo[a]pyrene
BbCHR	Benzo[b]chrysene
BbFA	Benzo[b]fluoranthene
BbFL	Benzo[b]fluorene
BbNTP	Benzo[b]naphto[2,1-d]thiophene

Peak	Name
BbPer	Benzo[b]peryelene
BbTP	Benzo[b]triphenylene
BcFL	7H-benzo[c]fluorene
BeP	Benzo[e]pyrene
BghiP	Benzo[g,h,i]perylene
BjFA	Benzo[j]fluoranthene
BkFA	Benzo[k]fluoranthene
Cor	Coronene
DBaeP	Dibenzo[a,e]pyrene

Peak	Name
DBahA	Dibenzo[a,h]anthracene
DBahP	Dibenzo[a,h]pyrene
DBaiP	Dibenzo[a,i]pyrene
DBalP	Dibenzo[a,l]pyrene
IP	Indeno[1,2,3-cd]pyrene
6MC	6-Methylchrysene
5MC	5-Methylchrysene
Per	Perylene
Pic	Picene

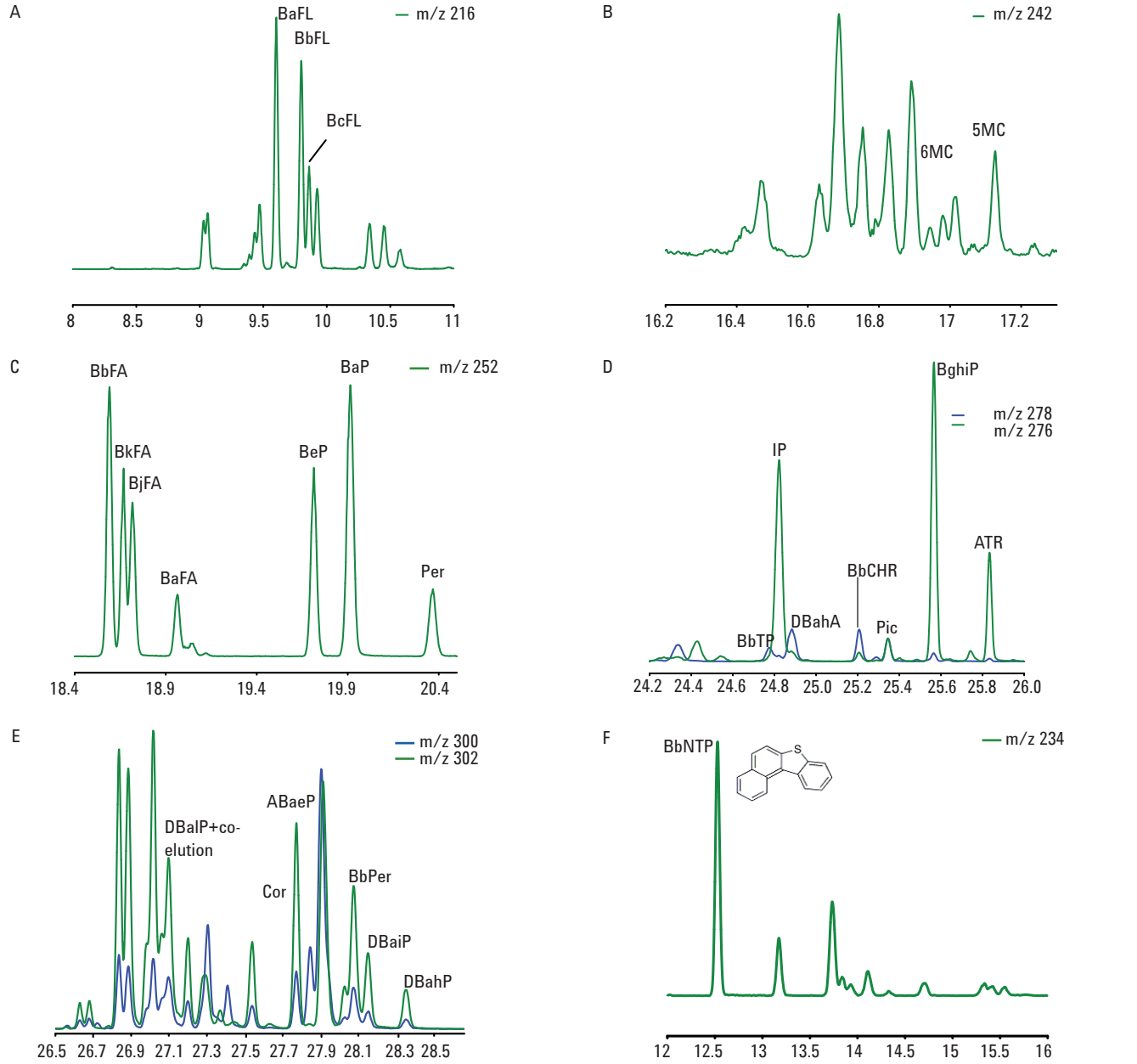

Figure 3. GC/MS analysis of 1 µL of SRM 1597a in SIM mode on Select PAH . Some examples of critical separations are shown: A. Benzofluorenes, B. Methylchrysenes, C. Benzofluoranthenes and isomers, D. Dibenzoanthracenes and isomers, E. Dibenzopyrenes and isomers, F. Benzo[b]naphtho[ ] thiophene isomers

Conclusion

The Select PAH column separated target PAHs in a complex mixture of coal tar in a single run with a run time of 29 min. Together with an optimized oven program, this unique column is the only easy-to-use, single-shot solution that separates all regulated PAH isomers, with no false positives or inaccurate results. With high speed, high resolution and high temperature stability it is the perfect column for accurate PAH analysis.

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Published in UK, April 12, 2011
SI-02279

Agilent Technologies

Fast Separation of 16 US EPA 610 Regulated PAHs on Agilent J&W Select PAH GC Columns

Application Note

Author

John Oostdijk
Agilent Technologies, Inc.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds that contain two or more aromatic rings. They are formed during incomplete combustion or pyrolysis of organic matter, industrial processes, and cooking and food processing. PAHs are therefore analyzed in environmental and food samples. The difficulty in determining PAHs is that several of their isomers have the same mass. This makes the separation of PAHs with GC/MS rather difficult, and so column selectivity and an optimized oven program are necessary for their resolution. This application note describes the interference-free analysis of 16 PAHs listed in EPA 610 using the J&W Select PAH GC column.

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Conditions

Technique:	GC/MS, Triple Quad
Column:	Select PAH, 30 m x 0.25 mm, df = 0.15 µm (part number CP7462)
Sample:	SRM 1647c, concentration appr. 0.8-21 µg/mL (www.nist.com)
Injection Volume:	1 µL
Temperature:	70 °C (0.80 min), 60 °C/min, 180 °C, 20 °C/min 350 °C (5 min)
Carrier Gas:	Helium, constant flow 2.0 mL/min
Injector:	300 °C, Splitless mode, 0.75 min @ 50 mL/min
Detector:	Triple Quad, EI in SIM mode, ion source 275 °C, transfer line 300 °C

Results and Discussion

When performing this analysis, the conditions are optimized to give a fast separation of the 16 EPA PAHs. The most important isomers to resolve are phenanthrene and anthracene (m/z 178), benzo[b and k]fluoranthene (m/z 252), and indeno[1,2,3-c,d]pyrene and dibenz[a,h]anthracene with m/z 276 and 278. These compounds are all resolved by the Select PAH column, as shown in Figure 1. Figures 2 and 3 provide detailed information on the 16 EPA PAHs.

Table 1. Peak identification for Figure 1

Peak	MW	Compound	CAS
1	128	Naphthalene	91-20-3
2	152	Acenaphthylene	208-96-8
3	154	Acenaphthene	83-32-9
4	166	Fluorene	86-73-7
5	178	Phenanthrene	85-01-8
6	178	Anthracene	120-12-7
7	202	Fluoranthene	206-44-0
8	202	Pyrene	129-00-0
9	228	Benz[a]anthracene	56-55-3
10	228	Chrysene	218-01-9
11	252	Benzo[b]fluoranthene	205-99-2
12	252	Benzo[k]fluoranthene	207-08-9
13	252	Benzo[a]pyrene	50-32-8
14	278	Dibenz[a,h]anthracene	53-70-3
15	276	Indeno[1,2,3-c,d]pyrene	193-39-5
16	276	Benzo[g,h,i]perylene	191-24-2

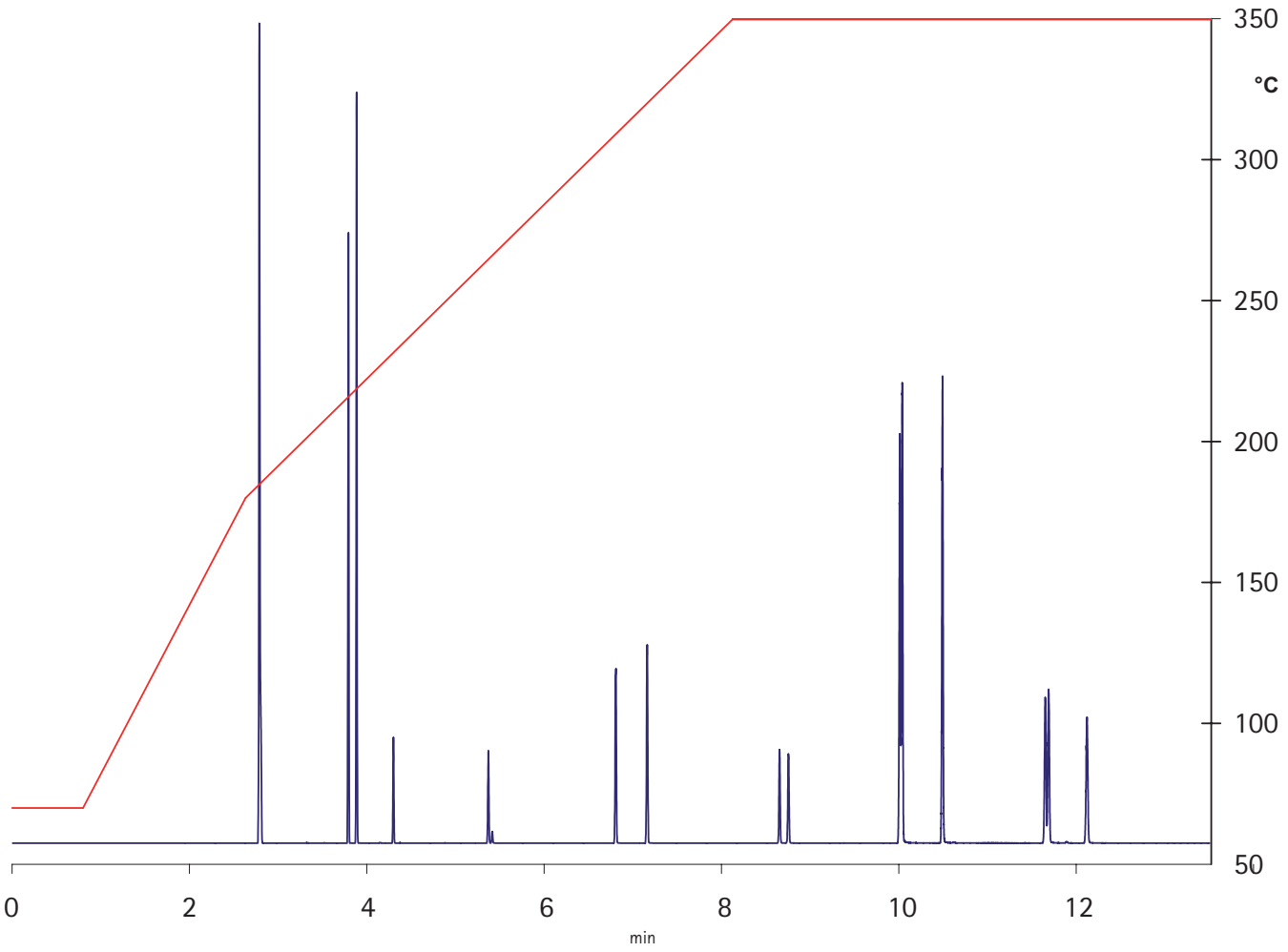

Figure 1. Fast GC/MS analysis of 16 US EPA PAHs on Select PAH, 30 m x 0.25 mm x 0.15 µm

Table 2. Peak Identification for Figure 2

Peak	MW	Compound	CAS
1	128	Naphthalene	91-20-3
2	152	Acenaphthylene	208-96-8
3	154	Acenaphthene	83-32-9
4	166	Fluorene	86-73-7
5	178	Phenanthrene	85-01-8
6	178	Anthracene	120-12-7
7	202	Fluoranthene	206-44-0
8	202	Pyrene	129-00-0
9	228	Benz[a]anthracene	56-55-3
10	228	Chrysene	218-01-9

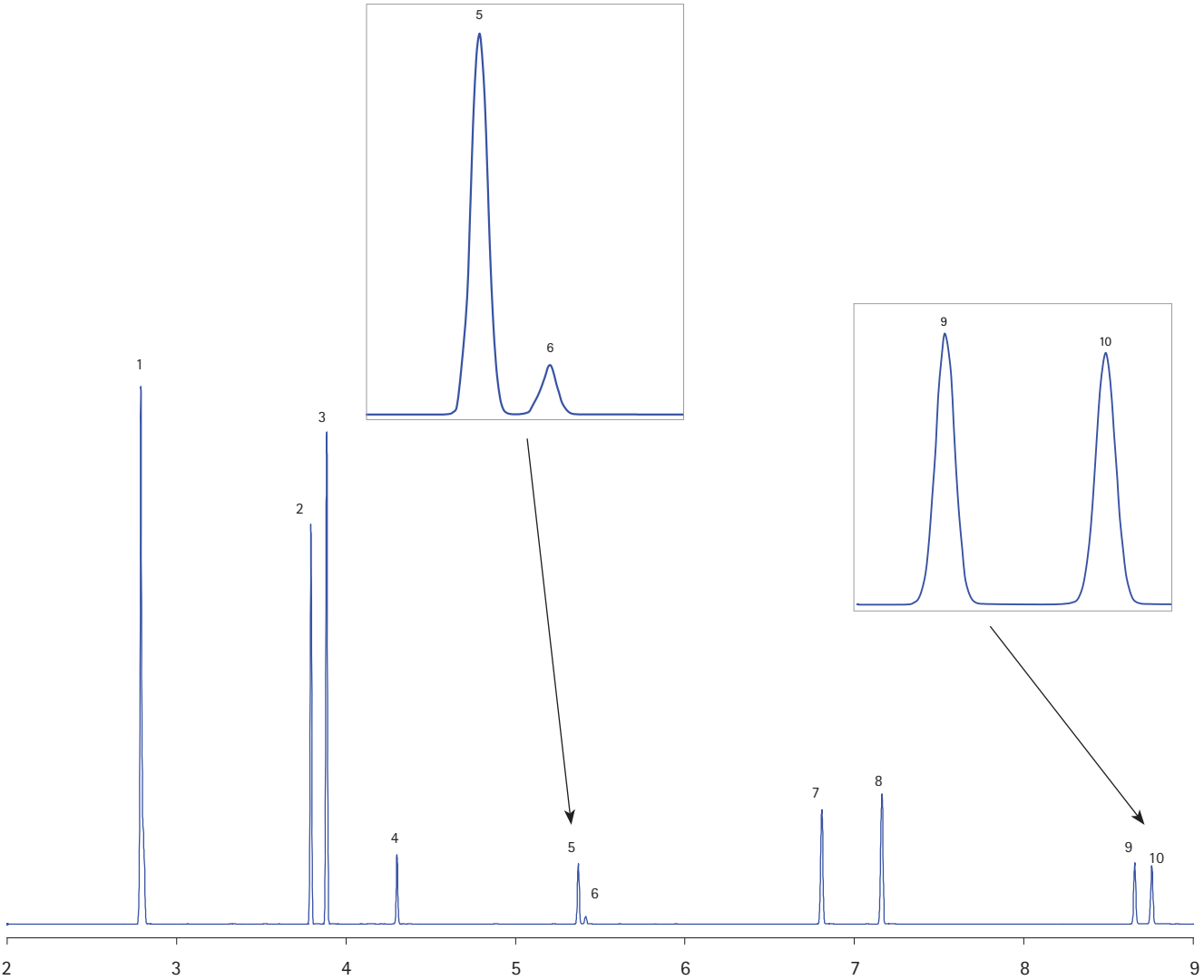

Figure 2. Details and identification of PAH peaks 1 –10

Table 3. Peak Identification for Figure 3

Peak	MW	Compound	CAS
11	252	Benzo[b]fluoranthene	205-99-2
12	252	Benzo[k]fluoranthene	207-08-9
13	252	Benzo[a]pyrene	50-32-8
14	278	Dibenz[a,h]anthracene	53-70-3
15	276	Indeno[1,2,3-c,d]pyrene	193-39-5
16	276	Benzo[g,h,i]perylene	191-24-2

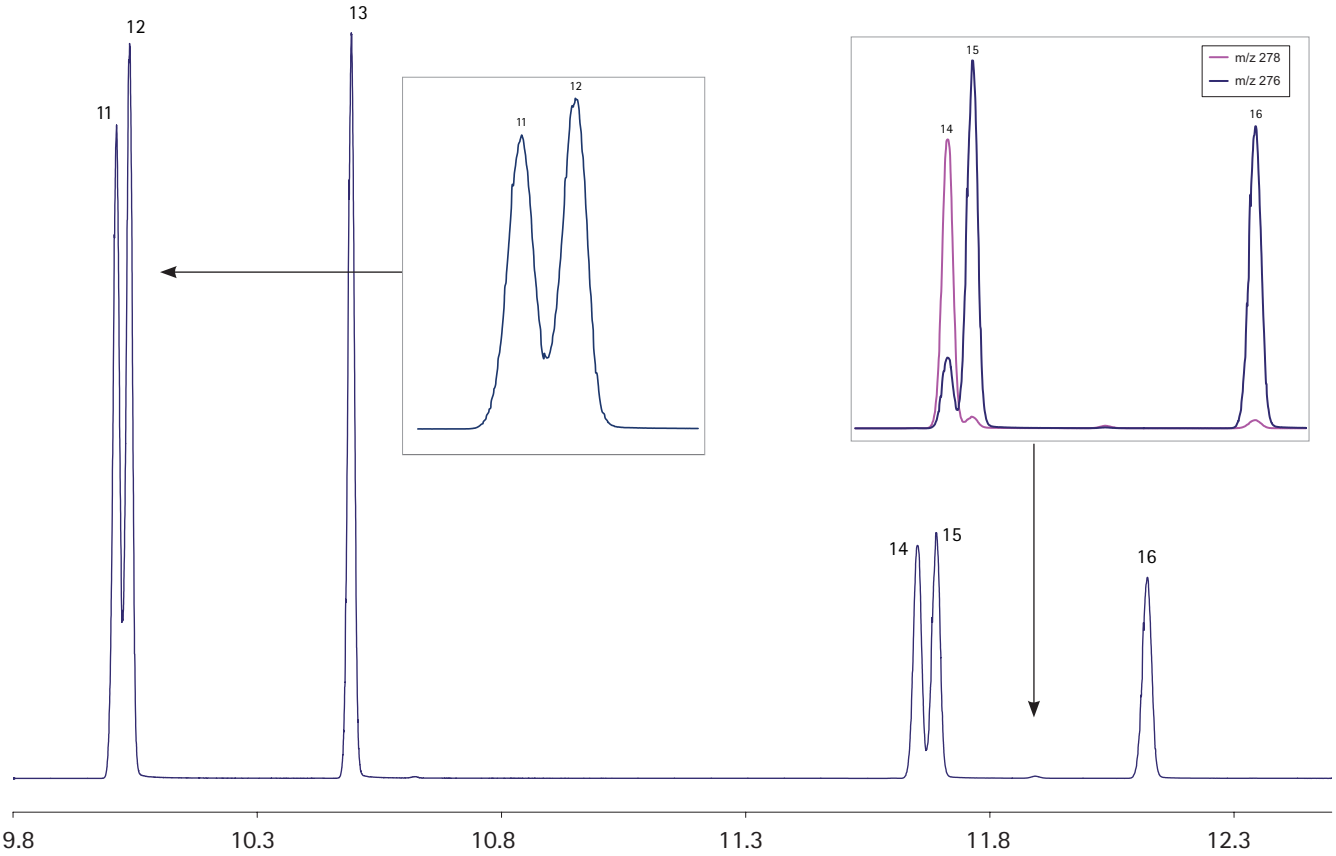

Figure 3. Details and identification of PAH peaks 11–16

Conclusion

Both GC column phase and oven program affect the separation of difficult to resolve PAH sets. With the optimized oven program described here, all 16 EPA PAHs are resolved in a single run with a runtime of less than 13 min. The Select PAH column also offers enhanced resolution of PAHs, preventing co-elution of interfering PAHs that can cause false positives and inaccurate results. Typical interferences are triphenylene on chrysene, and benzo[j]fluoranthene on benzo[k]fluoranthene. Application note SI-02232 describes the separation of 54 PAHs, including the 16 EPA PAHs and their interferences.

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Published in UK, October 11, 2010

SI-02263

Agilent Technologies

Fast Separation of EU and US EPA Regulated PAHs on Agilent J&W Select PAH GC Columns

Application Note

Author

John Oostdijk
Agilent Technologies, Inc.

Introduction

Several polycyclic aromatic hydrocarbons (PAHs) have the same mass, making them difficult to separate using GC/MS. To resolve these PAHs, the use of an appropriate column and an optimized oven program are necessary. In addition, speed of analysis is important for high productivity, but faster speeds often lead to lower resolution. With the 15 m x 0.15 mm ID version of the J&W Select PAH GC column, it is possible to perform high resolution PAH analysis at high speed with no loss of resolution. In this application note, we demonstrate high resolution analysis of 54 PAHs within 30 minutes.

PAHs are formed during incomplete combustion or pyrolysis of organic matter, during industrial processes and in cooking and food processing. Due to their carcinogenic properties, they are pollutants of concern and are subject to monitoring in food and environmental samples. However, different legislations describe different PAHs. The US Environmental Protection Agency (EPA) focuses on environmental PAH contamination whereas the EU legislation is more concerned with PAHs in foodstuffs.

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Conditions

Technique:	GC/MS, Triple Quad
Column:	Select PAH, 15 m x 0.15 mm, df = 0.10 µm (part number CP7461)
Sample Conc:	Approx. 0.1-0.3 µg/mL
Injection Volume:	1 µL
Temperature:	70 °C (0.4 min), 70 °C/min, 180 °C, 7 °C/min, 230 °C (7 min), 50 °C/min, 280 °C (7 min), 30 °C/min, 350 °C (4 min)
Carrier Gas:	Helium, constant flow 1.2 mL/min
Injector:	300 °C, Splitless mode, 0.5 min @ 100 mL/min
Detector:	Triple Quad, EI in SIM mode, ion source 275 °C, transfer line 300 °C

Results and Discussion

There are three groups of peaks that are difficult to resolve when analyzing PAHs. Benz[a]anthracene, cyclopenta[c,d] pyrene, chrysene and triphenylene have different masses, m/z 226 and 228. However, the compounds in this group with m/z 228 also contain some m/z 226, making resolution problematic using only MS. The same complication occurs when separating indeno[1,2,3-c,d]pyrene, benzo[b] triphenylene and dibenz[a,h]anthracene with m/z 276 and 278.

The third set of difficult to resolve PAHs are the benzofluoranthene isomers, benzo[b]fluoranthene, benzo[j] fluoranthene and benzo[k]fluoranthene, which have the same mass and, as before, cannot be resolved with MS only.

Therefore, resolution of these isomers must be done chromatographically. This is straightforward and fast using the Select PAH column and the optimized oven program, as shown in Figure 1 (all PAHs) and Figures 2-8 (details and information).

Table 1. Peak identification of Figure 1

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
1	136	Naphthalene-d8			1146-65-2
2	128	Naphthalene	x		91-20-3
3	142	2-Methylnaphthalene			91-57-6
4	142	1-Methylnaphthalene			90-12-0
5	152	Acenaphthylene	x		208-96-8
6	164	Acenaphthene-d10			15067-26-2
7	154	Acenaphthene	x		83-32-9
8	166	Fluorene	x		86-73-7
9	188	Phenanthrene-d10			1517-22-2
10	178	Phenanthrene	x		85-01-8
11	178	Anthracene	x		120-12-7
12	202	Fluoranthene	x		206-44-0
13	202	Pyrene	x		129-00-0
14	216	Benzo[a]fluorene			238-84-6
15	216	Benzo[b]fluorene			243-17-4
16	216	7H-Benzo[c]fluorene		x	205-12-9
17	234	Benzo[b]naphtho[2,1-d] thiophene			239-35-0
18	226	Benzo[g,h,i]fluoranthene			203-12-3
19	228	Benzo[c]phenanthrene			195-19-7
20	228	Benz[a]anthracene	x	x	56-55-3
21	226	Cyclopenta[c,d]pyrene		x	27208-37-3
22	240	Chrysene-d10			1719-03-5
23	228	Triphenylene			217-59-4
24	228	Chrysene	x	x	218-01-9
25	242	6-Methylchrysene			1705-85-7
26	242	5-Methylchrysene		x	3697-24-3
27	252	Benzo[b]fluoranthene	x	x	205-99-2
28	252	Benzo[k]fluoranthene	x	x	207-08-9
29	252	Benzo[j]fluoranthene		x	205-82-3
30	252	Benzo[a]fluoranthene			203-33-8
31	252	Benzo[e]pyrene			192-97-2
32	252	Benzo[a]pyrene	x	x	50-32-8
33	264	Perylene-d12			1520-96-3
34	252	Perylene			198-55-0
35	268	3-Methylcholanthrene			56-49-5
36	330	9,10-diphenylanthracene			216-105-1
37	279	Dibenzo[a,h]acridine			226-36-8
38	279	Dibenzo[a,j]acridine			224-42-0
39	278	Dibenz[a,j]anthracene			224-41-9
40	292	Dibenzo[a,h]anthracene D14			13250-98-1
41	278	Benzo[b]triphenylene			215-58-7
42	276	Indeno[1,2,3-cd]pyrene	x	x	193-39-5
43	278	Dibenz[a,h]anthracene	x	x	53-70-3
44	278	Benzo[b]chrysene			214-17-5
45	278	Picene			213-46-7
46	27	Benzo[g,h,i]perylene	x	x	191-24-2
47	276	Dibenzo[def,mno]chrysene			191-26-4
48	267	7H-Dibenzo[c,g]carbazole			194-59-2
49	302	Dibenzo[a,l]pyrene		x	191-30-0
50	302	Dibenzo[a,e]pyrene		x	192-65-4
51	300	Coronene			191-07-1
52	302	Benzo[b]perylene			197-70-6
53	302	Dibenzo[a,i]pyrene		x	189-55-9
54	302	Dibenzo[a,h]pyrene		x	189-64-0

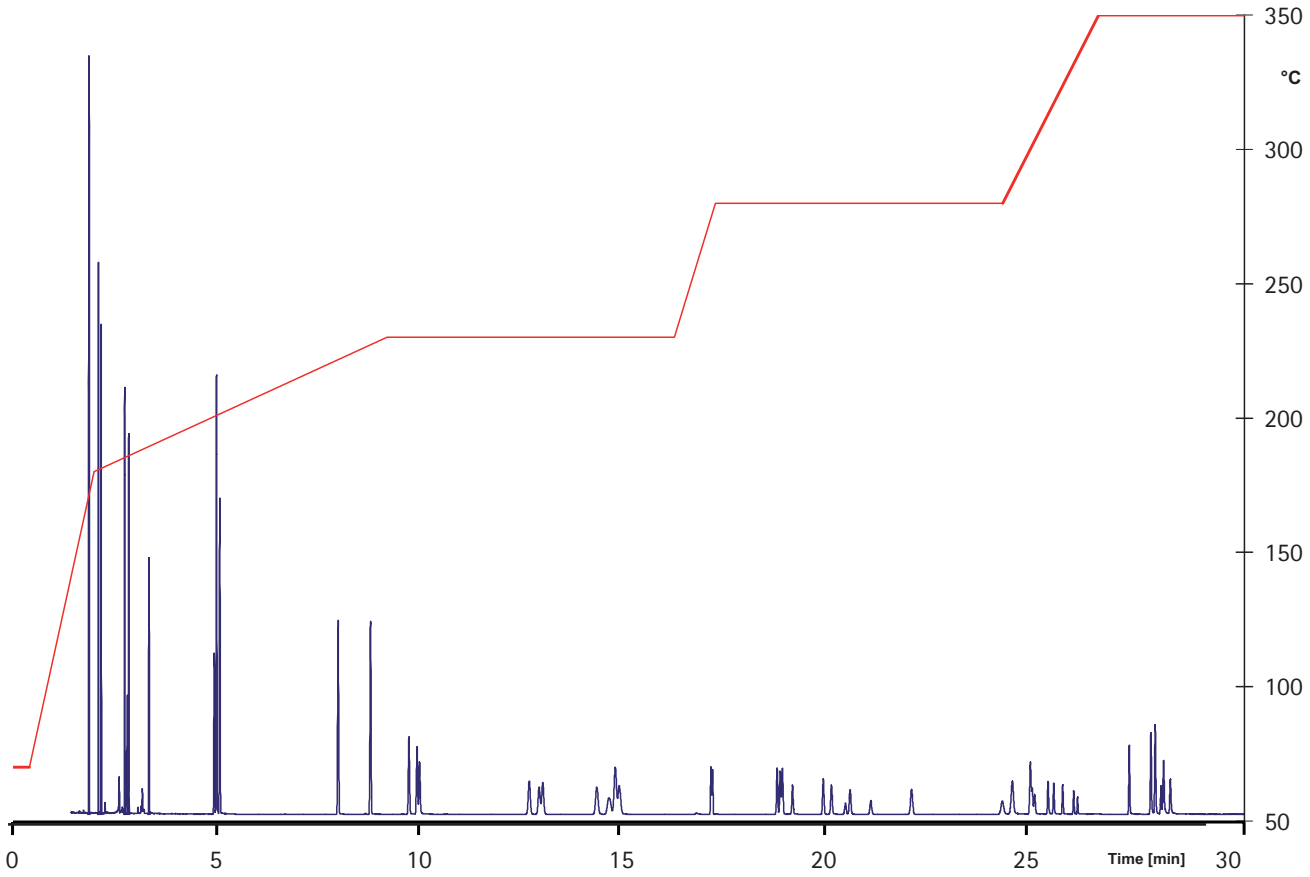

Figure 1. GC/MS analysis of EU and EPA PAHs on Select PAH 15 m x 0.15 mm x 0.10 µm

Table 2. Peak Identification for Figure 2

Peak	MW	Compound	EPA 610	CAS
1	136	Naphthalene-d8		1146-65-2
2	128	Naphthalene	x	91-20-3
3	142	2-Methylnaphthalene		91-57-6
4	142	1-Methylnaphthalene		90-12-0
5	152	Acenaphthylene	x	208-96-8
6	164	Acenaphthene-d10		15067-26-2
7	154	Acenaphthene	x	83-32-9
8	166	Fluorene	x	86-73-7

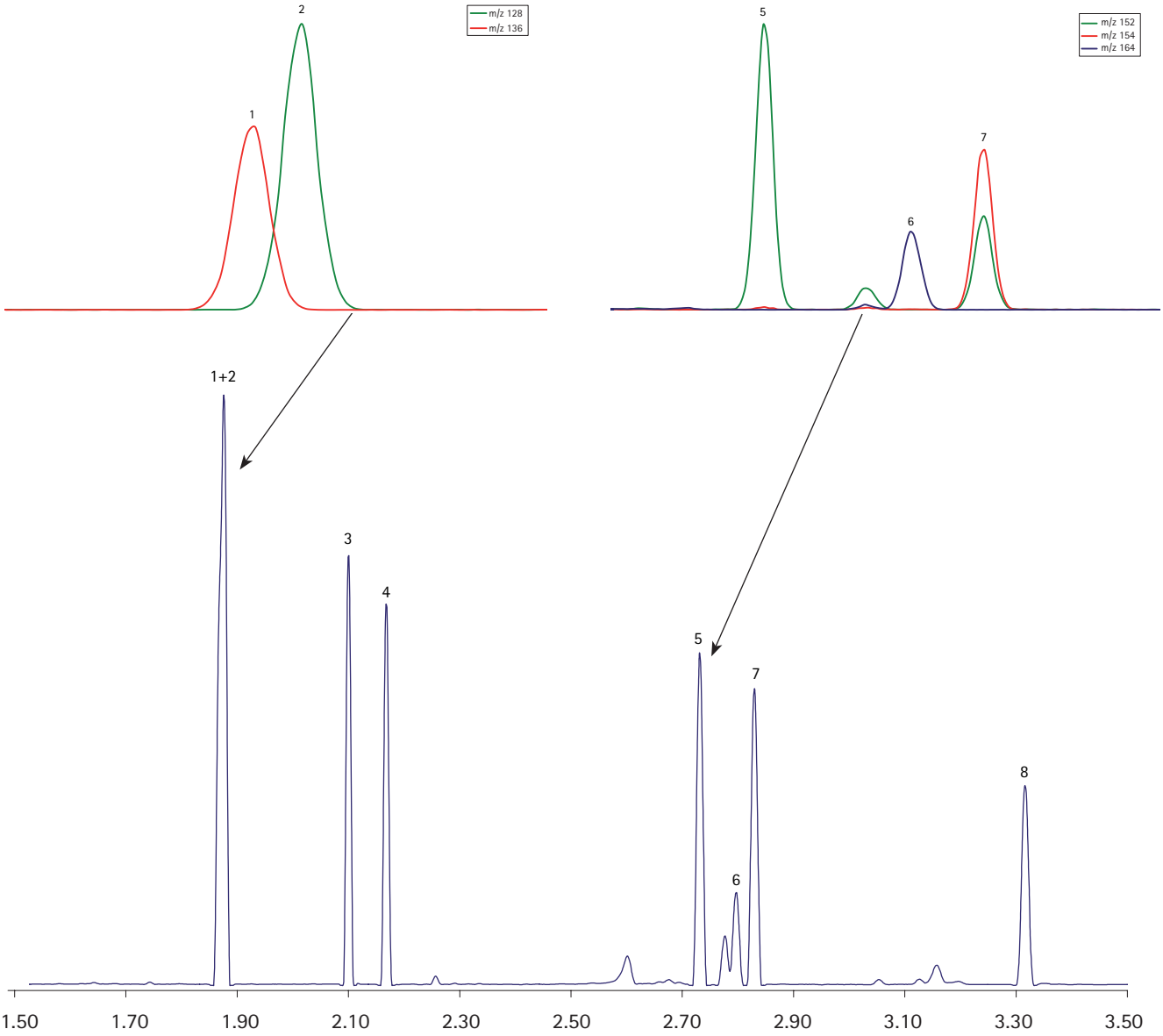

Figure 2. Details and identification of peaks 1 to 8

Table 3. Peak Identification for Figure 3

Peak	MW	Compound	EPA 610	CAS
9	188	Phenanthrene-d10		1517-22-2
10	178	Phenanthrene	x	85-01-8
11	178	Anthracene	x	120-12-7
12	202	Fluoranthene	x	206-44-0
13	202	Pyrene	x	129-00-0

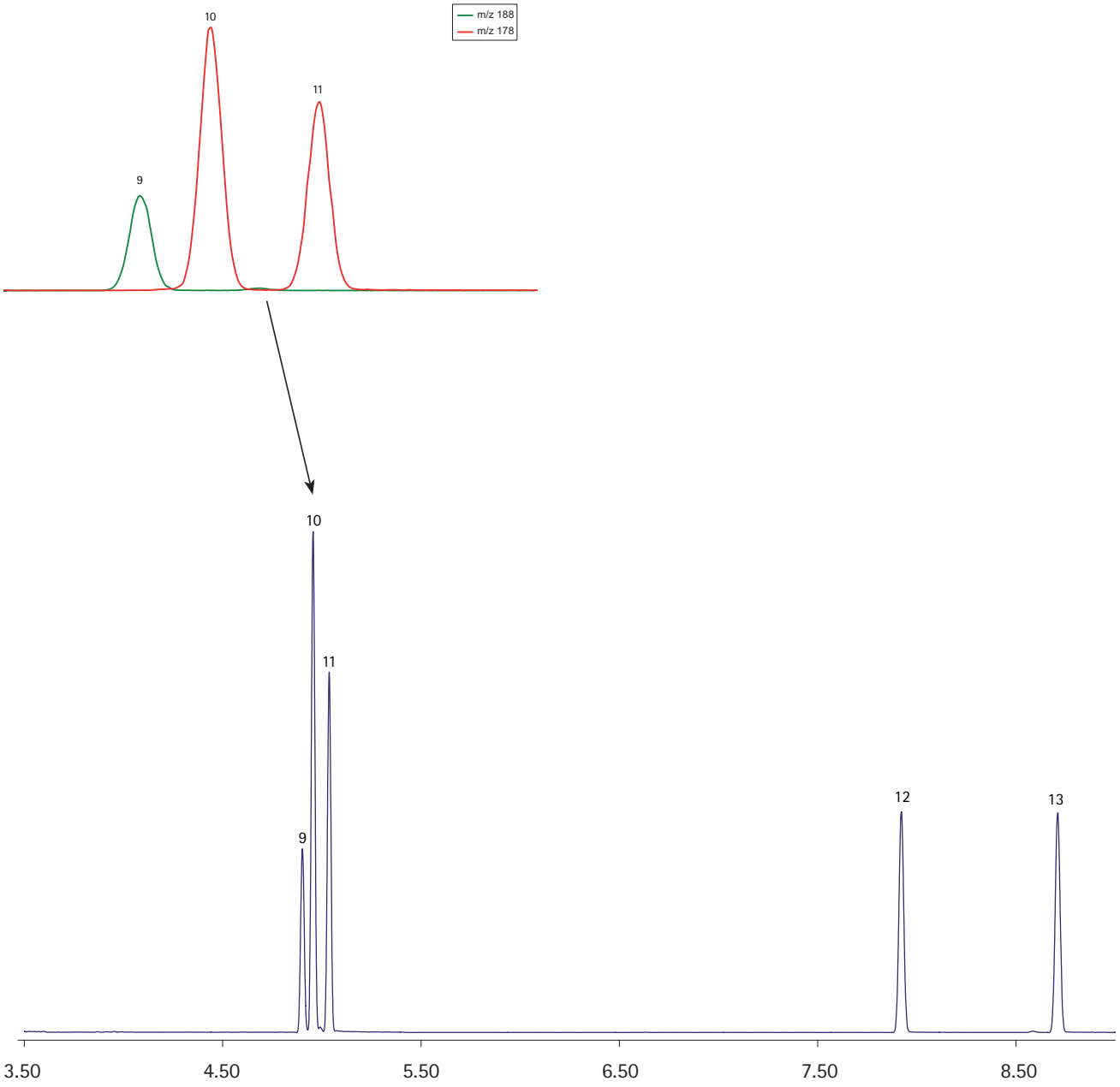

Figure 3. Details and identification of peaks 9 to 13

Table 4. Peak Identification for Figure 4

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
14	216	Benzo[a]fluorene			238-84-6
15	216	Benzo[b]fluorene			243-17-4
16	216	7H-Benzo[c]fluorene		x	205-12-9
17	234	Benzo[b]naphtho[2,1-d] thiophene			239-35-0
18	226	Benzo[g,h,i]fluoranthene			203-12-3
19	228	Benzo[c]phenanthrene			195-19-7
20	228	Benz[a]anthracene	x	x	56-55-3
21	226	Cyclopenta[c,d]pyrene		x	27208-37-3
22	240	Chrysene-d10			1719-03-5
23	228	Triphenylene			217-59-4
24	228	Chrysene	x	x	218-01-9

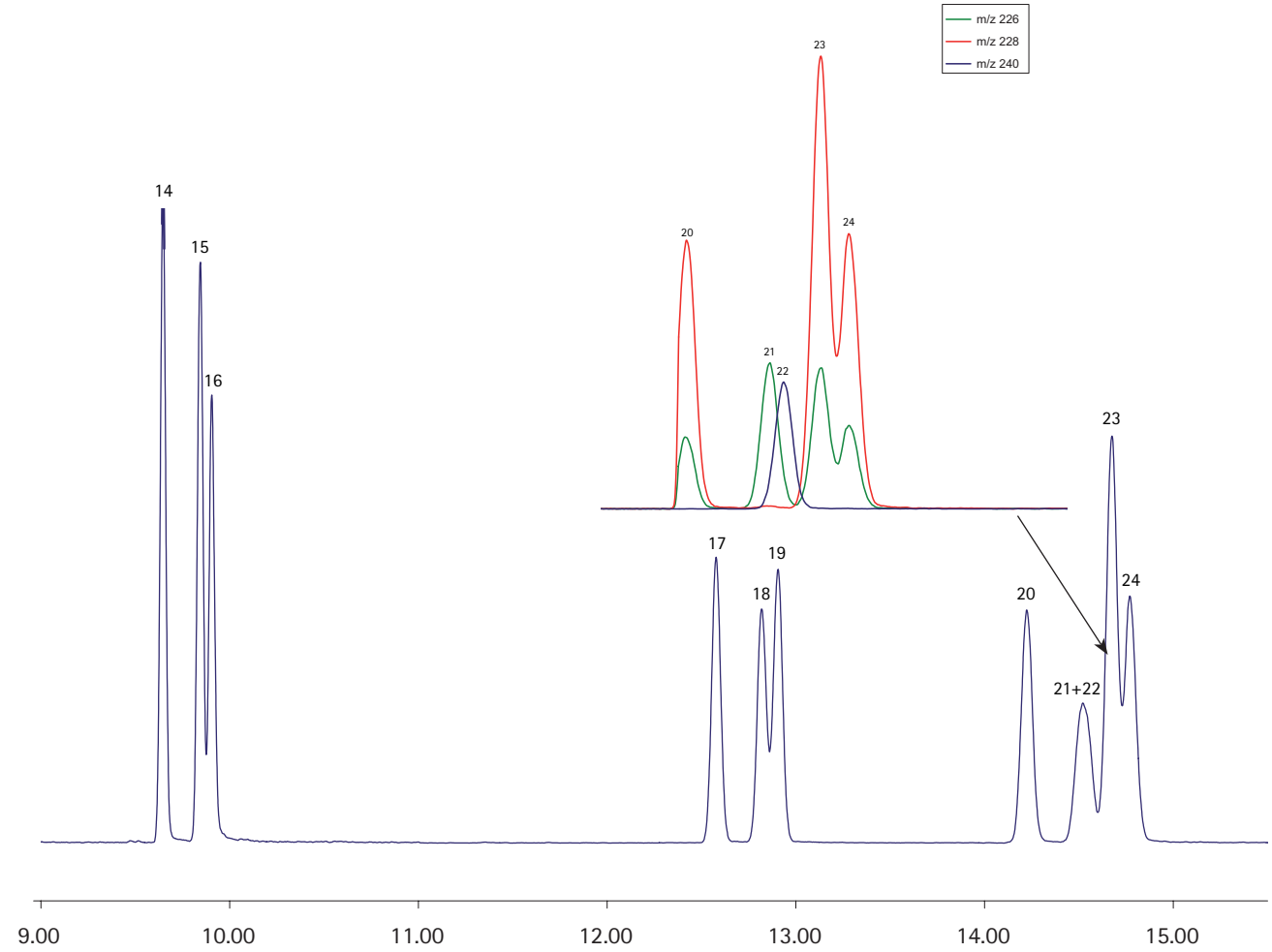

Figure 4. Details and identification of peaks 14 to 24

Table 5. Peak Identification for Figure 5

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
25	242	6-Methylchrysene			1705-85-7
26	242	5-Methylchrysene		x	3697-24-3
27	252	Benzo[b]fluoranthene	x	x	205-99-2
28	252	Benzo[k]fluoranthene	x	x	207-08-9
29	252	Benzo[j]fluoranthene		x	205-82-3
30	252	Benzo[a]fluoranthene			203-33-8
31	252	Benzo[e]pyrene			192-97-2
32	252	Benzo[a]pyrene	x	x	50-32-8
33	264	Perylene-d12			1520-96-3
34	252	Perylene			198-55-0
35	268	3-Methylcholanthrene			56-49-5
36	330	9,10-diphenylanthracene			216-105-1

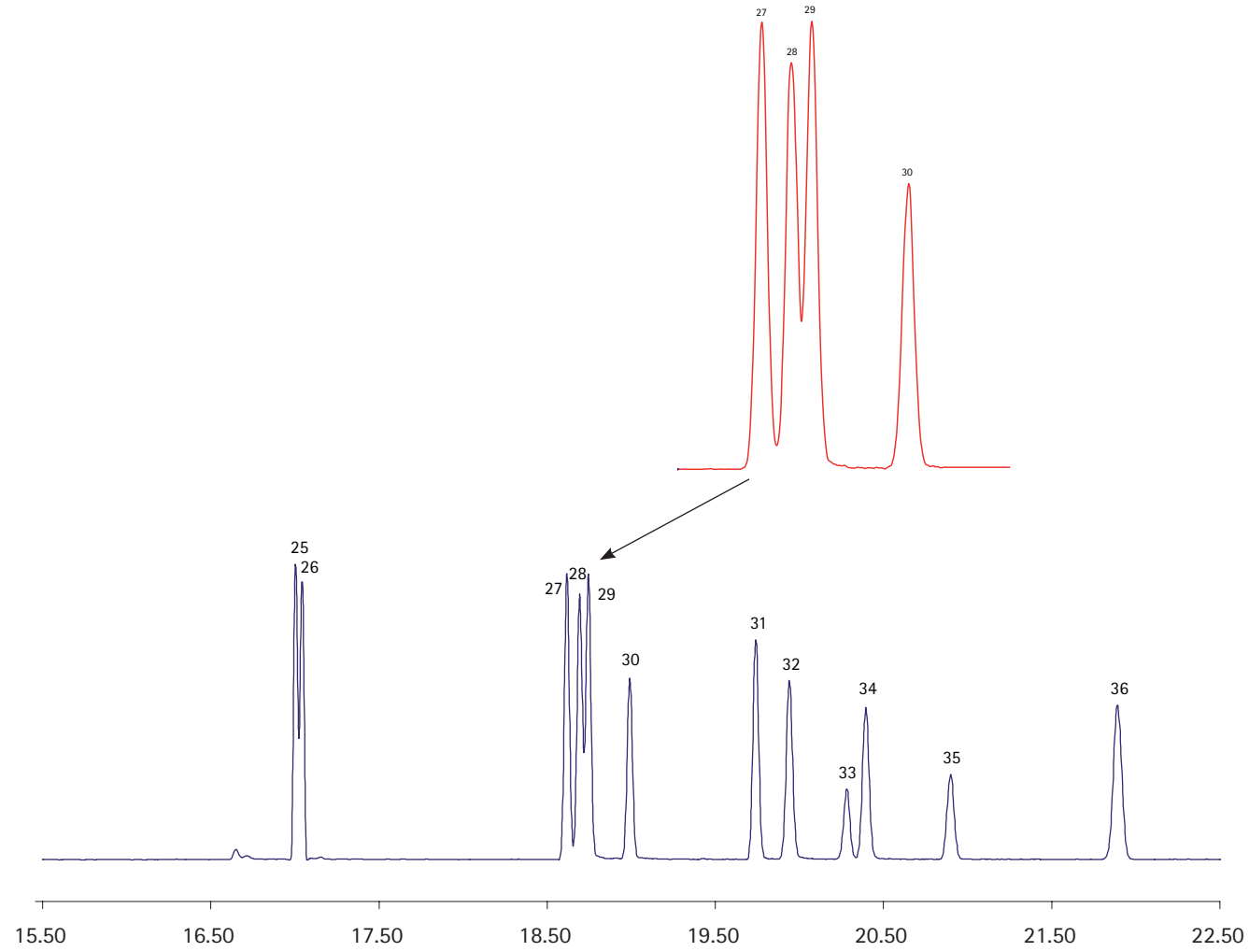

Figure 5. Details and identification of peaks 25 to 36

Table 6. Peak Identification for Figure 6

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
37	279	Dibenzo[a,h]acridine			226-36-8
38	279	Dibenzo[a,j]acridine			224-42-0
39	278	Dibenz[a,j]anthracene			224-41-9
40	292	Dibenzo[a,h]anthracene D14			13250-98-1
41	278	Benzo[b]triphenylene			215-58-7
42	276	Indeno[1,2,3-c,d]pyrene	x	x	193-39-5
43	278	Dibenz[a,h]anthracene	x	x	53-70-3
44	278	Benzo[b]chrysene			214-17-5
45	278	Picene			213-46-7
46	27	Benzo[g,h,i]perylene	x	x	191-24-2
47	276	Dibenzo[def,mno]chrysene			191-26-4
48	267	7H-Dibenzo[c,g]carbazole			194-59-2
49	302	Dibenzo[a,l]pyrene		x	191-30-0
50	302	Dibenzo[a,e]pyrene		x	192-65-4
51	300	Coronene			191-07-1
52	302	Benzo[b]perylene			197-70-6
53	302	Dibenzo[a,i]pyrene		x	189-55-9
54	302	Dibenzo[a,h]pyrene		x	189-64-0

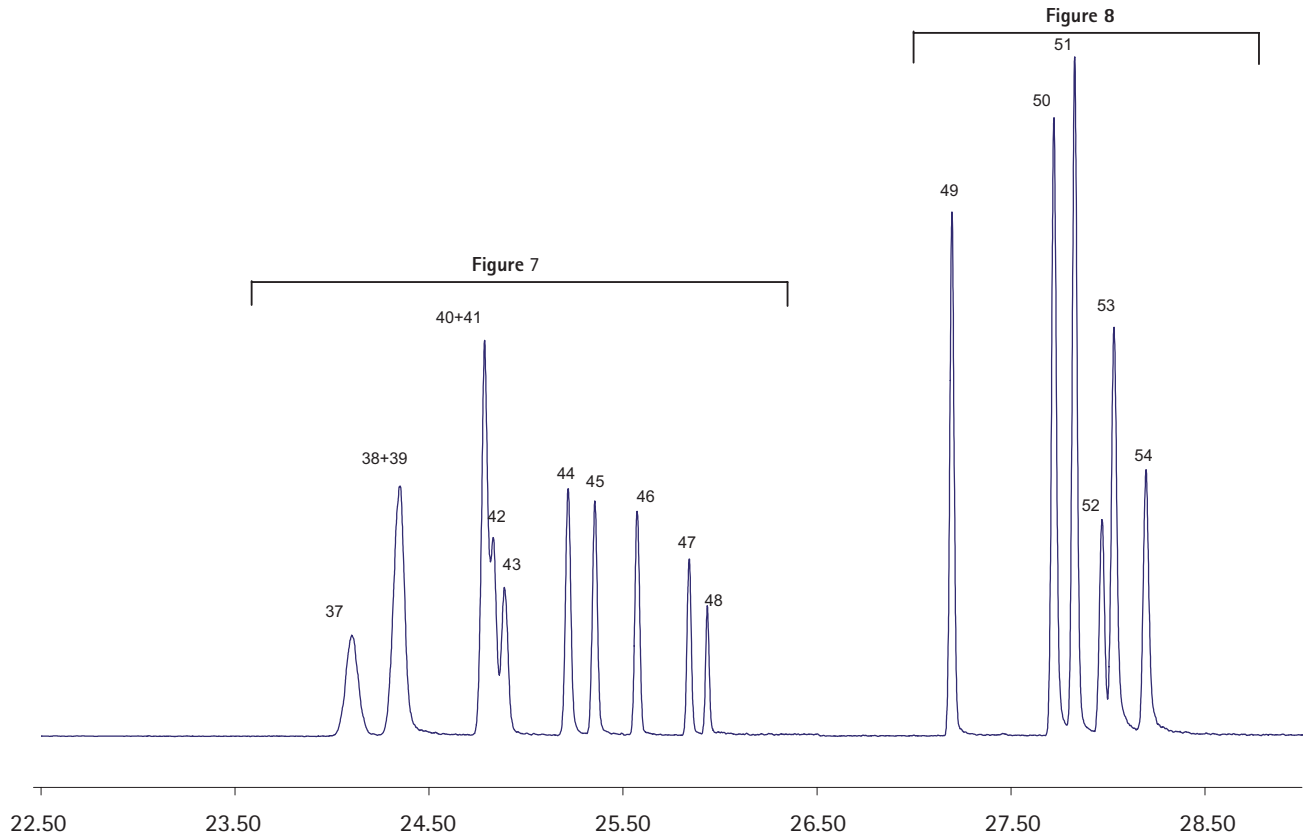

Figure 6. Details and identification of peaks 37 to 54

Table 7. Peak Identification for Figure 7

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
37	279	Dibenzo[a,h]acridine			226-36-8
38	279	Dibenzo[a,j]acridine			224-42-0
39	278	Dibenz[a,j]anthracene			224-41-9
40	292	Dibenzo[a,h]anthracene D14			13250-98-1
41	278	Benzo[b]triphenylene			215-58-7
42	276	Indeno[1,2,3-c,d]pyrene	x	x	193-39-5
43	278	Dibenz[a,h]anthracene	x	x	53-70-3
44	278	Benzo[b]chrysene			214-17-5
45	278	Picene			213-46-7
46	27	Benzo[g,h,i]perylene	x	x	191-24-2
47	276	Dibenzo[def,mno]chrysene			191-26-4
48	267	7H-Dibenzo[c,g]carbazole			194-59-2

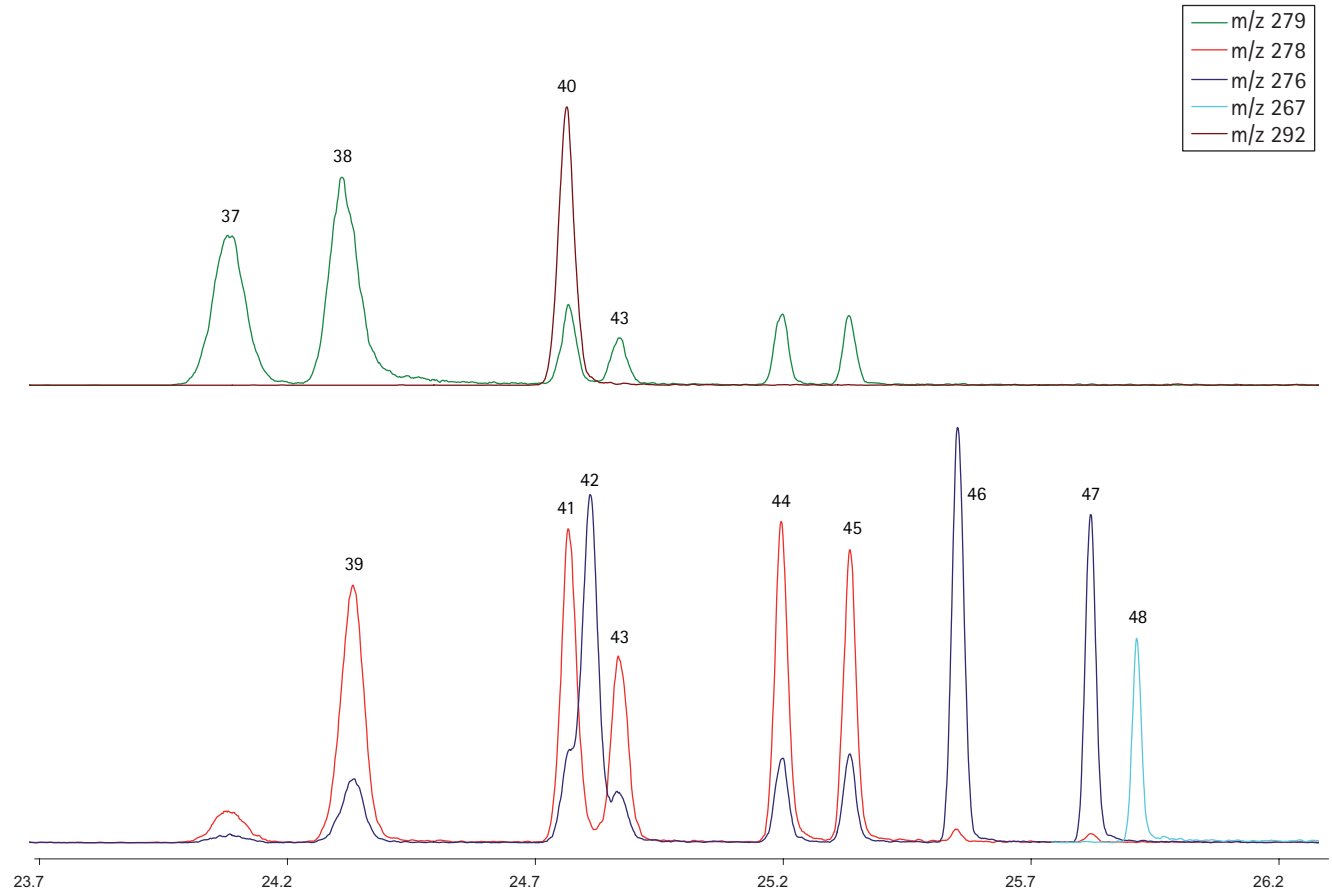

Figure 7. Details and identification of peaks 37 to 48

Table 8. Peak Identification for Figure 8

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
49	302	Dibenzo[a,l]pyrene		x	191-30-0
50	302	Dibenzo[a,e]pyrene		x	192-65-4
51	300	Coronene			191-07-1
52	302	Benzo[b]perylene			197-70-6
53	302	Dibenzo[a,i]pyrene		x	189-55-9
54	302	Dibenzo[a,h]pyrene		x	189-64-0

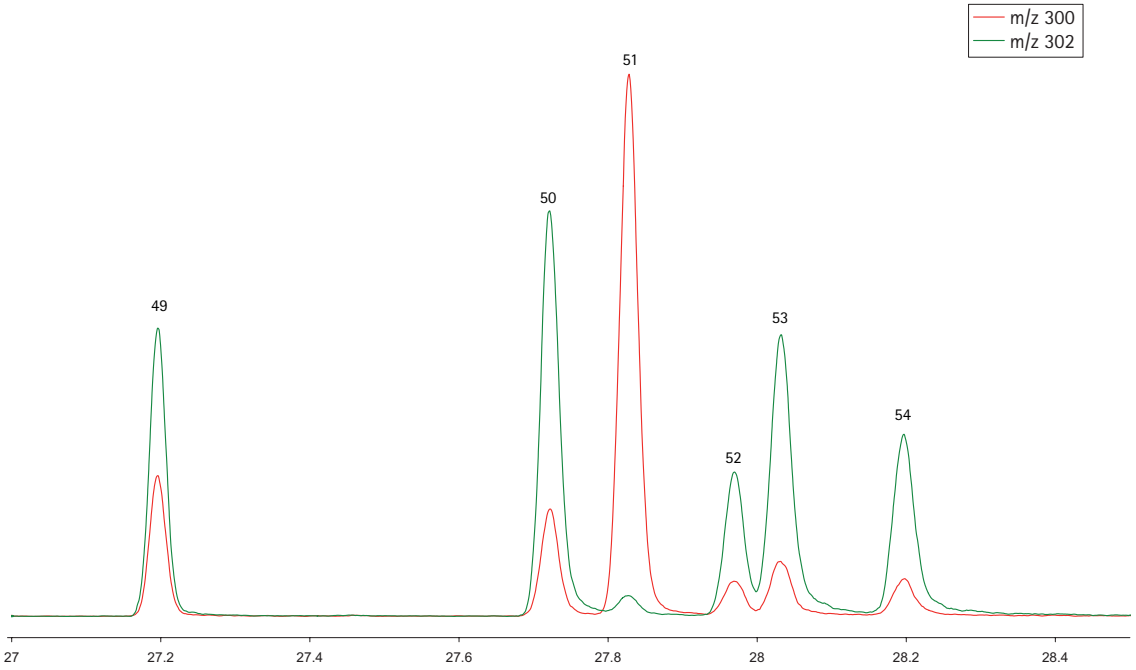

Figure 8. Details and identification of peaks 49 to 54

Conclusion

The J&W Select PAH column phase, as well as the GC oven program, affects the separation of difficult to resolve PAH sets. With the optimized oven program described in this application note, all EU and EPA PAHs, and known interferences, are resolved in a single run with a runtime of 29 min.

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Published in UK, October 11, 2010

SI-02259

Agilent Technologies

GC Analysis of Halogenated Pesticides using the Agilent J&W FactorFour VF-1701ms with EZ-Guard

Application Note

Author

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Introduction

Pesticides analyses can be challenging due to complex sample matrices. Guard columns are often used to protect the analytical column from non-volatile compounds in the sample matrix. While prolonging the lifetime of the analytical column, the guard can introduce the risk of leakage through the coupling, or adsorption of analytes due to the influence of the connector. Leakage decreases column lifetime and adsorption causes asymmetrical peaks, lower detection response, and decreases the sensitivity of the analysis.

EZ-Guard is an integrated guard column that eliminates the risk of leakage and adsorption. When the resolution or the analyte response diminishes, a coil is removed from the EZ-Guard column so that column performance will improve.

The VF-1701ms is often used as a conformation column for pesticide analysis. Now with EZ-Guard, the VF-1701ms can be used for such samples in complex matrices. This application note demonstrates the performance of the VF-1701ms with EZ-Guard in the separation of pesticides.

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Materials and Methods

Column:	VF-1701ms, 30 m x 0.25 mm ID, df=0.25 µm, + 10 m EZ-Guard (part number CP9177)
Sample Size:	2 µL, Split ratio 1:50
Sample Conc:	0.1% in hexane
Temp Program:	220 °C
Carrier Gas:	H2
Pressure:	90 kPa
Injector Temp:	275 °C, split
Detector Temp:	300 °C, ECD

Results and Discussion

As shown in the chromatogram (Figure 1) all compounds have excellent peak shape and high response, including unstable endrin and p, p'-DDT, demonstrating the inertness of the whole GC column.

Conclusion

The J&W FactorFour VF-1701ms with EZ-Guard offers improved column lifetimes and high performance in the analysis of pesticides in complex matrices. Peak shapes are excellent, maximizing the sensitivity of the analysis. In addition, EZ-Guard columns are fast and easy to install and operate, boosting productivity and delivering improved efficiency. Also, the last meter is uncoated, functioning as an integrated transfer line, providing faster detector stabilization.

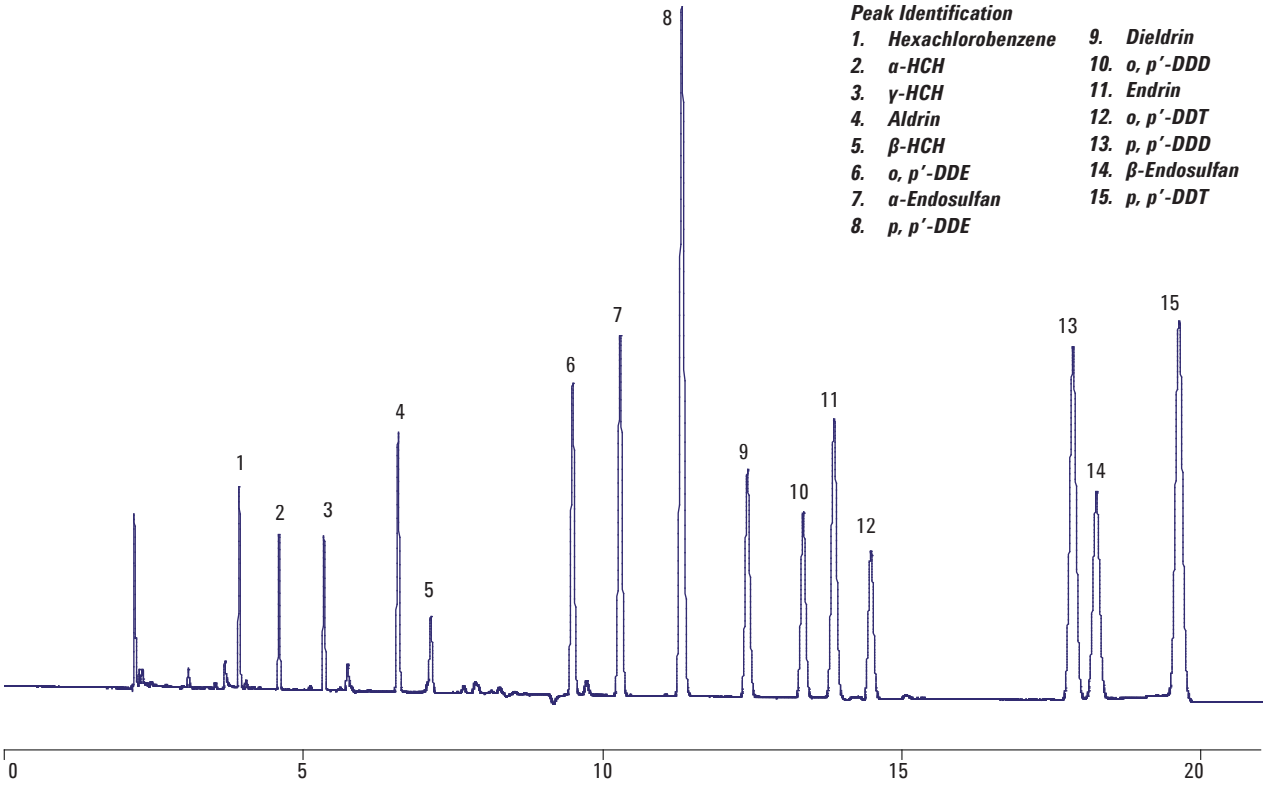

Figure 1. Excellent resolution of 12 halogenated pesticides on a VF-1701ms GC column with EZ-Guard

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Published in UK, October 11, 2010
SI-02247

Agilent Technologies

Separation of 54 PAHs on an Agilent J&W Select PAH GC Column

Application Note

Author

John Oostdijk
Agilent Technologies, Inc.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds that contain two or more aromatic rings. They are formed during incomplete combustion or pyrolysis of organic matter, industrial processes and cooking and food processing. PAHs are therefore analyzed in environmental and food samples.

The difficulty in analyzing PAHs lies in separating PAH isomers. These isomers have the same chemical structure and same ion fragment and therefore cannot be separated by mass spectrometers. The Select PAH capillary column has enhanced selectivity towards PAHs, separating the isomers and enabling accurate PAH analysis. This application note describes an optimized oven program for the Select PAH column. In PAH analysis, there is a difference between the European (EU) and American (EPA) legislation. The legislations both describe a different set of PAHs and address different matrix origins. Table 1 lists the PAH regulated by EU and EPA legislation, including the potential interferences. For this application note, we chose a sample containing 54 PAHs to demonstrate the unique selectivity of the Select PAH.

Agilent Technologies

Conditions

Technique:	GC/MS, Triple Quad
Column:	Select PAH, 30 m x 0.25 mm, df=0.15 µm (part number CP7462)
Sample Conc:	approx 0.1-0.3 µg/mL
Injection Volume:	1 µL
Temperature:	70 °C (0.7 min), 85 °C/min, 180 °C, 3 °C/min, 230 °C (7 min), 28 °C/min, 280 °C (10 min), 14 °C/min, 350 °C (3 min)
Carrier Gas:	Helium, constant flow 2 mL/min
Injector:	300 °C, Splitless mode, 1 min @ 50 mL/min
Detector:	Triple Quad, EI in SIM mode, ion source 275°C, transfer line 300°C

Results and Discussion

Figure 1 shows the separation of all 54 PAH isomers and Figures 2-6 reveal the finer details.

When performing this analysis, there are three sets of peaks that are difficult to resolve. The first set, comprising benzo[a]anthracene, cyclopenta[c,d]pyrene, chrysene and triphenylene, has different masses, m/z 226 and 228. The compounds with m/z 228 also contain some m/z 226, making it difficult to resolve this set by MS alone. The same problem occurs when separating indeno[1,2,3-c,d]pyrene, benzo[b]triphenylene and dibenzo[a,h]anthracene with m/z 276 and 278. The third set of PAH isomers difficult to resolve are the benzofluoranthenes. These three isomers, benzo[b] fluoranthene, benzo[j]fluoranthene and benzo[k]fluoranthene, have the same mass and cannot be resolved by MS.

However, the unique selectivity of Select PAH resolves all the PAH isomers and enables accurate quantification of all PAHs.

Peaks 1 and 2 show some tailing, which can be resolved by further optimization of the splitless injection conditions.

Conclusion

The Select PAH column resolves 54 PAHs in a single GC/MS run, including the hard to separate PAH isomers. The optimized GC oven program performs the run time in less than 45 minutes.

Table 1. Peak Identification for Figure 1

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
1	136	Naphthalene-d8			1146-65-2
2	128	Naphthalene	X		91-20-3
3	142	2-Methylnaphthalene			91-57-6
4	142	1-Methylnaphthalene			90-12-0
5	152	Acenaphthylene	X		208-96-8
6	164	Acenaphthene-d10			15067-26-2
7	154	Acenaphthene	X		83-32-9
8	166	Fluorene	X		86-73-7
9	188	Phenanthrene-d10			1517-22-2
10	178	Phenanthrene	X		85-01-8
11	178	Anthracene	X		120-12-7
12	202	Fluoranthene	X		206-44-0
13	202	Pyrene	X		129-00-0
14	216	Benzo[a]fluorene			238-84-6
15	216	Benzo[b]fluorene			243-17-4
16	216	7H-Benzo[c]fluorene		X	205-12-9
17	234	Benzo[b]naphto[2,1-d]-thiophene			239-35-0
18	226	Benzo[g,h,i]-fluoranthene			203-12-3
19	228	Benzo[c]phenanthrene			195-19-7
20	228	Benz[a]anthracene	X	X	56-55-3
21	226	Cyclopenta[c,d]pyrene		X	27208-37-3
22	240	Chrysene-d10			1719-03-5
23	228	Triphenylene			217-59-4
24	228	Chrysene	X	X	218-01-9
25	242	6-Methylchrysene			175-85-7
26	242	5-Methylchrysene		X	3697-24-3
27	252	Benzo[b]fluoranthene	X	X	205-99-2
28	252	Benzo[k]fluoranthene	X	X	207-08-9
29	252	Benzo[j]fluoranthene		X	205-82-3
30	252	Benzo[a]fluoranthene			203-33-8
31	252	Benzo[e]pyrene			192-97-2
32	252	Benzo[a]pyrene	X	X	50-32-8
33	264	Perylene-d12			1520-96-3
34	252	Perylene			198-55-0
35	268	3-Methylcholanthrene			56-49-5
36	330	9,10-Diphenylanthracene			216-105-1
37	279	Dibenzo[a,h]acridine			226-36-8
38	279	Dibenzo[a,j]acridine			224-42-0
39	278	Dibenzo[a,j]anthracene			224-41-9
40	292	Dibenzo[a,h] anthracene-d14			13250-98-1
41	278	Benzo[b]triphenylene			215-58-7
42	276	Indeno[1,2,3-c,d]pyrene	X	X	193-39-5
43	278	Dibenzo[a,h]anthracene	X	X	53-70-3
44	278	Benzo[b]chrysene			214-17-5
45	278	Picene			213-46-7
46	276	Benzo[g,h,i]perylene	X	X	191-24-2
47	276	Dibenzo[def,mno]-chrysene			191-26-4
48	267	7H-Dibenzo[c,g]-carbazole			194-59-2
49	302	Dibenzo[a,l]pyrene		X	191-30-0
50	302	Dibenzo[a,e]pyrene		X	192-65-4
51	300	Coronene			191-07-1
52	302	Benzo[b]perylene			197-70-6
53	302	Dibenzo[a,i]pyrene		X	189-55-9
54	302	Dibenzo[a,h]pyrene		X	189-64-0

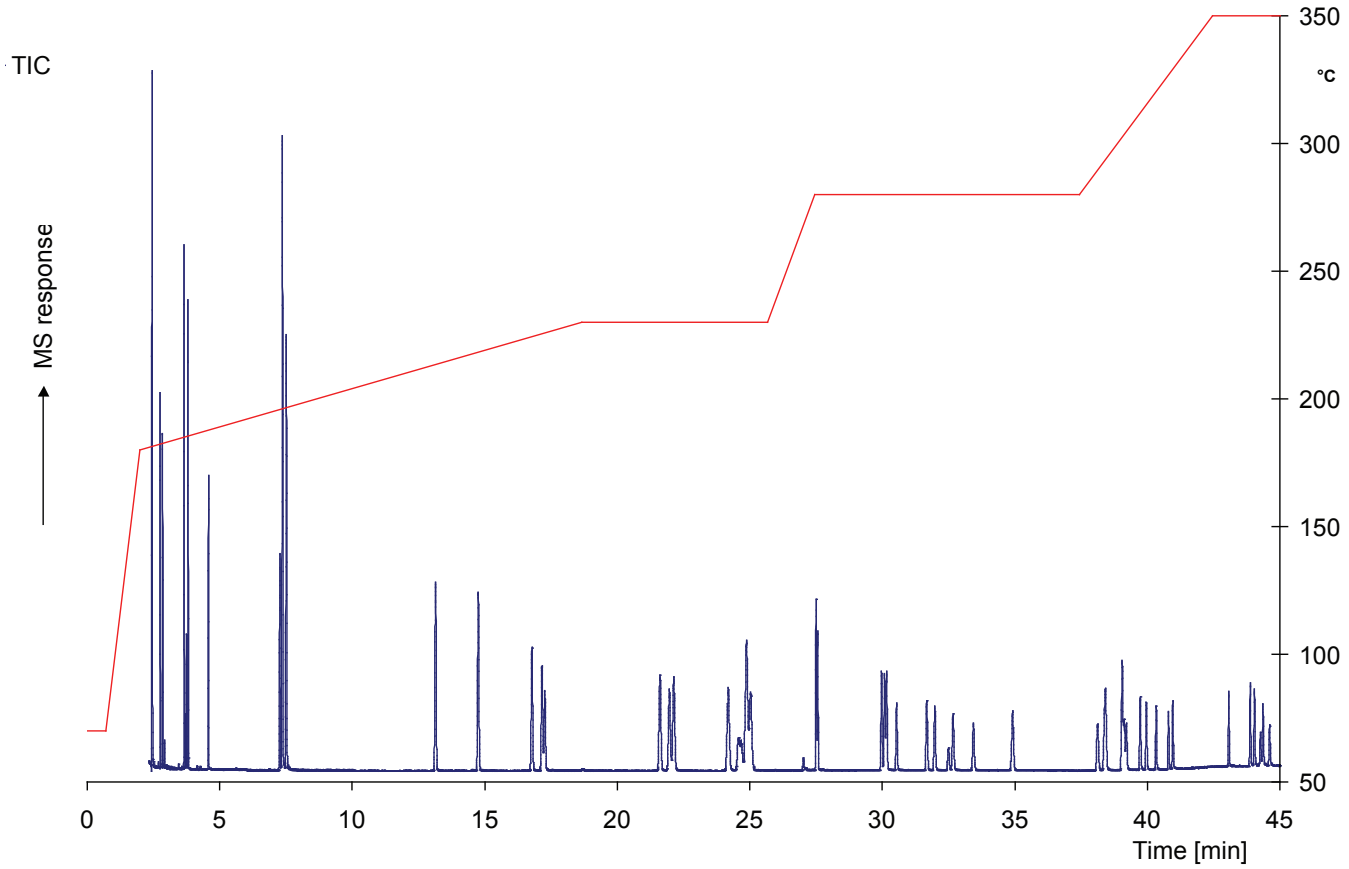

Figure 1. GC/MS analysis of EU and EPA PAHs on Select PAH column, 30 m x 0.25 mm x 0.15 µm

Table 2. Peak Identification for Figure 2

Peak	MW	Compound	EPA 610	CAS
1	136	Naphthalene-d8		1146-65-2
2	128	Naphthalene	X	91-20-3
3	142	2-Methylnaphthalene		91-57-6
4	142	1-Methylnaphthalene		90-12-0

Peak	MW	Compound	EPA 610	CAS
5	152	Acenaphthylene	X	208-96-8
6	164	Acenaphthene-d10		15067-26-2
7	154	Acenaphthene	X	83-32-9
8	166	Fluorene	X	86-73-7

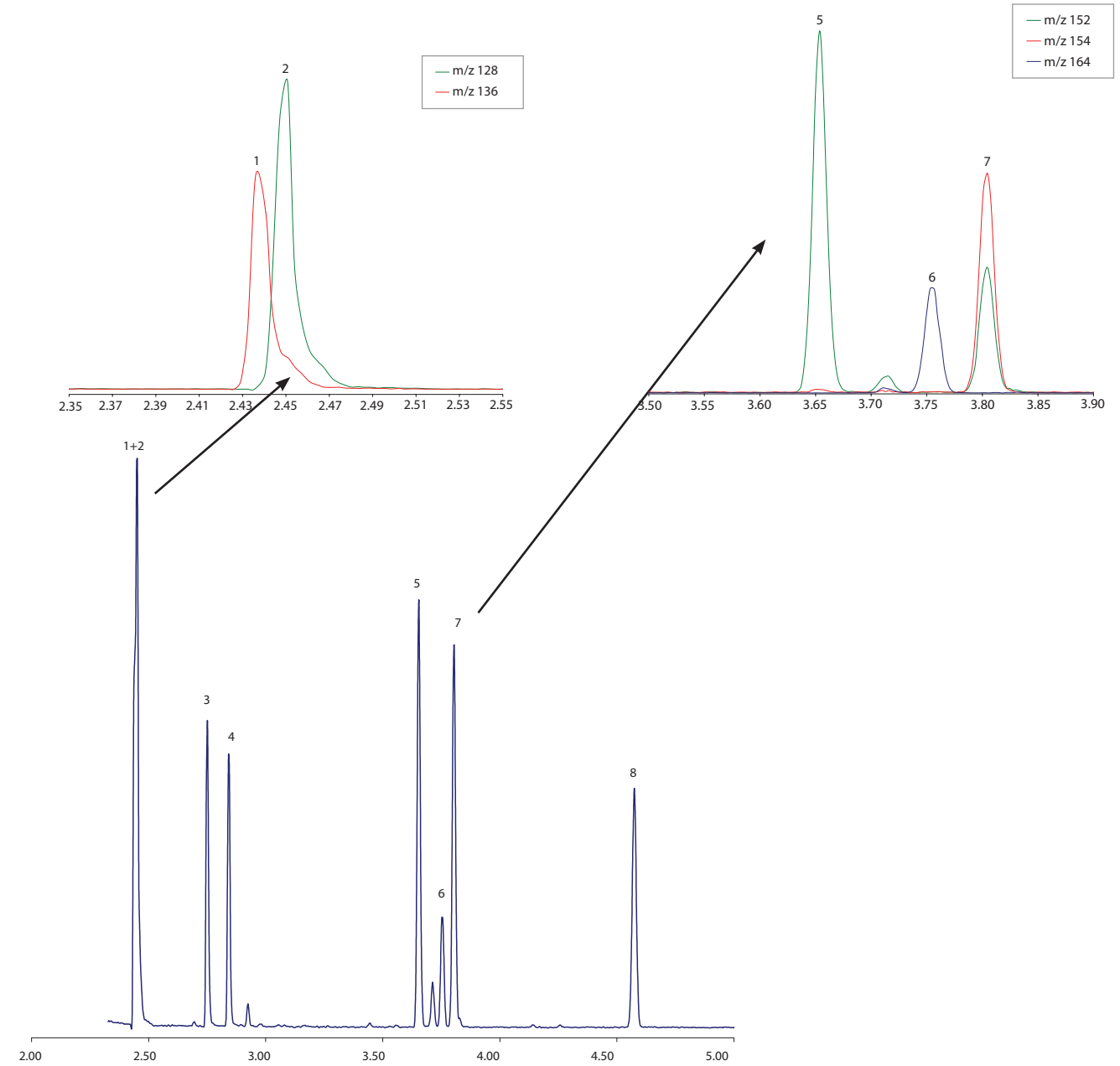

Figure 2. Details and identification of peaks 1 to 8

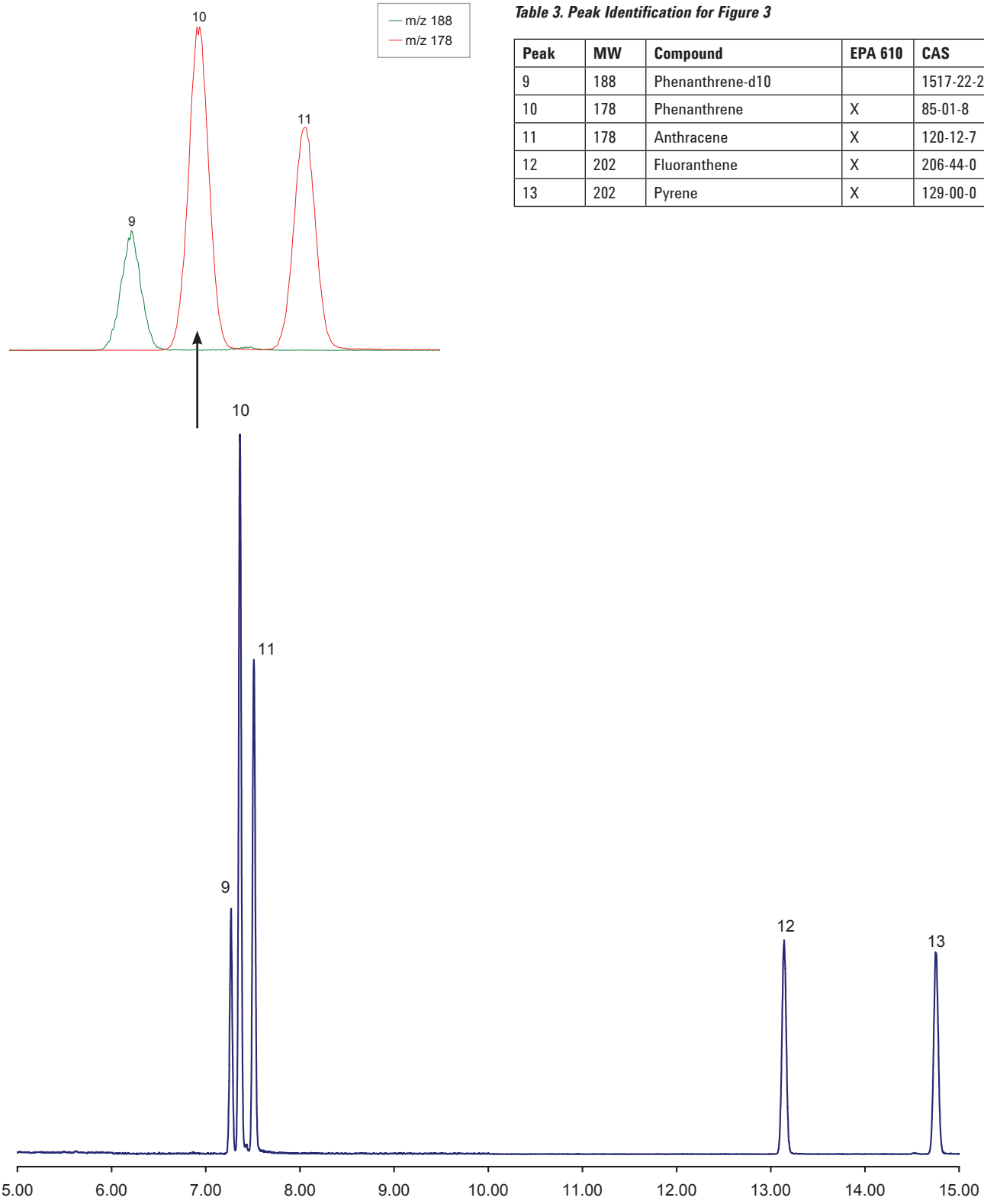

Figure 3. Details and identification of peaks 9 to 13

Table 3. Peak Identification for Figure 3

Peak	MW	Compound	EPA 610	CAS
9	188	Phenanthrene-d10		1517-22-2
10	178	Phenanthrene	X	85-01-8
11	178	Anthracene	X	120-12-7
12	202	Fluoranthene	X	206-44-0
13	202	Pyrene	X	129-00-0

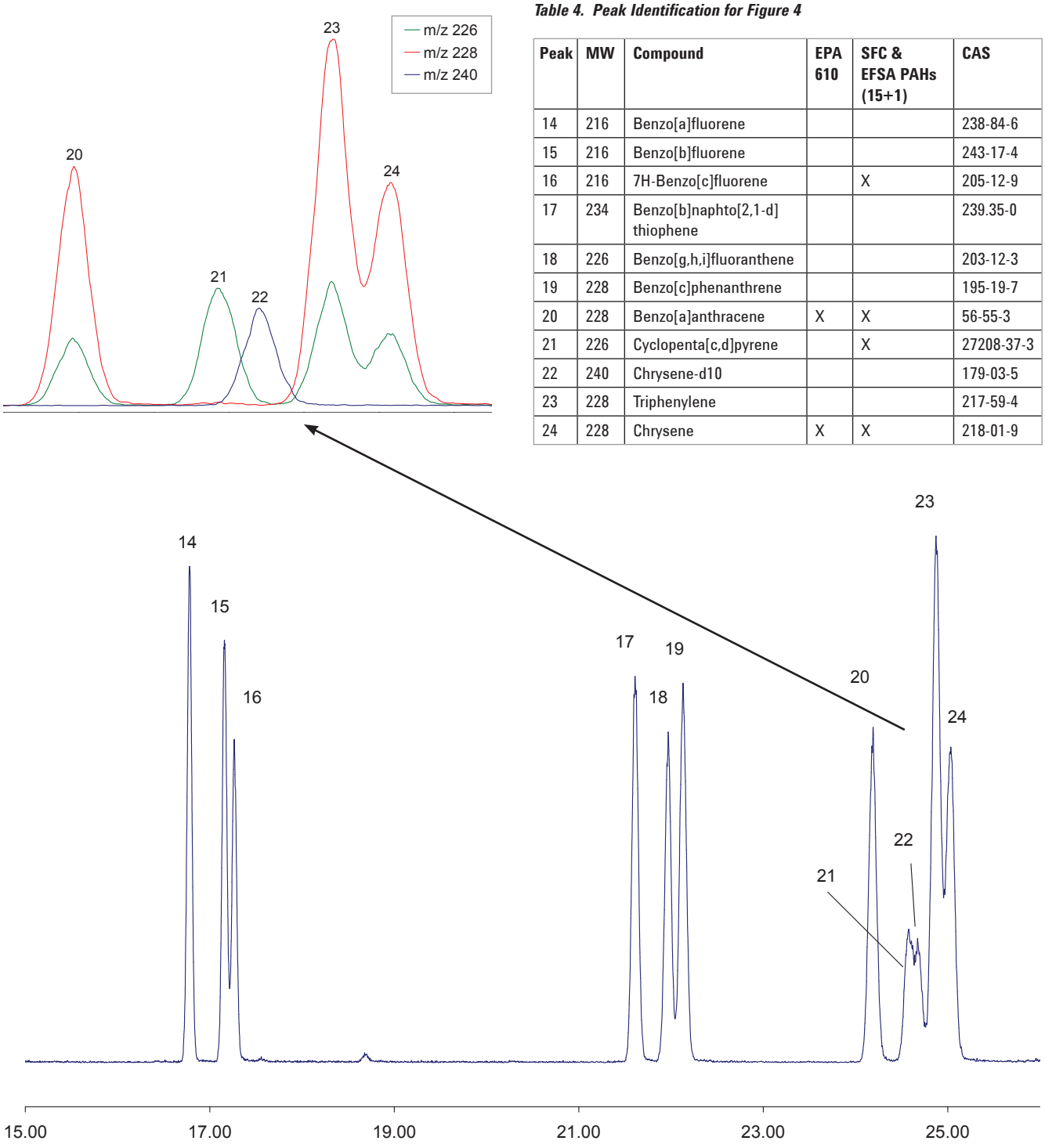

Figure 4. Details and identification of peaks 14 to 24

Table 4. Peak Identification for Figure 4

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
14	216	Benzo[a]fluorene			238-84-6
15	216	Benzo[b]fluorene			243-17-4
16	216	7H-Benzo[c]fluorene		X	205-12-9
17	234	Benzo[b]naphto[2,1-d] thiophene			239.35-0
18	226	Benzo[g,h,i]fluoranthene			203-12-3
19	228	Benzo[c]phenanthrene			195-19-7
20	228	Benzo[a]anthracene	X	X	56-55-3
21	226	Cyclopenta[c,d]pyrene		X	27208-37-3
22	240	Chrysene-d10			179-03-5
23	228	Triphenylene			217-59-4
24	228	Chrysene	X	X	218-01-9

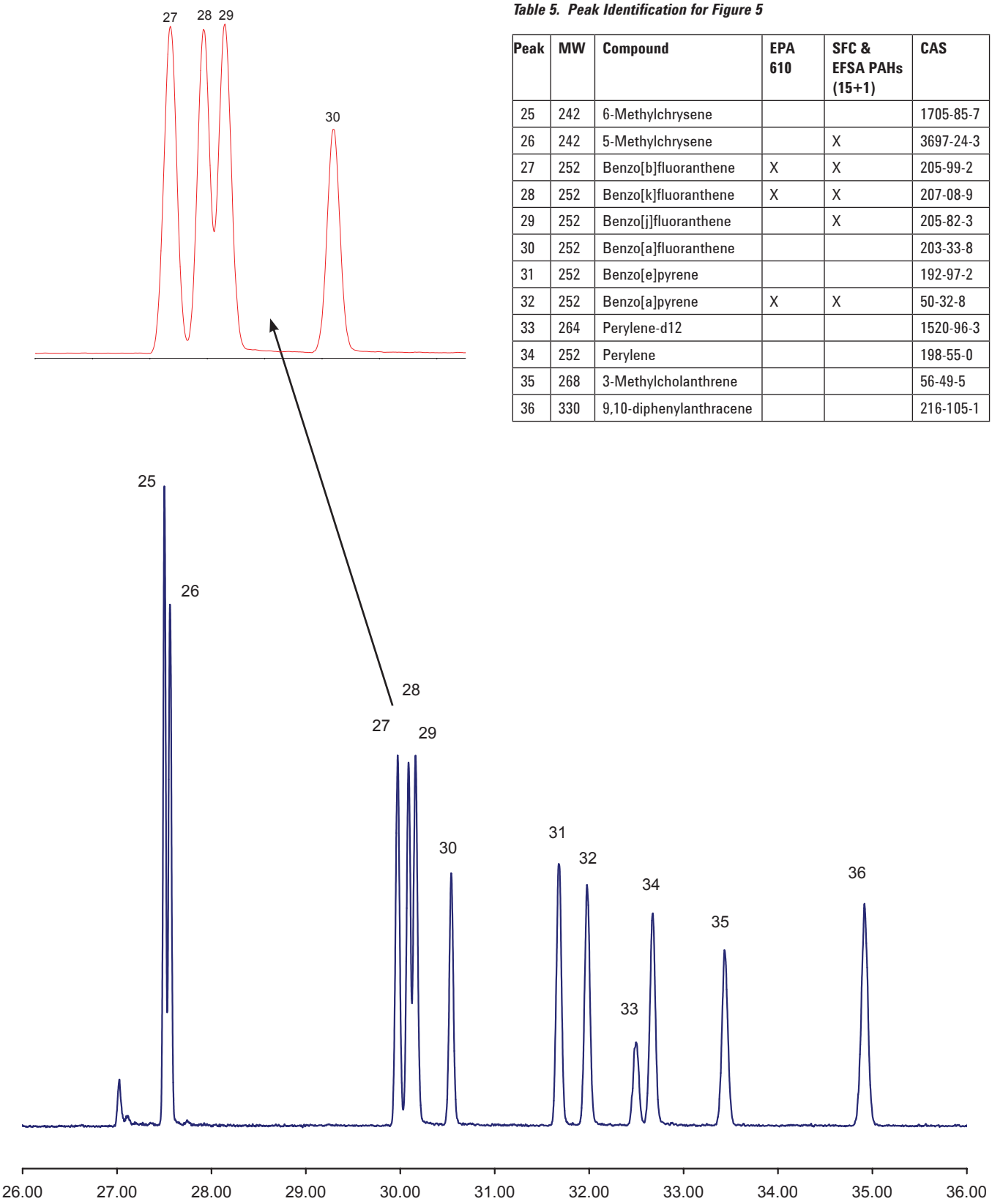

Figure 5. Details and identification of peaks 25 to 36

Table 5. Peak Identification for Figure 5

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
25	242	6-Methylchrysene			1705-85-7
26	242	5-Methylchrysene		X	3697-24-3
27	252	Benzo[b]fluoranthene	X	X	205-99-2
28	252	Benzo[k]fluoranthene	X	X	207-08-9
29	252	Benzo[j]fluoranthene		X	205-82-3
30	252	Benzo[a]fluoranthene			203-33-8
31	252	Benzo[e]pyrene			192-97-2
32	252	Benzo[a]pyrene	X	X	50-32-8
33	264	Perylene-d12			1520-96-3
34	252	Perylene			198-55-0
35	268	3-Methylcholanthrene			56-49-5
36	330	9,10-diphenylanthracene			216-105-1

Table 6. Peak Identification for Figure 6

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
37	279	Dibenzo[a,h]acridine			226-36-8
38	279	Dibenzo[a,j]acridine			224-42-0
39	278	Dibenzo[a,j]anthracene			224-41-9
40	292	Dibenzo[a,h] anthracene-d14			13250-98-1
41	278	Benzo[b]triphenylene			215-58-7
42	276	Ideno[1,2,3-c,d]pyrene	X	X	193-39-5
43	278	Dibenzo[a,h]anthracene	X	X	53-70-3
44	278	Benzo[b]chrysene			214-17-5
45	278	Picene			213-46-7

Peak	MW	Compound	EPA 610	SFC & EFSA PAHs (15+1)	CAS
46	276	Benzo[g,h,i]perylene	X	X	191-24-2
47	276	Dibenzo[def,mno]chrysene			191-26-4
48	267	7H-Dibenzo[c,g]carbazole			194-59-2
49	302	Dibenzo[a,l]pyrene			191-30-0
50	302	Dibenzo[a,e]pyrene		X	192-65-4
51	300	Coronene			191-07-1
52	302	Benzo[b]perylene			197-70-6
53	302	Dibenzo[a,i]pyrene		X	189-55-9
54	302	Dibenzo[a,h]pyrene		X	189-64-0

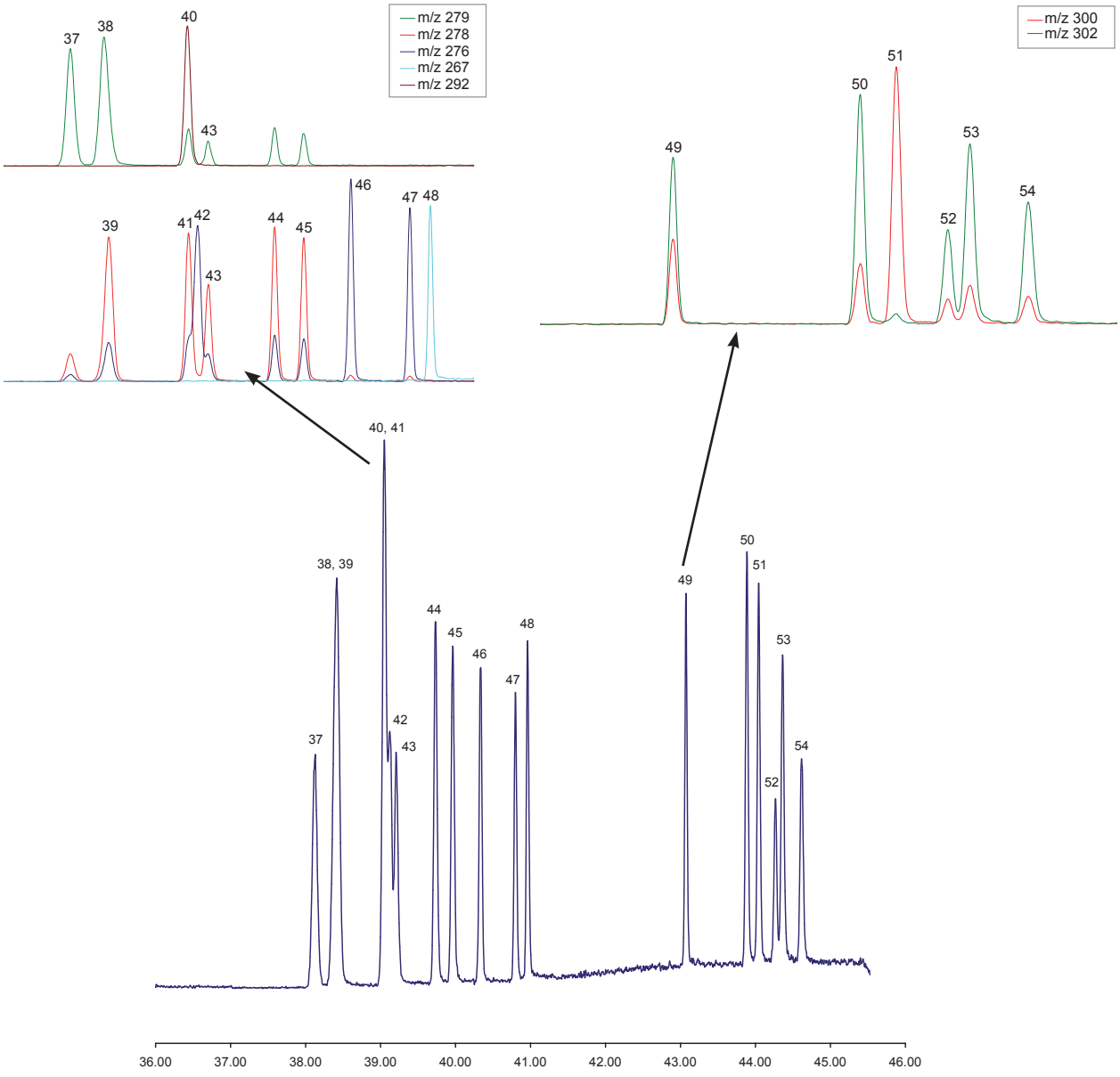

Figure 6. Details and identification of peaks 37 to 54

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Published in UK, October 11, 2010

SI-02232

Agilent Technologies

GC Analysis of PAHs using an Agilent J&W FactorFour VF-17ms Column with EZ-Guard

Application Note

Author

Peter Heijnsdijk
Agilent Technologies, Inc.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) can be formed during incomplete combustion or pyrolysis of organic matter, industrial processes and cooking and food processing. US and European Union legislation require the monitoring of pollutant PAHs in air, waste, sludge, drinking water and food because of their carcinogenic properties. The matrix of the sample can be complex, depending on the nature of the sample. In particular, food, waste water and sludge can introduce non-volatiles into the column, despite sample preparation. These non-volatile components originating from the sample matrix will adhere to the analytical column and diminish separation and detector response.

By using an EZ-Guard in front of the analytical column, the non-volatile components are trapped on the integrated guard. When column performance diminishes, a meter of the EZ-Guard can be cut off to restore column performance with no loss of resolution in the analytical column.

The VF-17ms GC column is widely used for analyzing PAHs, being chosen for its high selectivity towards certain PAH isomers. This application note demonstrates the selectivity of the VF-17ms with EZ-Guard in the analysis of these important pollutants.

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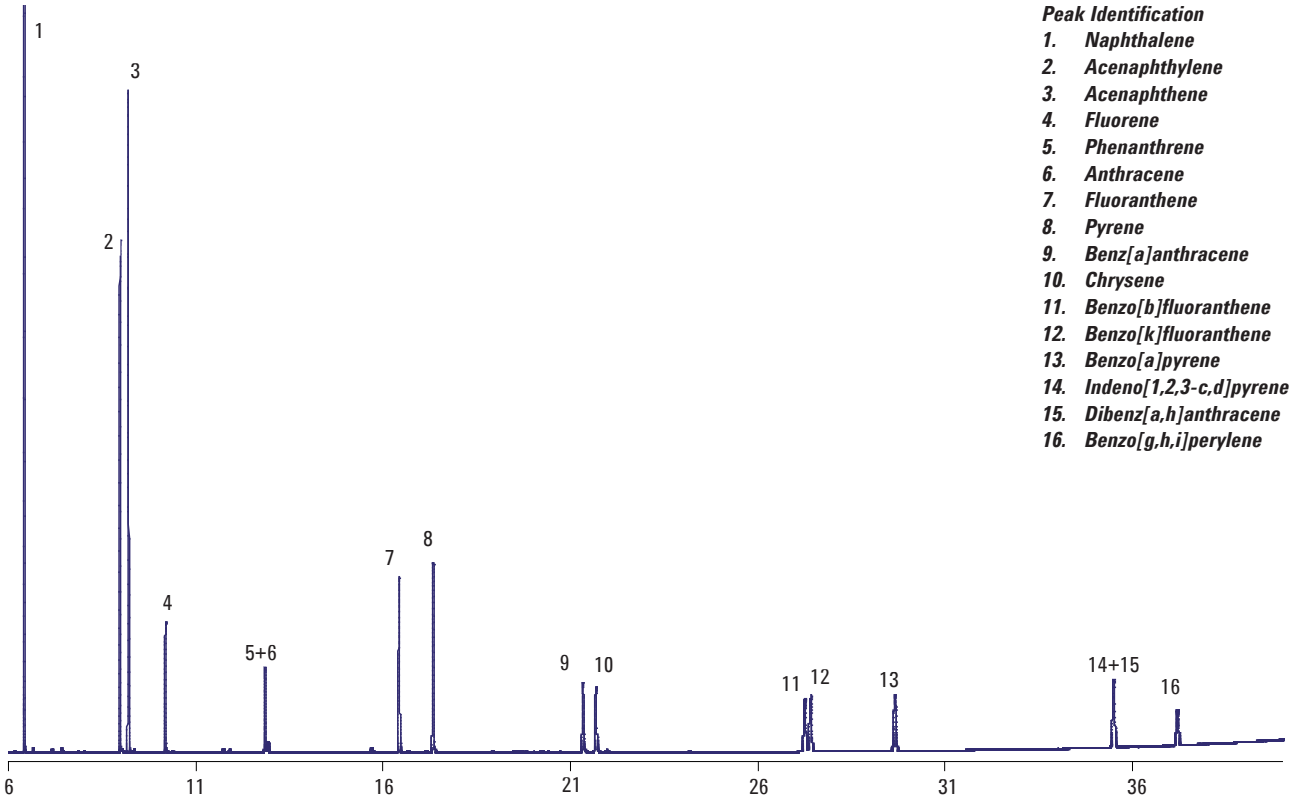

Analysis of polycyclic aromatic hydrocarbons using a VF-17ms column with EZ-Guard

Materials and Methods

Technique:	GC-FID
Column:	FactorFour VF-17ms, 30 m x 0.25 mm ID, df=0.25 µm, + 10 m EZ-Guard (part number CP9025)
Temp Program:	70 °C (1.7 min) to 180 °C (20 °C/min) to 280 °C (8 °C/min) 5 min isothermal, to 350 °C (4 °C/min), 10 min isothermal
Carrier Gas:	He
Pressure:	2.0 bar
Injector Temp:	275 °C
Detector Temp:	325 °C, FID
Splitless Time:	1 min (split 100 ml/min)
Sample Size:	2 µL
Sample Info:	4-27 ug/mL, solvent acetonitrile

Results and Discussion

The figure above shows the performance of the VF-17ms with EZ-Guard for PAH analysis. EZ-Guard does not interfere with the column's inertness, as shown by the excellent peak shapes.

Conclusion

With the VF-17ms with EZ-Guard, Agilent introduces a GC column ideal for PAH analysis in complex matrices.

The EZ-Guard extends the lifetime of the GC column by trapping the non-volatile components arising from the sample matrix. In addition, EZ-Guard columns are quick, and easy to install and operate, boosting productivity and delivering improved efficiency as the integrated transfer line provides faster detector stabilization.

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Published in UK, October 08, 2010
SI-02221

Agilent Technologies

Analysis of all 136 tetra- through octa- polychlorinated dibenzo-p-dioxins and dibenzofurans using an Agilent J&W FactorFour VF-Xms GC column

Application Note

Authors

Johan Kuipers
Agilent Technologies, Inc.

Vyacheslav N. Fishman
The Dow Chemical Company

Introduction

Out of the 136 polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) congeners (tetra- through octa-), there are 17 congeners with chlorines in the 2378-positions that are considered to be of toxicological significance and are typically used for Toxic Equivalence (TEQ) calculations.

In order to establish TEQ values, an accurate determination of isomer-specific concentrations of all 17 2378-substituted dioxins and furans is required. Most of the regulatory methods for the PCDD/PCDF analysis have been developed based upon a dual column approach using a combination of non-polar and polar GC columns.

In this note, data is presented for the elution order of all 136 tetra- through octa- polychlorinated dioxins and furans, including the separation of 16 out of 17 2378-substituted dioxins and furans from other close-eluted isomers. We used a high Si-arylene modified liquid phase column, VF-Xms, with no need for a second column.

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Materials and Methods

Column:	VF-Xms Fused Silica, 60 m x 0.25 mm, df = 0.25 µm (part number CP8809)
Carrier Gas:	He, constant pressure at 290 kPa or 42 psi
Oven Temp:	160 ºC for 1 min 160-230 ºC at 35 ºC/min, hold for 18 min 230-250 ºC at 10 ºC/min, hold for 25 min 250-310 ºC at 10 ºC/min, hold for 6 min
Interface Temp:	290 ºC
Injection:	Splitless, 270 ºC, 1 µL
Detector:	MAT-95XP double focusing high raesolution magnetic sector mass spectrometer, EI 42eV, Ion source 270 °C, Acceleration voltage 4700V, SIM mode at resolution > 10,000 (10% valley)
Sample:	128 qualitative standards (tetra- through hexa-) PCDD/PCDF with an approximate concentration of 25 ng/mL in nonane. (Cambridge Isotope Laboratories Andover, MA, USA). In addition, native HpCDD, HpCDF, OCDD and OCDF were added to the above mixture

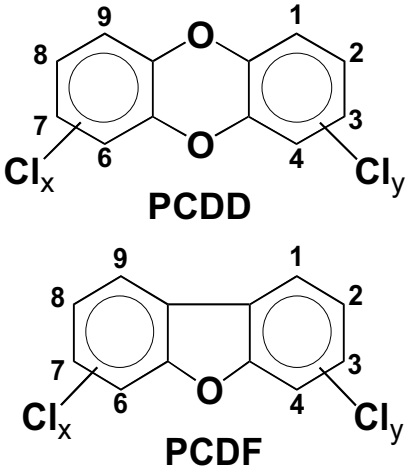

Figure 1. Chemical structures of PCDD and PCDF congeners

Table 1. Number of isomers of the PCDD and PCDF

Chlorines (x+y)	Acronyms	PCDD number of isomers	PCDF number of isomers
4	TCDD/TCDF	22	38
5	PnCDD/ PnCDF	14	28
6	HxCDD/ HxCDF	10	16
7	HpCDD/ HpCDF	2	4
8	OCDD/OCDF	1	1
4-8	Total	49	87

Results

Figures 2 and 3 show the separation of PCCD/PCDF where quantitative results can be easily achieved on 16 out of 17 2378-substituted congeners. Note that critical 2378-TCDD and 2378-TCDF separations can be easily achieved with better than 25% valley as typically required by regulatory methods.

Conclusion

The VF-Xms liquid phase provides superior performance towards dioxins and furans separations compared to other GC columns, delivering a high degree of quantification accuracy for 2378-substituted congeners. The ability to separate 2378-TCDF at the baseline from other closely eluting isomers means there is no need for confirmatory analysis by a complementary GC column when strictly following US and Canadian regulatory methods.

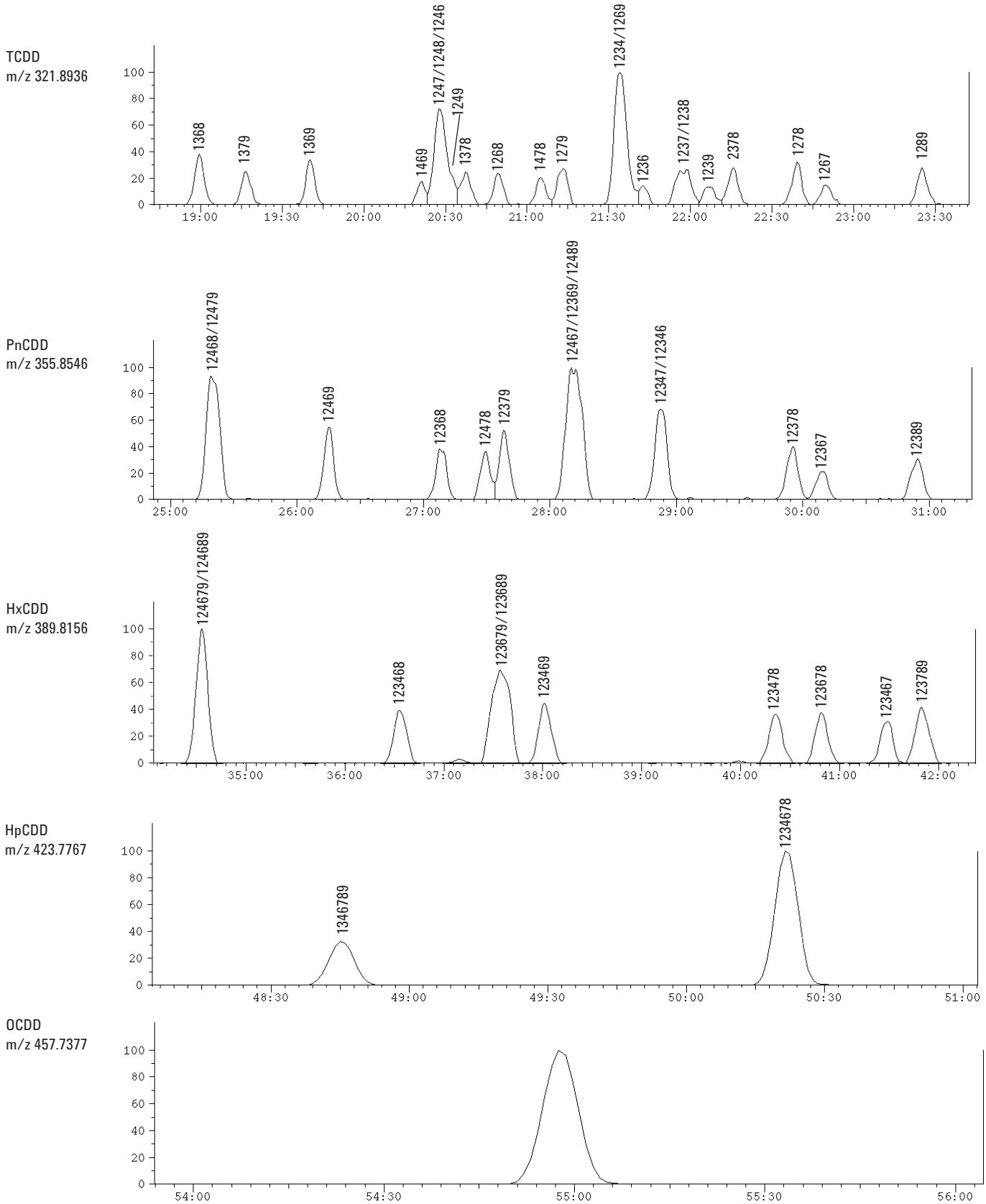

Figure 2. Separations of polychlorinated dibenzo-p-dioxins on a VF-Xms GC column

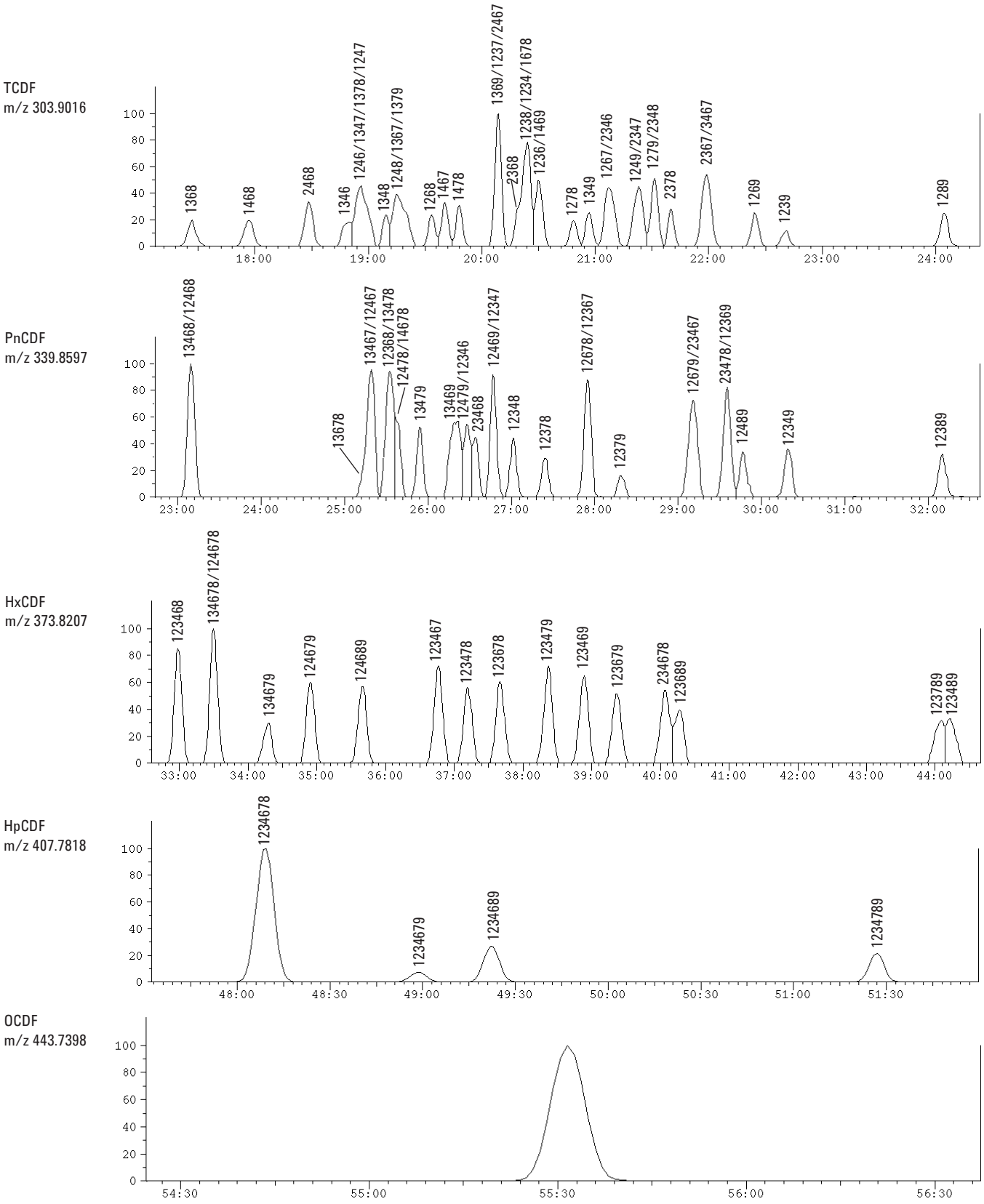

Figure 3. Separations of polychlorinated dibenzofurans on a VF-Xms GC column

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Published in UK, October 08, 2010
SI-02196

Agilent Technologies

Fast GC Analysis of EPA and EU Regulated PAHs

Application Note

Authors

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Agilent Technologies, Inc.

Andrea Agostini, Paolo Altemura, Marco Vatteroni, Valeria Filippi and Pierluigi Bardini
Agenzia Regionale per la Protezione Ambientale della Toscana

Introduction

After assessing 33 PAHs, the European Commission Scientific Committee on Food (SCF) in 2002 found that due to their toxicity, 15 of them were of major concern to human health and should therefore be monitored. The FAO/WHO Expert committee on Food Additives identified benzo(c)fluorine in 2005 as an additional compound that should be analyzed. These 15+1 EU priority PAHs differ from the 16 PAHs covered under the US EPA regulations.

With the increase of the number of target PAH analytes and the fact that many PAHs and their isomers exhibit identical MS mass fragmentation, the chromatographic separation and selectivity of the stationary phase for the analysis of PAHs has become more important.

This application note illustrates the successful use of an Agilent J&W FactorFour VF-17ms column from Agilent Technologies, Inc. for the fast GC/MS analysis of most PAHs listed in the EU and EPA regulations (Figure 1).

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Conditions

Technique:	GC/MS
Column:	VF-17ms 20 m x 0.15 mm df = 0.15 mm (p/n CP5884), retention gap 2.5 m x 0.53 mm (p/n CP8009)
Temperature:	90 °C (1 min), 78 °C/min, 130 °C (0.4 min), 21 °C/min, 190 °C (1.90 min), 15.7 °C/min, 320 °C (12 min)
Carrier Gas:	Helium, constant flow 1 mL/min
Injector:	Splitless mode, pulsed pressure 50 psi for 0.2 min, 280 °C
Detector:	Quadrapole MS, EI in SIM, ion source 230 °C, transfer line 280 °C
Sample:	Concentration 5 ppm, isotope ISTDs 0.5 ppm, solvent toluene
Injection Vol:	1 µL

Results

Critical peak pairs such as the benzofluoranthene isomers (m/z 252) are almost baseline resolved (Figure 3). Although the VF-17ms stationary phase exhibits an excellent selectivity towards difficult to separate PAHs, the coelution of some peak pairs cannot be avoided. Indeno[1,2,3-cd]pyrene (m/z 276) and dibenzo[a,h]anthracene (m/z 278) coelute but can be distinguished by their difference in molecular masses (Figure 4). Crysene cannot be unambiguously quantified due to the possible coelution with triphenylene and identical quantifying ions (m/z 228), but is well resolved from benzo(a) anthracene (Figure 5). The four EU priority dibenzopyrenes (m/z 302) are well separated by VF-17ms (Figure 6). However, due to the large number of isomers with the same molecular mass, possible interferences may influence results.

The temperature program was carefully optimized to obtain optimal results in terms of separation and speed of analysis.

Discussion

The VF-17ms column efficiently separates all 16 US regulated PAHs as well as the 15+1 EU priority PAHs. The 50% phenyl groups incorporated in the liquid phase provide additional selectivity based on π/π stacking interactions and electrostatic supported separation mechanisms. The smaller 0.15 mm ID column delivers fast analysis within 25 minutes without compromizing the resolution efficiency needed for the separation of critical PAH peak pairs. Quantification of the 23 PAHs of this particular analysis is carried out by means of isotope dilution using deuterium labelled PAH internal standards. Perylene D12 is used as an additional standard to correct for response variations between standard and sample analysis due to matrix influences during injection.

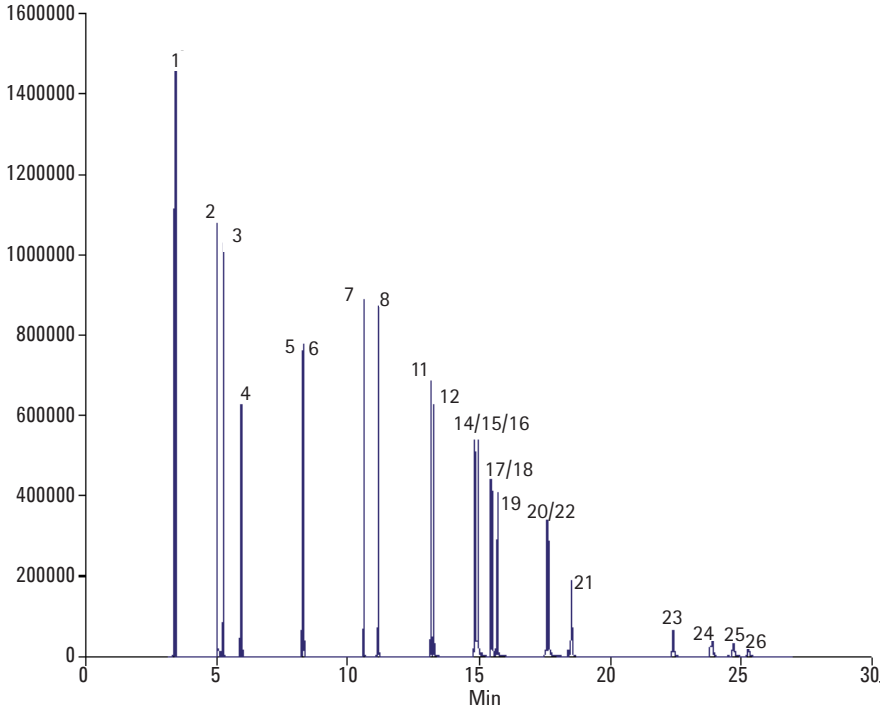

Figure 1. Fast GC/MS analysis of EU and EPA PAHs on VF-17ms 20 m x 0.15 mm x 0.15 µm

Peak Identification

	PAH	Abbre-viation	MW	EU PAHs	EPA PAHs
1	Napthalene	Nap	128		x
2	Acenaphthylene	Acy	152		x
3	Acenaphtene	Acp	154		x
4	Fluorene	Flr	166		x
5	Phenanthrene	Phen	178		x
6	Anthracene	Ant	178		x
7	Fluoranthene	Flt	202		x
8	Pyrene	Pyr	202		x
9	Benzo[c]fluorene	B[c]F	216	x	
10	Cyclopenta[cd]pyrene	CCP	226	x	
11	Benzo[a]anthracene	BaA	228	x	x
12	Chrysene	Chr	228	x	x
13	5-Methylchrysene	5-MC	242	x	
14	Benzo[b]fluoranthene	BbF	252	x	x
15	Benzo[k]fluoranthene	BkF	252	x	x
16	Benzo[j]fluoranthene	BjF	252	x	
17	Benzo[e]pyrene	BeP	252		
18	Benzo[a]pyrene	BaP	252	x	x
19	Perylene	Per	252		
20	Indeno[1,2,3-cd]pyrene	IcdP	276	x	x
21	Benzo[ghi]perylene	BghiP	276	x	x
22	Dibenzo[a,h]anthracene	DahA	278	x	x
23	Dibenzo[a,l]pyrene	DalP	302	x	
24	Dibenzo[a,i]pyrene	DaiP	302	x	
25	Dibenzo[a,e]pyrene	DaeP	302	x	
26	Dibenzo[a,h]pyrene	DahP	302	x	

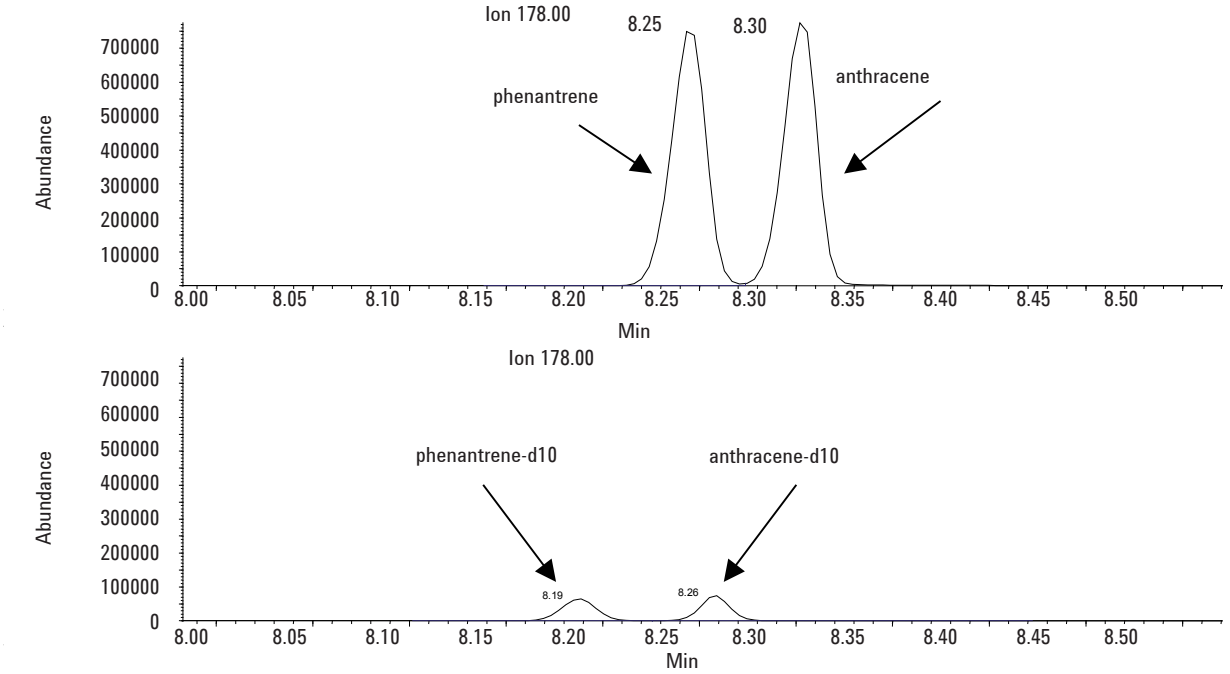

Figure 2. Phenanthrene and Fluoranthene both with m/z 178 are efficiently separated

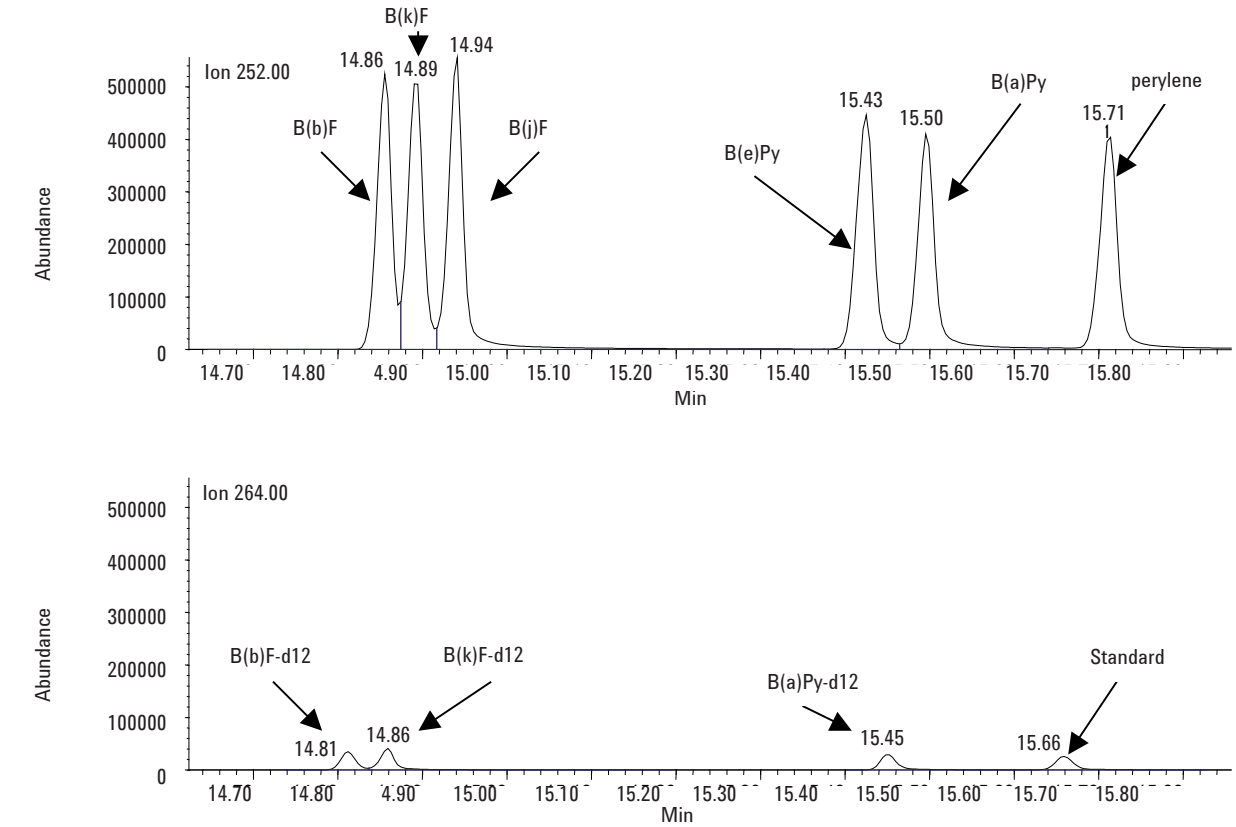

Figure 3. The most critical separations of the PAHs with m/z 252. The benzofluoranthenes isomers are almost baseline resolved

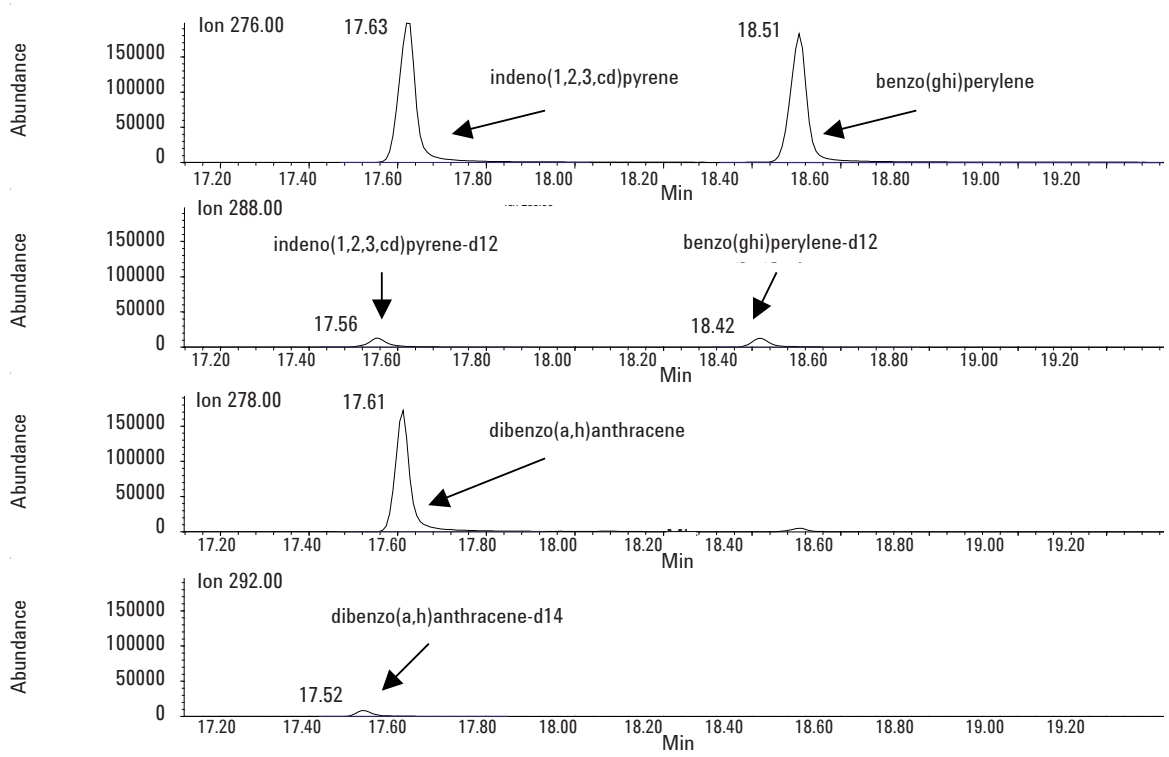

Figure 4. The VF-17ms is able to separate indeno(1,2,3,cd) pyrene from dibenzo(a,h)anthracene using different temperature programs, in this analysis they are discriminated by their difference in molecular masses

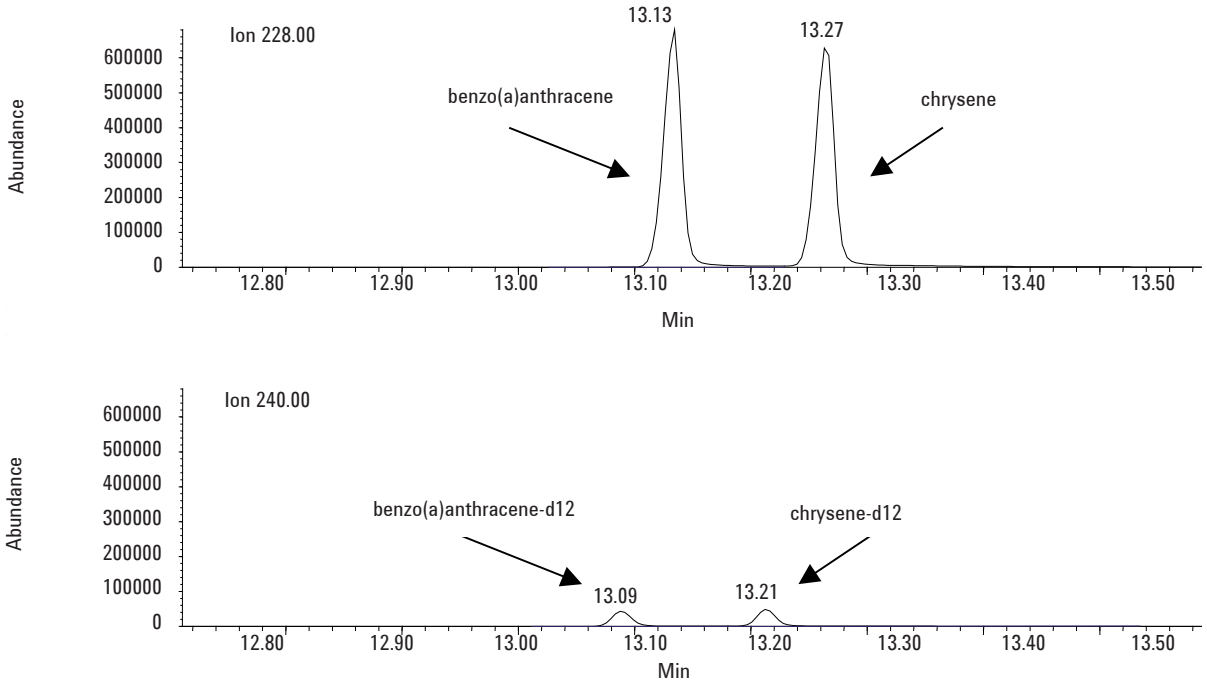

Figure 5. Benzo(a)anthracene and chrysene are well resolved

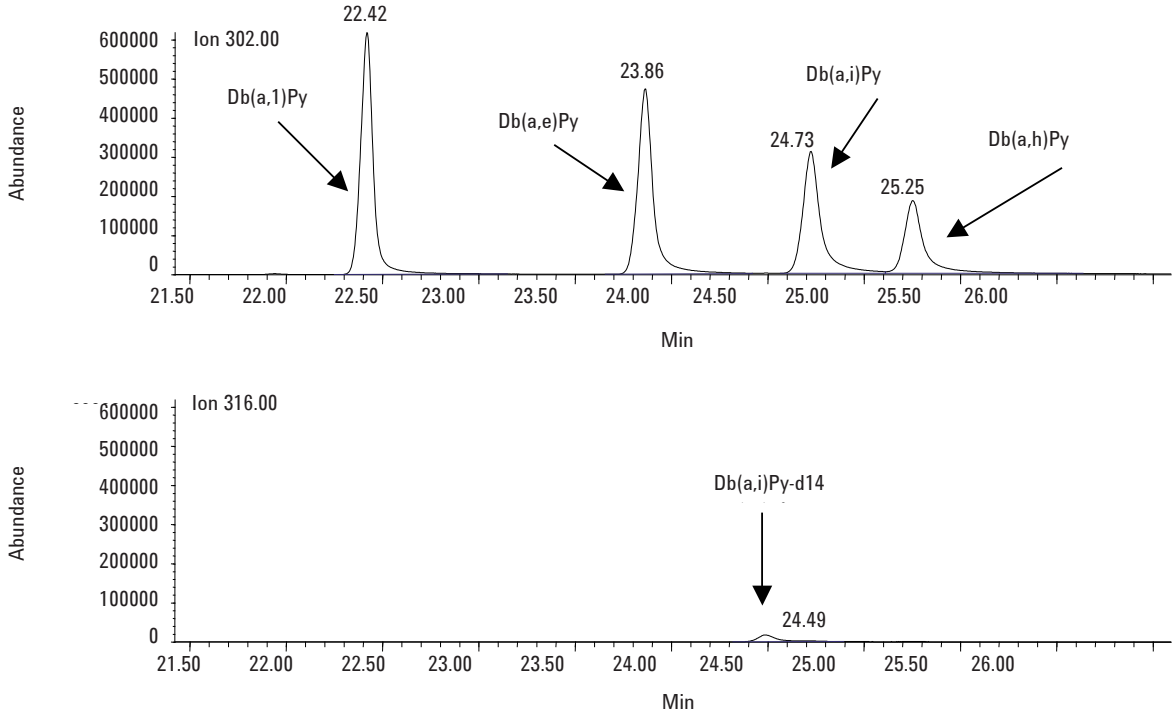

Figure 6. The last target compound dibenzo(a,h)pyrene elutes in about 25 minutes

References

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Published in UK, October 08, 2010

SI-01853

Agilent Technologies

New SPE Sorbent for Clean-up of Fusarium Toxin-contaminated Cereals & Cereal-based Foods, Bond Elut Mycotoxin

Application Note

Fusarium Fungi, Cereals

Authors

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Introduction

Fusarium fungi are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia. A variety of fusarium fungi produce different toxins of the class of trichothecenes. More than 140 of these compounds have been isolated from fungi cultures and this number is still increasing[1]. The toxic effects of fusarium toxins on humans and animals are well-documented[2,3] and reliable and sensitive analysis methods, which comply with the European regulations for mycotoxin determination in food and feed, are required[4, 5]. Traditional sample preparation for trichothecene analysis typically involves extraction with acetonitrile/water and clean-up via charcoal-alumina columns[6]. As the trichothecenes differ considerably in polarity and solubility, recoveries of the more polar analytes are often compromised with this approach. Another approach is the use of immunoaffinity columns (IAC)[7]. These provide highly selective extractions with high recoveries, however, separate IAC columns are needed for each toxin. To overcome the limitations of these methods, there was a need to develop an extraction and clean-up method for the simultaneous determination of several trichothecenes with high recoveries for polar toxins by minimizing the matrix effects.

This application note shows the optimized extraction and clean-up step of 12 type A- and B-trichothecenes and zearalenone (ZEA) in cereals and cereal-based food on Bond Elut Mycotoxin, a newly developed extraction sorbent. Structures and names of the 12 toxins investigated in this application are shown in Figure 1.

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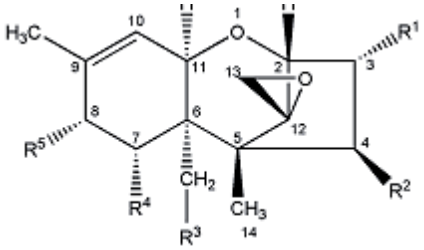
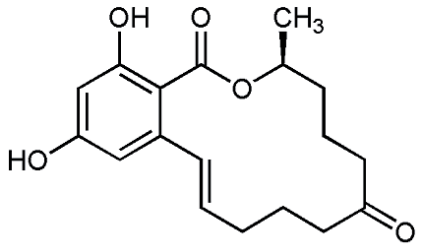
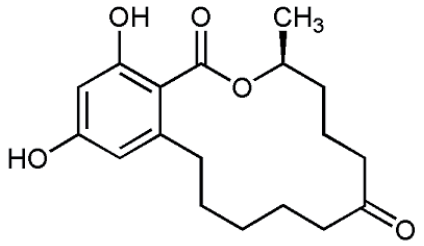


Trichothecenes



Zearalenone



Zearalanone

A healthy wheat head (left) next to one showing severe symptoms of Fusarium head blight disease (right) (Photo by Keith Weller, ARS US Department of Agriculture)

	Trichothecene	R1	R2	R3	R4	R5
Type A	Neosolaniol (NEO)	OH	OAc	OAc	H	OH
	HT-2 toxin (HT-2)	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
	T-2 toxin (T-2)	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
	T-2 triol	OH	OH	OH	H	$OCOCH_2CH(CH_3)_2$
	T-2 tetraol	OH	OH	OH	H	OH
	Monoacetoxyscirpenol (MAS)	OH	OH	OAc	H	H
	Diacetoxyscirpenol (DAS)	OH	OAc	OAc	H	H
Type B	Deoxynivalenol (DON)	OH	H	OH	OH	=O
	3-Acetyl-DON (3ADON)	OAc	H	OH	OH	=O
	15-Acetyl-DON (15DON)	OH	H	OAc	OH	=O
	Nivalenol (NIV)	OH	OH	OH	OH	=O
	Fusarenon-X (FUS)	OH	OAc	OH	OH	=O

Figure 1. Chemical structure of type A- and B-trichothecenes, zearalenone (ZEA) and zearalanone (ZAN). Type A trichothecenes have various groups at ring position 8, type B-trichothecenes have a carbonyl function at position 8

Extraction and Clean-up

Clean-up methods of trichothecenes and ZEA from cereals and cereal-based foods widely use commercially available polar clean-up columns. Analytical interfering substances are retained while trichothecenes are not adsorbed on the packing material. This purification method, however, gives low recoveries for the polar toxins NIV, T-2 tetraol and DON. New studies[9] point out that one possible reason for the low recoveries for the polar toxins might be the low water content in the acetonitrile/water mixture (ACN/H_20; 84/16; v/v), which is used for the extraction of the mycotoxins from the matrix. Furthermore, to elute these polar compounds from the column containing polar adsorbents like alumina, a hydrophilic solvent is needed. When using more polar extraction mixtures like ACN/H_20 (75/25; v/v), the recoveries of the polar toxins NIV, T-2 tetraol and DON could be raised. However, the higher the content of water in the extraction solvent resulted in co-extraction of more matrix compounds and led therefore to strong ion suppression in the LC-MS analysis.

To address these problems, we optimized the extraction step by marginally increasing the polarity of the extraction solvent to ACN/H_20 (80/20; v/v) and used the Bond Elut Mycotoxin cartridge to clean up the extracts. Trials with the polar DON reference material from Food Analysis Performance Assessment Scheme (FAPAS) confirm that the best recovery data were achieved with the Bond Elut Mycotoxin method (Table 1).

Table 1. Recovery comparison of Food Analysis Performance Assessment Scheme (FAPAS) certified reference material for DON applying LC-MS/MS.

Reference Material	Certified Value (µg/kg)	Method 1 (µg/kg)	Method 2 (µg/kg)
FAPAS T2210	463 ± 167	495 ± 5	395 ± 15

Method 1: Clean-up on Bond Elut Mycotoxin
Method 2: Clean-up on competitor column (charcoal-alumina)

To calculate the amount of ZEA, the extracted matrices were spiked with a defined amount of zearalanone (ZAN) standard solution before the clean-up step on Bond Elut Mycotoxin. The measured value of ZEA was corrected by the value of ZAN, as previously described by Berthiller et al[8]. Using ZAN as internal standard, the recovery of ZEA was about 100%.

Bond Elut Mycotoxin Method

1. Extract 25 g of finely ground sample with a solution of 100 mL acetonitrile/water (80/20; v/v) by blending at high speed for 3 minutes. For simultaneous determination of zearalenone (ZEA), spike extract at a level of 50 ng/g sample with zearalanone (ZAN) solution in acetonitrile as internal standard.

2. Filter.

3. Pass 4 mL of the filtrate through a Bond Elut Mycotoxin column (part number 12165001B).

4. Evaporate 2 mL of the eluate to dryness at 50 °C under a gentle stream of nitrogen.

5. Reconstitute in 0.5 mL acetonitrile/water (20/80; v/v). Inject 10 µL into LC-MS/MS for analysis.

Results and Discussion

The Bond Elut Mycotoxin product provides a single column method for the clean-up of 12 type A- and B-trichothecenes plus ZEA (corrected via internal standard ZAN).

Table 2 shows the average recoveries and RSDs obtained for 12 trichothecenes and ZEA from spiked wheat, corn, durum, oats, bread, muesli and cereal infant food samples after clean-up with Bond Elut Mycotoxin columns. By combining an increased polarity of the extraction solvent with the clean-up step on Bond Elut Mycotoxin, recoveries, especially for the polar toxins DON, NIV, 3ADON and T-2 tetraol, were increased up to 31% when compared to the extraction method on charcoal-alumnia cartridges[9].

Table 3 shows the trichothecene content of 6 naturally contaminated samples after 3 different clean-up methods. Up to 43% higher values were achieved in the analysis of naturally contaminated samples for the polar toxins DON, NIV, 3ADON, 15ADON and T-2 tetraol in comparison to the charcoal-alumina based method. If the determination of DON alone is of interest, then the highest content can be achieved with an extraction of 100% water and clean-up with IAC; however, for the determination of 12 trichothecenes with different polarities, the Bond Elut Mycotoxin provides comparable results.

Conclusion

As the performance of the Bond Elut Mycotoxin cartridges is similar or better, and the columns are more cost effective, the new clean-up procedure is a very good alternative to other standardized methods commonly used.

Table 2. Average recovery and RSD in percentage obtained for 12 trichothecenes and ZEA from spiked wheat, corn, durum, oats, bread, muesli and cereal infant food samples (spiking levels of 50/100, 200/400 and 500/1000 ng/g for trichothecenes/DON and 50 ng/g for ZEA and ZAN), after clean-up with Bond Elut Mycotoxin columns, (n=3). Data reported by Klötzel et al[10].

Toxin	Recovery [%] ± RSD [%], 3 levels, n = 3						
	Wheat	Corn	Durum	Oats	Bread	Muesli	Infant Food
DON	90 ± 5.2	93 ± 2.8	98 ± 3.8	96 ± 5.1	87 ± 1.7	87 ± 3.7	88 ± 12
NIV	67 ± 5.9	74 ± 2.5	67 ± 6.3	73 ± 10	65 ± 5.7	71 ± 13	66 ± 10
3ADON	89 ± 9.3	88 ± 7.6	97 ± 6.6	93 ± 11	100 ± 5.5	101 ± 7.1	91 ± 9.4
15ADON	92 ± 13	87 ± 15	89 ± 11	89 ± 11	96 ± 9.5	98 ± 8.3	96 ± 6.6
FUS	91 ± 10	94 ± 4.2	91 ± 7.8	91 ± 7.8	98 ± 8.5	97 ± 6.4	96 ± 4.3
T-2	87 ± 7.6	88 ± 8.8	84 ± 2.2	84 ± 2.2	83 ± 8.2	75 ± 11	70 ± 7.3
HT-2	82 ± 7.3	91 ± 3.3	85 ± 5.0	85 ± 5.0	79 ± 3.3	70 ± 7.7	74 ± 0
NEO	91 ± 2.6	78 ± 11	68 ± 18	68 ± 18	80 ± 2.0	104 ± 10	71 ± 6.3
DAS	82 ± 8.3	89 ± 3.6	85 ± 5.2	85 ± 5.2	75 ± 3.7	82 ± 6.8	68 ± 4.6
MAS	86 ± 13	85 ± 12	93 ± 4.2	93 ± 4.2	86 ± 11	88 ± 16	91 ± 14
T-2 triol	69 ± 9.1	66 ± 1.2	83 ± 2.8	83 ± 2.8	76 ± 9.3	82 ± 3.3	71 ± 7.9
T-2 tetraol	69 ± 12	75 ± 6.8	73 ± 10	73 ± 10	65 ± 11	67 ± 17	70 ± 16
ZEA	110 ± 5.9	113 ± 5.0	108 ± 4.8	108 ± 4.8	111 ± 6.0	102 ± 2.7	116 ± 6.7

Table 3. Trichothecene contents of six naturally contaminated samples analyzed with DONPrep IAC, MycoSep 227 and Bond Elut Mycotoxin cartridges, (n=3). Data reported by Klötzel et al[10].

Sample	Clean-up	DON [ng/g]	NIV [ng/g]	15ADON [ng/g]	HT-2 [ng/g]	T-2 [ng/g]	T2 tetraol [ng/g]
Bread	IAC	690 ± 18					
Bread	Mycosep	557 ± 19					
Bread	Bond Elut Mycotoxin	648 ± 21					
Corn	IAC	368 ± 8.4					
Corn	Mycosep	333 ± 14	12 ± 0	69 ± 2.0			
Corn	Bond Elut Mycotoxin	356 ± 3.8	14 ± 0	99 ± 2.5			
Wheat	IAC	488 ± 5.5					
Wheat	Mycosep	421 ± 16					
Wheat	Bond Elut Mycotoxin	468 ± 19					
Oats	IAC	299 ± 11					
Oats	Mycosep	220 ± 5.3	22 ± 3.3	7.0 ± 0.4	93 ± 12	15 ± 4.1	91 ± 6.2
Oats	Bond Elut Mycotoxin	264 ± 13	19 ± 1.2	7.7 ± 0.1	78 ± 4.9	12 ± 4.3	106 ± 3.2
Wheat	IAC	1680 ± 32					
Wheat	Mycosep	1590 ± 40	39 ± 3.7	24 ± 2.0			
Wheat	Bond Elut Mycotoxin	1750 ± 120	64 ± 1.4	42 ± 2.2			
Durum	IAC	512 ± 15					
Durum	Mycosep	407 ± 25	25 ± 4.1				
Durum	Bond Elut Mycotoxin	456 ± 42	22 ± 1.3				

Ordering Information

Part number	Description
12165001B	Bond Elut Mycotoxin 1 gm in JR cartridge, 100 cartridges/pk
12131009	Reservoir 6 mL 100 tubes/pk
12131015	Reservoir 6 mL with 2 x 20 µm polypropylene filter pre-installed 100/pk
12131021	20 µm polypropylene filter for 6 mL cartridges
12234105	Vac Elut 20 Manifold SPE Cartridge Processing Station with collection rack for 10x75 mm test tubes

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Published in UK, August 17, 2010

SI-00295

Agilent Technologies

Multi-Residue Confirmation of Pesticides in Honey using Solid Supported Liquid Extraction

Application Note

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University of Liege

Introduction

In recent years, several Belgian beekeepers were asked to shut down their hives due to withering of the hive. The cause of the withering was unknown. A multi-factoral study was initiated, which included a multi-class pesticide residue study from honey, to determine if pesticides were the cause of the decline. The extensive distribution of pesticides causes bees that have been fed on contaminated blossoms to transfer pesticide residues into honey. Multi-residue confirmation methods to identify and quantify widely used pesticides, which could have been the source of the bees decline, needed to be developed. Previously published papers already report determination methods for pesticides in honey, however, most of them analyze only one or two pesticide groups, such as organochlorine or organophosphorous residues. This application note shows the development and validation of 17 pesticides and metabolites of different chemical classes:

Insecticides: Carbofuran (Ca), Methiocarb (Mh), Pirimicarb (Pi), Dimethoate (Dm), Fipronil (Fi), Imidacloprid (Im)

Herbicides: Amidosulfuron (Am), Rimsulfuron (Ri), Atrazine (At), Simazine (Si), Chlorotoluron (Ch), Linuron (Li), Isoxaflutole (Is), Metosulam (Mo)

Fungicides: Diethofencarb (De)

Metabolites: Methiocarb sulfoxide (Mhs), 2-Hydroxytertbutylazine (TOH)

The application described here is based on solid supported liquid-liquid extraction method (SLE) followed by LC/MS/MS. The results are compared to data from standard liquid-liquid extraction (LLE) to check extraction efficiency and appropriateness of the SLE method. More detailed information on the method development and validation has been published previously[1].

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Chem Elut - the solid support

The solid support consists of specially processed, wide-pore, diatomaceous earth packed into clean polypropylene cartridges. The aqueous sample is applied to the dry Chem Elut sorbent. The sample is distributed as a thin film over the chemically inert support, which acts as a stationary phase. Subsequently, elution takes place using immiscible organic solvents. The lipophilic substances are extracted from the aqueous into the organic phase, while the aqueous phase remains on the Chem Elut sorbent. A phase-separation filter is incorporated into the cartridge as a safeguard to ensure that organic eluents remain uncontaminated by aqueous matrix. The extraction on Chem Elut is carried out with gravity only - no vacuum is required (Figure 1).

Sample Preparation and Clean Up

SLE procedure on 5 mL Chem Elut cartridge (part number 12198006)

Method:

1. Spike 1 g honey sample with 20 µL of surrogate standard solution (concentrations listed in Figure 2).

2. Mix with 1.25 mL water and 2.5 mL acetone.

3. Add 1.25 mL of NaCl solution (20 g/100 mL).

4. Apply sample to Chem Elut cartridge by gravity flow.

5. Allow 15 mins for complete adsorption to take place.

6. Elute twice with 10 mL ethyl acetate.

7. Evaporate at 30 °C.

8. Reconstitute with 200 µL acetonitrile/water (10/90).

9. Inject 20 µL into LC/MS/MS.

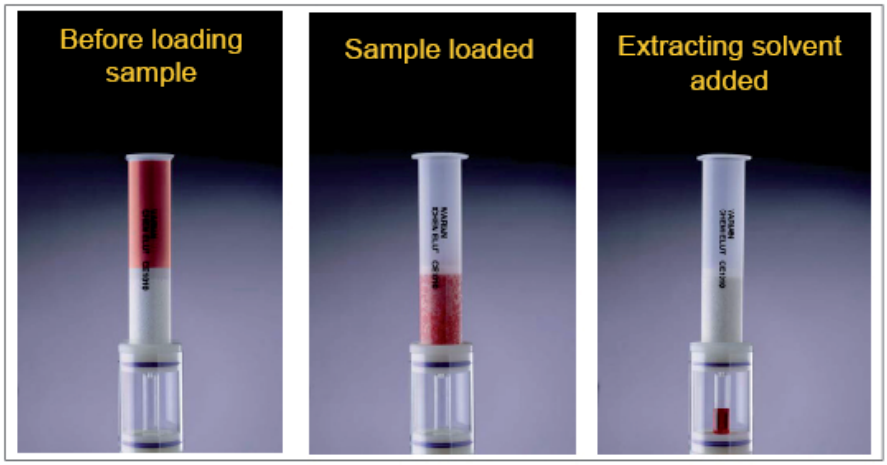


Figure 1. Solid Supported Extraction on Chem Elut cartridges

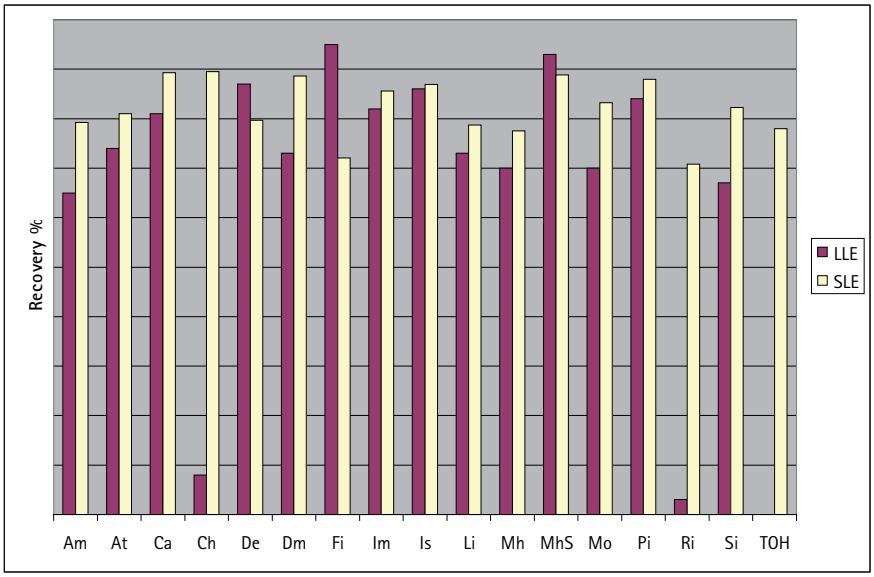
Analysis Conditions

The method is based on HPLC coupled to mass spectrometry (MS) operating in tandem mode (MS/MS) according to EU advice 2002/657/EC [2].

Column:	Polaris C18-A 3 µm, 2.0 x 150 mm (part number A2001150X020)
Temperature:	40 °C
Mobile Phase A:	Water + 0.1% acetic acid
Mobile Phase B:	Acetonitrile + 0.1% acetic acid
Linear Gradient Conditions:	held 10% B for 1 min, to 80% B in 14 mins, to 100% B in 2 mins, held 100% B for 2 mins
Flow Rate:	0.4 mL/min

Results and Discussion

Figure 2 shows the comparison between recoveries obtained after SLE on Chem Elut and classical LLE. The LLE was performed by mixing 1 g honey with 2 mL water and 6.5 mL acetonitrile for 30 mins. After centrifugation the organic layer is evaporated to 100 µL and 100 µL of water is added prior to LC/MS. The comparison shows that the Chem Elut extraction provides similar or even higher extraction efficiency than LLE for most compounds. The greatest advantage of SLE is that the SLE technique avoids emulsion formation, which is standard in LLE, significantly easing the extraction procedure. Key advantages of Chem Elut cartridges are their ease of use and the wide range of compounds that can be extracted efficiently.



Pesticide (ng/mL):
Am 0.4, At 0.4, Ca 0.4, Ch 20.0, De 2.0, Dm 2.0, Fi 10.0, Im 2.0, Is 2.0, Li 2.0, Mh 10.0, MhS 20.0, Mo 2.0, Pi 0.4, Ri 0.4, Si 2.0, TOH 1.0

Figure 2. Recovery comparison of pesticides between solid supported liquid-liquid extraction (SLE) on Chem Elut and classical liquid-liquid extraction (LLE)

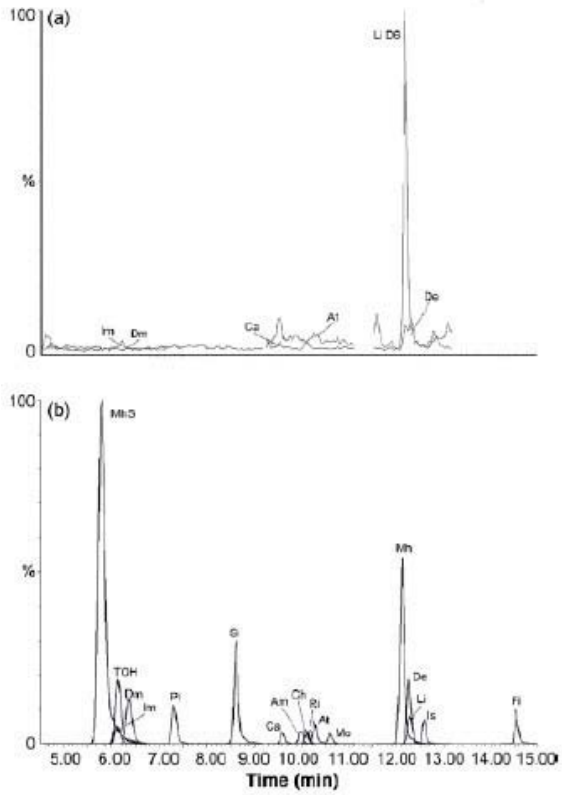


Figure 3. Chromatogram obtained for a honey blank matrix (a) and for a methanolic standard solution (b) using the Polaris C18-A column. Concentrations between 0.4 ng/mL and 20 ng/mL

Figure 3 shows the chromatogram of a methanolic standard solution with pesticide concentration between 0.4 and 20 ng/mL and a blank honey matrix. The total analysis time was 23 mins. The Polaris C18-A column is based on ultra-pure silica with a polar group placed between the primary C18 chain and the silica surface. The resultant packing material contains a surface, which is easily "wetted" with polar eluents and shows unique selectivity for a broad range of chemically different compounds. Furthermore, the polar group shields reactive silanols from polar silanophilic compounds, which improves peak symmetry and minimizes the "collapse" of the C18 chains in high-aqueous eluents. Seventeen pesticides were separated by optimizing the LC gradient, and the co-eluted pesticides with different masses were identified using MRM mode.

Conclusion

A rapid, reliable, time- and resource-saving analytical method is reported for the measurement of 17 pesticides of different chemical classes used in apiculture or in the surrounding agriculture in the context of a bee mortality study. The multi-residue analytical procedure developed in this study was based on a solid supported liquid-liquid extraction step using diatomaceous earth as inert solid support. Extracts were analyzed without further purification by LC/MS/MS in ESI mode. The SLE with Chem Elut cartridges has proven to be efficient for a wide range of pesticides, nearly independent of their polarity.

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Published in UK, August 20, 2010
SI-01002

Agilent Technologies

GC/μECD Analysis and Confirmation of PCBs in Fish Tissue with Agilent J&W DB-35ms and DB-XLB GC Columns

Application Note

Food Analysis

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Abstract

A fish sample obtained from a local grocery store was analyzed for 19 PCB (Polychlorinated Biphenyl) compounds using a GC with μECD. A QuEChERS procedure (Quick, Easy, Cheap, Effective, Rugged, and Safe) with dispersive solid phase extraction (dSPE) cleaned the sample prior to analysis. A dual μECD and dual capillary GC column approach performed simultaneous primary and confirmatory analysis. The primary column, Agilent J&W DB-35ms 30 m × 0.25 mm, 0.25 μm and confirmatory column Agilent J&W DB-XLB 30 m × 0.25 mm, 0.50 μm effectively resolved all 19 PCBs. The method was calibrated at the 10, 20, 50, 100, 250, and 400 ng/mL PCB levels, yielding excellent linearity and reproducibility. Spiked recoveries ranged between 72 and 116% at 50 and 200 ng/mL levels in the fish matrix.

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Introduction

Omega-3 fatty acids lower cholesterol, cancer risks, and blood pressure levels. The human body does not produce this fatty acid that is mainly obtained through diet or supplements. Fish is a good source of omega-3 fatty acids, which is especially high in fatty fish. However, even though fish has many beneficial aspects, fish may also contain contaminants such as heavy metals, PCBs or other pollutants.

Even though polychlorinated biphenyls (PCBs) were banned in the late 1970s in the United States and other countries, PCB contamination remains a concern. PCBs are slow to break down and are persistent in the environment, collecting in the sediment of rivers and lakes [1,2]. PCBs are highly lipophilic and bioaccumulate in the fatty tissue of fish throughout the food chain. Consumption of contaminated fish is a significant source of human exposure [3]. Many agencies such as the EPA and local state governments have issued fish advisories recommending monthly or annual fish consumption limits [4].

A dual column, dual µECD system with an Agilent J&W DB-35ms 30 m × 0.25 mm, 0.25 µm primary analysis column and an Agilent J&W DB-XLB 30 m × 0.25 mm, 0.50 µm confirmatory column is shown to separate and analyze the PCBs in the fish sample. Continuous improvements and stringent process control with respect to column activity make this column pair a particularly good choice for analysis of PCBs in a challenging fish tissue matrix.

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) AOAC sample preparation approach is used for extraction and cleanup of 19 PCB congeners in fish tissue. This approach involves an initial extraction in a buffered aqueous and acetonitrile system, an extraction and partitioning step following a salt addition, and a cleanup step using dispersive solid phase extraction [5].

Experimental

An Agilent 7890A GC system equipped with dual µECD detection was used for this series of experiments enabling simultaneous identification and confirmation from a single injection. The GC was also fitted with an unpurged two-way splitter capillary flow technology (CFT) device, simplifying maintenance and reducing system downtime. 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

GC:	Agilent 7890A equipped with dual µECD detection
Sampler:	Agilent 7873B 5.0 µL syringe (Agilent p/n 5181-1273)
CFT device:	2-way unpurged splitter capillary flow technology (Agilent p/n G3181B)
Carrier:	Hydrogen 85 cm/s, constant flow 3.5 mL/min
Injection:	1.0 µL splitless; 250 °C, Purge flow 50 mL/min at 0.3 min, Gas saver 50 mL/min at 2 min
Column 1:	Agilent J&W DB-35 ms 30 m × 0.25 mm, 0.25 µm (Agilent p/n 122-3832)
Column 2:	Agilent J&W DB-XLB 30 m × 0.25 mm, 0.50 µm (Agilent p/n 122-1236)
Oven:	110 °C (0.1 min), 25 °C/min to 200 °C (0.5 min), 10 °C/min to 240 °C (0.5 min), 30 °C/min to 325 °C (1.5 min)
Injection:	1µL, 250 °C splitless, purge 50 mL/min at 0.3 min, gas saver 50 mL/min on at 2 min
Dual µECD:	350 °C, N_2 makeup; constant column + makeup = 30 mL/min

Table 2. Flow Path Supplies

Vials:	Amber screw top glass vials (Agilent p/n 5183-2072)
Vial caps:	Blue screw caps (Agilent p/n 5182-0717)
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 seal:	Gold plated inlet seal (Agilent p/n 5188-5367)
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)
CFT fittings:	Internal nut (Agilent p/n G2855-20530)
CFT ferrules:	SilTite ferrules, 0.25 mm id (Agilent p/n 5188-5361)
20x magnifier :	20x Magnifier loop (Agilent p/n 430-1020)

Reagents and Chemicals

All reagents and solvents were HPLC or Ultra Resi grade. Acetonitrile (ACN) was from Honeywell (Muskegon, MI, USA), acetic acid (HAc) was from Sigma-Aldrich (St. Louis, MO, USA), and acetone was from VWR International (West Chester, PA, USA). The PCB congeners standard (RPCM-8082) and surrogate standard (ISM-320) were purchased from Ultra Scientific (N. Kingstown, RI, USA).

Solutions and Standards

A 1% acetic acid solution in ACN was prepared by adding 10 mL of acetic acid to 1 L of ACN.

The PCB stock standard solution (100 µg/mL of 19 congeners) was diluted in acetone to yield spiking solutions of 1 and 5 µg/mL. A 10 µg/mL surrogate spiking solution was prepared by diluting the stock surrogate (200 µg/mL) solution in acetone. The spiking solutions were used to prepare the calibration curves in the matrix blank extract by appropriate dilution.

Sample Preparation

A Swai fish sample was purchased from a local grocery store. The fish was chopped into small cubes and frozen at –80 °C overnight. The samples were then comminuted thoroughly to achieve sample homogeneity. The sample extraction method used the QuEChERS method followed by dSPE [5]. Figure 1 illustrates the sample preparation procedure graphically in a flow chart.

A 3.0 g sample of fish was weighed into a centrifuge tube. QC samples were fortified with appropriate amount of PCB spiking solution to yield QC samples with concentrations of 10, 50, and 200 ng/mL. A 150-µL amount of surrogate spiking standard (10 µg/mL) was added to each QC sample to yield a 100 ng/mL concentration. Each sample received a 12.0-mL aliquot of deionized water and 15-mL aliquot of 1% HAc in ACN. The samples were vortexed at 1500 rpm for 1 minute. Two ceramic bars (Agilent p/n 5982-9313) were added to each sample to aid in sample extraction. An Agilent SampliQ QuEChERS AOAC extraction salt packet (Agilent p/n 5982-5755) containing 6 g of $MgSO_4$ and 1.5 g sodium acetate was added to each centrifuge tube. The capped tubes were shaken on a Geno/Grinder at 1500 rpm for 1 minute. The samples were centrifuged at 4000 rpm for 5 minutes.

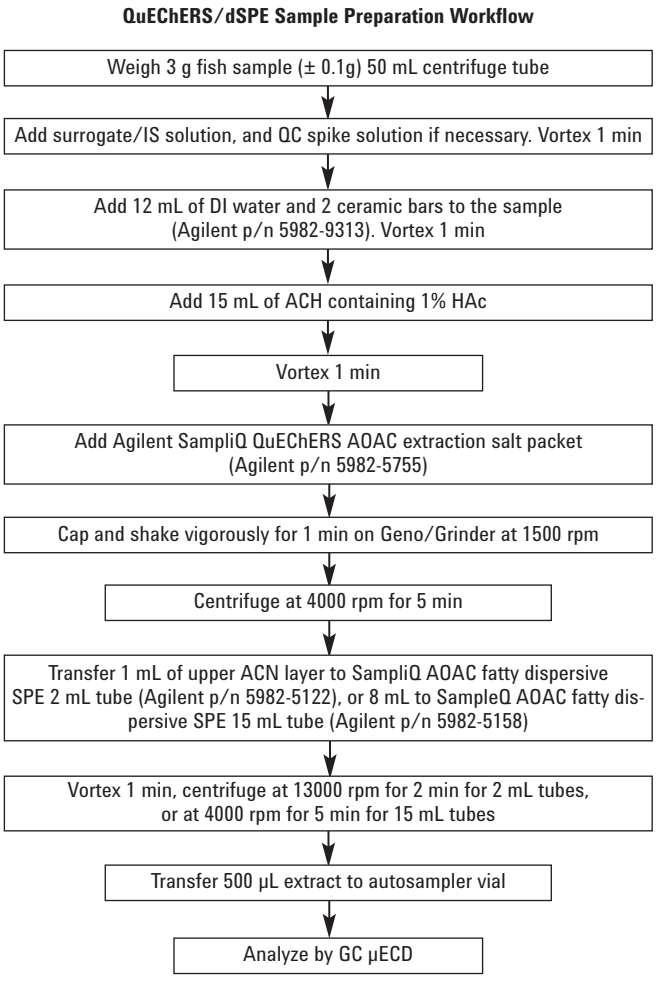


Figure 1. Flow chart of the Agilent SampliQ QuEChERS modified AOAC extraction procedure for fish sample [5].

An 8-mL aliquot of the upper layer was transferred to an Agilent SampliQ QuEChERS fatty sample dispersive SPE 15 mL tube (Agilent p/n 5982-5158). The dSPE tube was vortexed for 1 minute and then centrifuged at 4000 rpm for 5 minutes to complete the sample extraction. The liquid from the dSPE tube was transferred to a GC vial and analyzed by GC-µECD using the chromatographic conditions listed in Table 1.

Extractions of water and acetonitrile aliquots were prepared in the same manner as the samples and served as reagent blanks.

Table 3. r^2 Values for the PCB Congeners Calibration Standards over the 10 ng/mL to 400 ng/mL Range of this Study

Analytes	DB-35ms r^2	DB-XLB r^2
IUPAC 1	0.9994	0.9994
Tetrachloro-m-xylene (surrogate)	0.9913	0.9923
IUPAC 5	0.9993	0.9998
IUPAC 18	0.9998	0.9995
IUPAC 31	0.9980	0.9984
IUPAC 52	0.9986	0.9992
IUPAC 44	0.9988	0.9993
IUPAC 66	0.9990	0.9993
IUPAC 101	0.9992	0.9994
IUPAC 87	0.9984	0.9991
IUPAC 110	0.9939	0.9991
IUPAC 151	0.9998	0.9996
IUPAC 153	0.9981	0.9993
IUPAC 141	0.9993	0.9998
IUPAC 138	0.9984	0.9994
IUPAC 187	0.9989	0.9996
IUPAC 183	0.9993	0.9998
IUPAC 180	0.9994	0.9998
IUPAC 170	0.9993	0.9997
IUPAC206	0.9995	0.9996
Decachlorobiphenyl (surrogate)	0.9910	0.9895

Results and Discussion

The PCB and surrogate standards were resolved on the DB-35ms 30 m × 0.25 mm, 0.25 µm primary analysis column in less than 12 min. Figure 2 shows the separation of a 50 ng/mL PCB standard solution (100 ng/mL surrogate standard). Figure 3 shows a chromatogram of the same 50 ng/mL PCB standard (100 ng/mL surrogate standard) injection on the DB-XLB 30 m x 0.25 mm, 0.50 µm confirmatory analysis column.

The performance of the dual column set yielded acceptable linearity and recovery over the calibration range of this study. The method limit of quantitation (MLQ) of 10 ppb is substantially lower than the current regulatory guideline set by the FDA of 2,000 ppb for PCBs in food grade fish, and below the Agency for Toxic Substance and Disease Registry (ATSDR) maximum residue limit (MRL) of 0.02 mg/kg/day for these analytes [6]. The linearity of the column set as defined by the r^2 values of the PCB congeners standard curve ranged from 0.9939-0.9998. The individual PCB congener values are shown in Table 3. The lowest calibration standard on the column set also achieved excellent signal-to-noise ratios as shown in Figure 4.

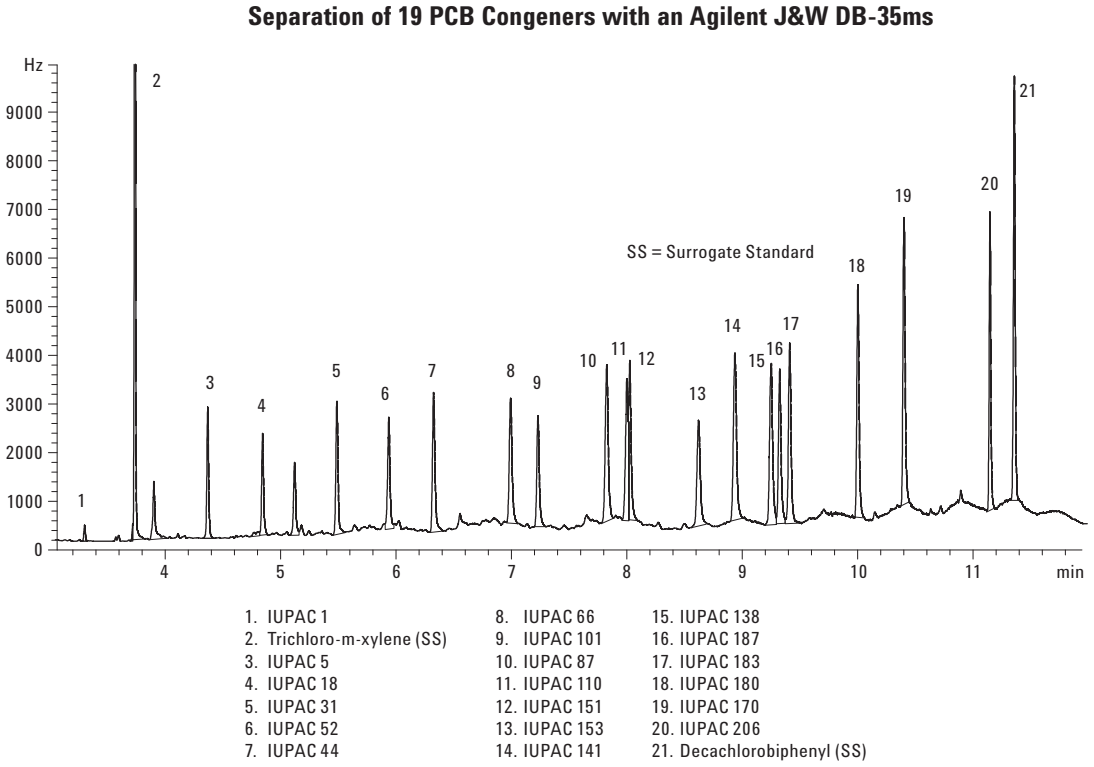


Figure 2. GC/µECD chromatogram of the 50 ng/mL PCB congeners standard analyzed on an Agilent J&W DB-35ms 30 m × 0.25 mm, 0.25 µm capillary GC column (Agilent p/n 122-3832). Chromatographic conditions are listed in Table 1.

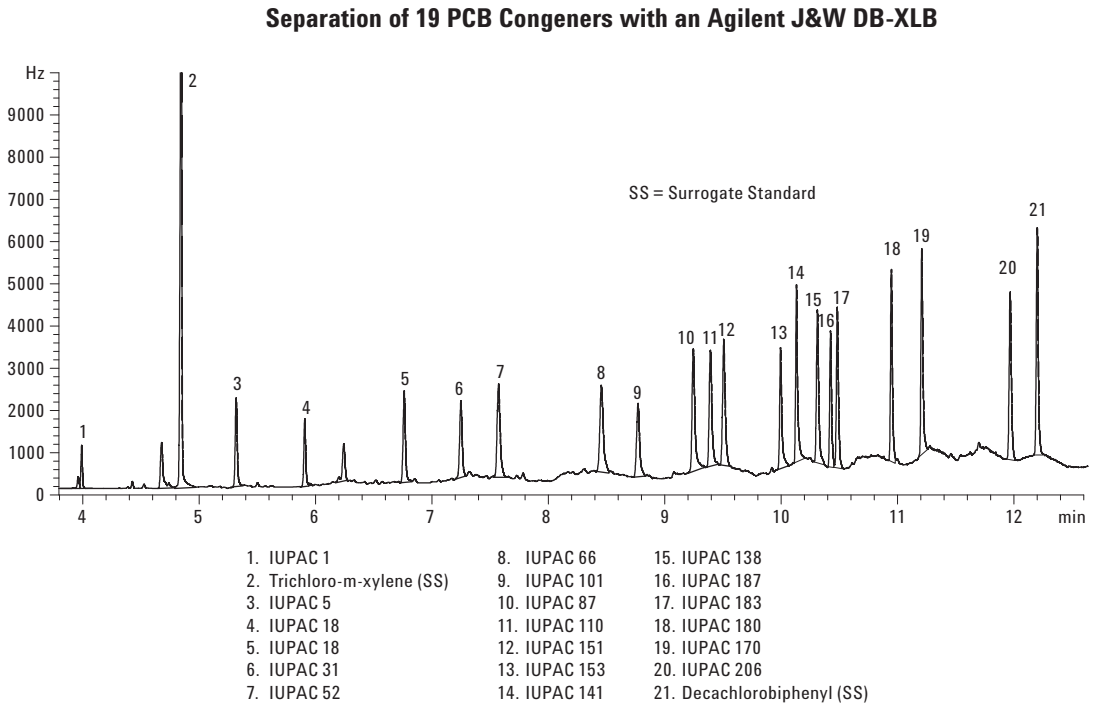


Figure 3. GC/µECD chromatogram of the 50 ng/mL PCB congeners standard analyzed on an Agilent J&W DB-XLB 30 m × 0.25 mm, 0.50 µm capillary GC column (Agilent p/n122-1236). Chromatographic conditions are listed in Table 1.

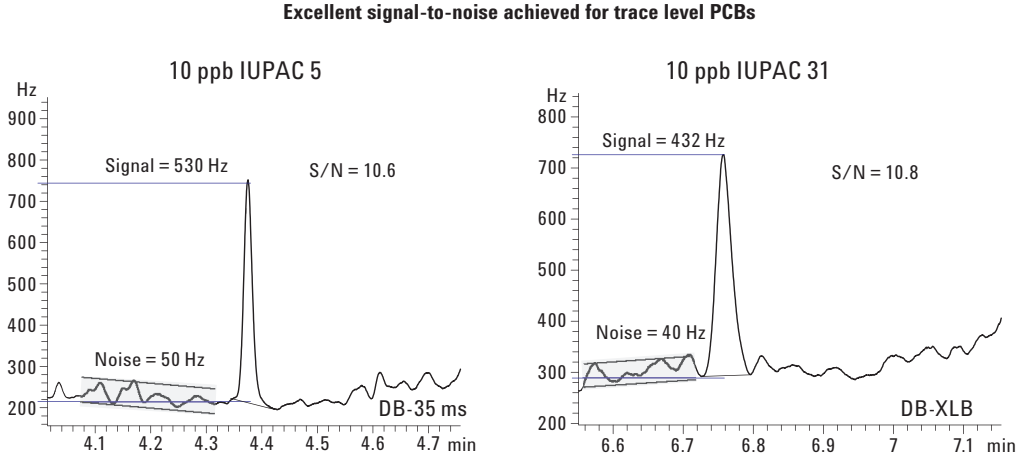
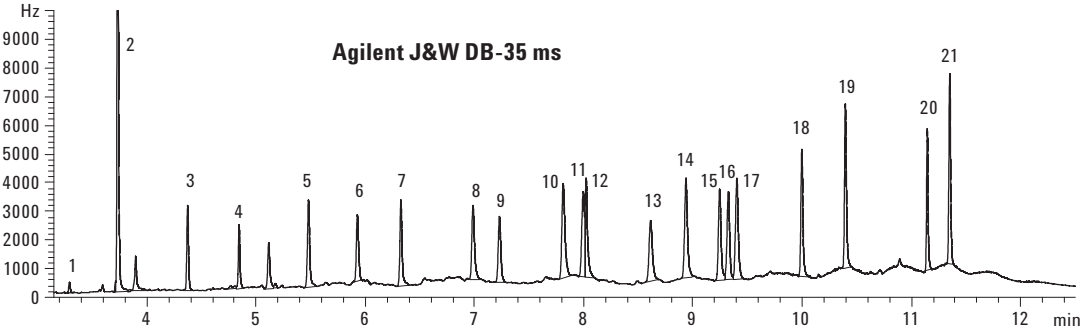


Figure 4 Enlarged view chromatogram of two individual congeners in the 10 ng/mL PCB calibration standard analyzed on the Agilent J&W DB-35ms and DB-XLB capillary columns. Chromatographic conditions are listed in Table 1.

The extraction process using the QuEChERS followed by dispersive SPE was effective in retaining the PCBs in the spiked fish sample and providing sufficient cleanup of the sample matrix for GC-µECD analysis. Figure 5 shows the separation of the extracted PCBs in a spiked fish sample on the primary and confirmation column set.

50 ppb PCBs in fish after Agilent's SampliQ QuEChERS extraction and dispersive SPE for fatty samples



1. IUPAC 1
2. Trichloro-m-xylene (SS)
3. IUPAC 5
4. IUPAC 18
5. IUPAC 31
6. IUPAC 52
7. IUPAC 44
8. IUPAC 66
9. IUPAC 101
10. IUPAC 87
11. IUPAC 110
12. IUPAC 151
13. IUPAC 153
14. IUPAC 141
15. IUPAC 138
16. IUPAC 187
17. IUPAC 183
18. IUPAC 180
19. IUPAC 170
20. IUPAC 206
21. Decachlorobiphenyl (SS)

Figure 5. GC/µECD chromatogram of the 50 ng/mL fortified fish extract analyzed on Agilent's J&W DB-35ms and DB-XLB GC columns. Chromatographic conditions are listed in Table 1.

The recoveries were determined at the 10, 50, and 200 ng/mL PCB levels. Recoveries for the individual PCBs on each column are shown in Tables 4 and 5. The mid and high level recovery ranges were excellent with the DB-35ms column (72 to 112%), and with the DB-XLB column (72 to 116%) for all PCBs investigated.

Lower recoveries were noted for three of the 19 congeners in the low level 10 ppb QC sample. The co-elution of IUPAC 110 and IUPAC 151 on the DB-35ms column contributed to the lower recovery seen for the IUPAC 110, but both congeners were resolved on the XLB column yielding recoveries over 72%.

Because the sensitivity of the ECD is relative to the amount of chloro substituents present, the response for the PCBs generally increases with increased chlorine content. Since IUPAC 1 is a monochlorinated biphenyl (2-chlorobiphenyl) it exhibits poor ECD sensitivity. This was found to contribute to the low recovery for IUPAC 1 at the 10 ng/mL level, however recovery and reproducibility were excellent at 50 ng/mL (average recovery 111%, average reproducibility 2.4%).

Table 4. Recovery and Repeatability of PCBs in Fortified Swai Fish with Agilent J&W DB-35ms column (Agilent p/n122-3832)

	10 ng/mL fortified QC		50 ng/mL fortified QC		200 ng/mL fortified QC	
Analytes	**%Recovery**	**RSD (n=6)**	**%Recovery**	**RSD (n=6)**	**%Recovery**	**RSD (n=6)**
IUPAC 1	43.2	4.0	111.9	2.4	99.0	1.6
Tetrachloro-m-xylene (surrogate)	107.5	1.2	110.4	1.4	103.9	1.8
IUPAC 5	68.5	1.3	107.0	1.8	99.6	2.2
IUPAC 18	99.3	2.9	108.5	1.9	95.1	2.5
IUPAC 31	56.7	3.3	110.5	2.0	101.8	2.3
IUPAC 52	75.7	3.4	91.8	2.1	100.5	1.9
IUPAC 44	61.4	1.7	107.8	2.2	100.6	2.1
IUPAC 66	66.9	2.0	97.2	2.8	96.2	2.4
IUPAC 101	65.8	2.5	99.5	2.8	94.7	2.2
IUPAC 87	75.7	2.8	99.1	1.5	101.4	2.4
IUPAC 110	29.7	2.6	100.0	2.6	102.8	2.8
IUPAC 151	100.1	2.1	99.8	2.6	94.5	1.9
IUPAC 153	49.9	1.9	89.9	1.6	91.5	2.9
IUPAC 141	67.7	2.1	93.1	1.5	92.2	2.3
IUPAC 138	52.2	2.9	95.9	2.4	93.4	2.2
IUPAC 187	57.0	3.4	92.1	2.3	89.5	2.2
IUPAC 183	62.1	3.2	87.3	2.2	85.7	2.2
IUPAC 180	63.1	3.2	88.6	1.1	84.0	2.6
IUPAC 170	65.4	4.2	91.0	1.6	86.7	2.3
IUPAC 206	58.8	3.2	77.7	1.5	72.5	2.4
Decachlorobiphenyl (surrogate)	75.0	2.9	81.1	2.4	75.9	2.7

Table 5. Recovery and Repeatability of PCBs in Fortified Swai Fish with Agilent J&W DB-XLB column (Agilent p/n 122-1236)

	10 ng/mL fortified QC		50 ng/mL fortified QC		200 ng/mL fortified QC	
Analytes	**%Recovery**	**RSD (n=6)**	**%Recovery**	**RSD (n=6)**	**%Recovery**	**RSD (n=6)**
IUPAC 1	56.5	3.2	116.2	2.2	99.4	2.0
Tetrachloro-m-xylene (surrogate)	108.0	1.6	110.9	1.3	105.1	1.9
IUPAC 5	87.4	2.5	112.1	1.5	100.7	1.9
IUPAC 18	69.9	1.6	112.3	2.1	100.8	2.5
IUPAC 31	63.1	3.5	108.3	1.8	103.2	2.3
IUPAC 52	59.6	3.5	104.7	2.6	100.5	1.8
IUPAC 44	75.7	2.9	105.0	1.9	101.7	2.0
IUPAC 66	83.1	2.9	101.5	2.7	98.2	2.0
IUPAC 101	73.0	2.4	98.4	1.9	96.6	2.0
IUPAC 87	60.7	2.7	109.2	1.8	100.7	1.6
IUPAC 110	72.9	2.6	103.0	2.3	100.1	1.6
IUPAC 151	75.1	3.6	93.9	2.9	93.5	2.6
IUPAC 153	36.0	6.9	104.5	3.0	91.1	1.9
IUPAC 141	74.4	2.6	98.0	1.6	91.9	2.3
IUPAC 138	65.4	3.3	98.9	2.5	93.1	2.1
IUPAC 187	68.4	2.2	94.6	1.0	88.7	2.0
IUPAC 183	72.6	3.7	92.2	2.0	86.1	2.4
IUPAC 180	76.0	3.2	92.2	2.0	84.5	2.7
IUPAC 170	74.9	8.7	94.6	2.2	87.5	2.3
IUPAC 206	60.1	3.2	78.3	2.4	72.6	2.7
Decachlorobiphenyl (surrogate)	74.6	2.4	81.4	1.6	76.7	2.3

Conclusions

This application note shows a robust, inexpensive, analytical method that sufficiently monitors PCBs in fish samples to address food safety concerns. This method demonstrates the feasibility of using a dual column µECD approach for routine fish screening as an alternative to GC/MS.

The Agilent SampliQ QuEChERS AOAC method for fatty samples followed by dSPE is effective at providing enough sample cleanup to avoid matrix interferences, while maintaining low level analyte detection.

The dual column set of an Agilent J&W DB-35ms primary analytical column and an Agilent J&W DB-XLB confirmatory column on one instrument allows simultaneous identification and confirmation of the presence of the PCBs. The DB-35ms primary analysis column and the DB-XLB confirmatory column with dual µECD detection were effective at analyzing 19 PCBs in a fish matrix following sample matrix cleanup. The single injection, dual column approach improves productivity by saving instrument and analyst time. Continuous improvements and stringent process control with respect to column activity make the DB-35ms and DB-XLB column pair an excellent choice for analysis of analytes such as PCBs.

The performance of the dual column set DB-35ms and DB-XLB with GC µECD had excellent linearity over the range of concentrations studied with r^2 values between 0.9939 and 0.9998 for the PCB compounds. Recovery and reproducibility was shown to be greater than 77% with an RSD below 3.0 at 50 ng/mL. The method limit of quantitation for the PCB congeners using this approach was significantly lower than currently regulated MRLs in fish. The results achieved with this method shows determination of PCBs by µECD as a reliable alternative to GC/MS.

Acknowledgements

The authors wish to thank Joan Stevens for her help and suggestions with the Agilent SampliQ QuEChERS AOAC extraction procedure.

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Printed in the USA
August 12, 2010
5990-6236EN

Agilent Technologies

A Low Femtogram Target Screen Method for Perfluorinated Compounds in Food Matrices and Potable Water Using the Agilent 6460 Triple Quadrupole LC/MS System Equipped with Agilent Jet Stream Technology

Application Note

Environmental, Food Safety

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Abstract

In this application note, we outline a viable method for reliably detecting low-femtogram levels of perfluorinated carboxylates (PFCA) and sulphonates (PFSA) without background component interference, with an inline contaminant trap LC modification. Good chromatographic resolution of all perfluorinated compounds was observed. A representative suite of PFCAs and PFSAs were analyzed herein and were all detected at on-column levels lower than 75 fg in drinking water matrices (S/N >3.) The most sensitive analytes PFHxS, PFDS and PFBS were detected at 2.6, 3.2 and 5 fg levels, respectively. Method detection limits for spiked pork liver matrix extract samples were below 600 fg on-column for the entire analyte suite. No detectable background contamination was observed in blank injections for any analyte in this study. Linearity for up to five orders of magnitude with R^2 values above 0.996 for the entire suite were recorded.

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Introduction

Exposure, bioaccumulation and potential toxicity continue to be issues in environmental biota and their food webs from emerging contaminants such as perfluoronated byproducts (PFC) from industrial processing. Since background levels of such analytes are significant and stable in the atmosphere already, it is difficult to obtain reliable and accurate low-level on-column measurements.

In this application note, we present a case in which a suite of perfluorinated carboxylates (PFCA) and sulfonates (PFSA) were screened at low fg on-column levels in a potable water matrix and in spiked (pork) liver samples with zero background interference using dynamic multiple reaction monitoring (dynamic MRM) [1]. This approach allowed us to gain reliable positive identifications and extremely low limits of detection. By utilizing an inline contaminant trap configuration we assured cleanliness of the HPLC system and allowed use of inline membrane degassing without compromising system dead-volume or analysis speed.

A comprehensive evaluation of a suite of PFCAs & PFSAs including isotopically labeled ISTDs was undertaken which examined sensitivity and linearity of each component. Appropriate dynamic MRM transitions were identified using automatic instrument optimizations of fragmentor (frag) and collision energy (CE) voltages and applied to the chromatographic method dynamically to maximize analyte signal quality at lower concentrations. Optimal settings for the Agilent Jet Stream Technology [2] were determined for the complete PFCA & PFSA suite effectively increasing the sensitivity to around 14x that of normal electrospray ionization (ESI) conditions.

Table 1. Compounds Analyzed for this Study

Target compounds	
perfluoro-1-butanesulfonate	(PFBS)
perfluoro-n-hexanoic acid	(PFHxA)
perfluoro-n-heptanoic acid	(PFHpA)
perfluoro-1-hexanesulfonate	(PFHxS)
perfluoro-n-octanoic acid	(PFOA)
perfluoro-n-nonanoic acid	(PFNA)
perfluoro-1-octanesulfonate	(PFOS)
perfluoro-n-undecanoic acid	(PFUA)
perfluoro-1-decanesulfonate	(PFDS)
perfluoro-n-dodecanoic acid	(PFDoA)
perfluoro-n-tridecanoic acid	(PFTriA)
perfluoro-n-tetradecanoic acid	(PFTA)

Experimental

This analysis was performed using an Agilent 6460A triple quadrupole LC/MS with an Agilent 1200SL Series LC system. The LC system consisted of a binary pump (G1312B), vacuum degasser (G1379B), a low carryover automatic liquid sampler (G1367D), thermostatted column compartment (G1316B) and MassHunter data system.

Sample Handling

Sample handling is a critical element in the measurement of trace amounts of perfluorinated carboxylates and sulphonates, since background levels can be prevalent and derived from laboratory consumables and protective lab-wear. The series of analyses outlined in this application note considered this and precautions were taken to eliminate any such cross-contamination. Silanized glass vials were used with aqueous diluents that had been passed through a solid-phase extraction. Nitrile rubber vial caps and non PTFE-containing pipette tips were used. Only nitrile rubber derived protective laboratory gloves were worn.

Instrumentation

Rapid Resolution HPLC Conditions and Configuration

Configuration:

Agilent 1200 Series Binary Pump SL:	(G1312B)
High Performance WP Sampler SL Plus:	(G1367D)
Sampler Thermostat:	(G1330B)
Thermostatted Column Compartment SL	(G1316B)

Method Conditions:

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 50 mm, 1.8 µm	
Column temperature:	55 °C	
Injection volume:	1 µL	
Autosampler temp:	4 °C	
Needle wash:	Flushport (100% methanol), 5 seconds	
Mobile phase:	A = 2 mM NH_4 acetate in water	
	B = 2 mM NH_4 acetate in methanol	
Gradient flow rate:	0.5 mL/min	
Gradient:	Time (min)	%B
	0	6
	0.5	6
	6	95
	8	95
Total run time:	9.0 min (including 1 min equilibration time)	

Mass Spectrometer Dynamic MRM Conditions and Configuration

Configuration:

Agilent 6460 Triple Quadrupole Mass Spectrometer equipped with Agilent Jet Stream Technology

Ion Source Conditions:

Ion mode:	ESI/Agilent Jet Stream, Negative
Capillary voltage:	3750 V
Nozzle voltage:	0 V
Drying gas (nitrogen):	4 L/min
Drying gas temperature:	320 °C
Nebulizer gas (nitrogen):	60 psi
Sheath gas temperature:	350 °C
Sheath gas flow:	12 L/min

Dynamic MRM acquisition:

Cycle time:	250 ms
Total dynamic MRMs:	29
Maximum concurrent MRMs:	12
Retention time window:	30 sec
Minimum/maximum dwell:	17.33/246.50 ms
Q1 and Q2 resolution:	0.7 amu [unit]
Delta EMV:	0 V

Dynamic MRM triple quad MS parameters are listed in Table 2. All fragmentor voltage (frag) settings, respective collision energies (CE), and most abundant MS/MS product ions per analyte were determined automatically using the Agilent MassHunter Optimizer software.

Ion Source Optimization

In order to achieve the optimal and most sensitive Agilent 6460 ESI MS source conditions for the complete suite of analytes, each dynamic MRM method transition was measured using a single mixed standard repetitively. In addition, each subsequent sample injection was also measured using a systematic and single source parameter change. This was to obtain the best and most sensitive method conditions for an optimized method, but only had to be undertaken once.

In reality, a single set of source parameter conditions are not necessarily the optimum settings for all analytes in a suite (or assay) so a compromise set of conditions were determined for the suite of perfluorinated analytes. A subsequent

Table 2. Dynamic MRM PFSA/PFCA Settings

Compound name	Precursor ion mass	Q1-resolution	Product ion mass	Q2-resolution	Fragmentor voltage	Collision energy (eV)	Retention time (min)	RT delta (min)	Ion polarity
PFBS	298.9	unit	80	unit	133	45	3.623	1	Negative
PFBS (Q)	298.9	unit	98.9	unit	133	29	3.623	1	Negative
PFDA	512.9	unit	469	unit	102	5	5.543	1	Negative
PFDA (C13)2	514.9	unit	469.9	unit	102	5	5.542	1	Negative
PFDoA	612.9	unit	569	unit	97	5	5.961	1	Negative
PFDoA (C13)2	614.9	unit	570	unit	97	5	5.961	1	Negative
PFDoA (Q)	612.9	unit	169	unit	97	25	5.961	1	Negative
PFDS	598.9	unit	80	unit	205	94	5.752	1	Negative
PFHpA	362.9	unit	319	unit	66	5	4.626	1	Negative
PFHpA (Q)	362.9	unit	169	unit	66	13	4.626	1	Negative
PFHxA	312.9	unit	268.9	unit	66	5	4.143	1	Negative
PFHxA (C13)2	314.9	unit	269.9	unit	66	5	4.141	1	Negative
PFHxS	398.9	unit	80	unit	174	49	4.671	1	Negative
PFHxS (O18)2	402.9	unit	83.9	unit	174	49	4.671	1	Negative
PFHxS (Q)	398.9	unit	99	unit	174	45	4.671	1	Negative
PFNA	462.9	unit	418.9	unit	66	5	5.296	1	Negative
PFNA (C13)5	467.9	unit	423	unit	66	5	5.296	1	Negative
PFNA (Q)	462.9	unit	169	unit	66	17	5.296	1	Negative
PFOA	412.9	unit	368.9	unit	86	5	5.003	1	Negative
PFOA (C13)4	416.9	unit	371.9	unit	86	5	5.001	1	Negative
PFOA (Q)	412.9	unit	169	unit	86	13	5.003	1	Negative
PFOS	498.9	unit	80	unit	210	50	5.302	1	Negative
PFOS (C13)4	502.9	unit	80	unit	210	50	5.301	1	Negative
PFOS (Q)	498.9	unit	99	unit	210	50	5.302	1	Negative
PFTA	712.9	unit	669	unit	112	9	6.255	1	Negative
PFTriA	662.9	unit	619	unit	102	9	6.117	1	Negative
PFUA (C13)2	564.9	unit	519.9	unit	92	5	5.764	1	Negative
PFUA	562.9	unit	519	unit	92	5	5.762	1	Negative
PFUA (Q)	562.9	unit	169	unit	92	21	5.762	1	Negative

technical note that details the complete source optimization of this suite of compounds will soon be published.

Results and Discussion

Inline Contaminant Trapping

For highly sensitive measurements of PFCAs and PFSAs at low femtogram on-column levels, it was necessary to ensure the removal of background PFC contamination derived from sample work-up, mobile phase impurities or instrument components. PFCAs and PFSAs are typically hard to break down naturally. Their precursors are widely released into the atmosphere which are degraded to terminal PFCAs and PFSAs.

One approach is to stop PFCAs and PFSAs from entering the high-pressure HPLC flow system by effectively trapping them using a small inline reverse phase column or cartridge immediately after the respective pump head, prior to the point at which the gradient mix is achieved. Figure 1 schematically shows this configuration with a low dead-volume binary pump setup.

The positioning of the inline contaminant trap [Agilent ZORBAX Eclipse Plus C18 (4.6 mm × 30 mm, 3.5 µm, p/n-959936-902)] was prior to the mixing point of the gradient pump and on the aqueous pump channel (in this case Pump A.) It was exposed to a 100% isocratic aqueous mobile phase and effectively trapped all PFCA and PFSA contaminants from entering the HPLC flow path. Further, since the inline trap was before the gradient mix point, it had zero dead-volume implications to the HPLC separations. *It must be noted that this is a nonstandard configuration and as such may not be supported by Agilent Technologies.*

Moreover, extreme care regarding sample handling techniques was observed so that PFCAs and PFSAs were not introduced artificially during the preparation process. For example, careful choice of silanized glass vials with rubber septa were a necessity as were the use of non PTFE-containing pipette tips and protective clothing (nitrile rubber gloves). Sample diluent was also isocratically pumped through a C18 flash column to remove background PFCAs and PFSAs prior to use.

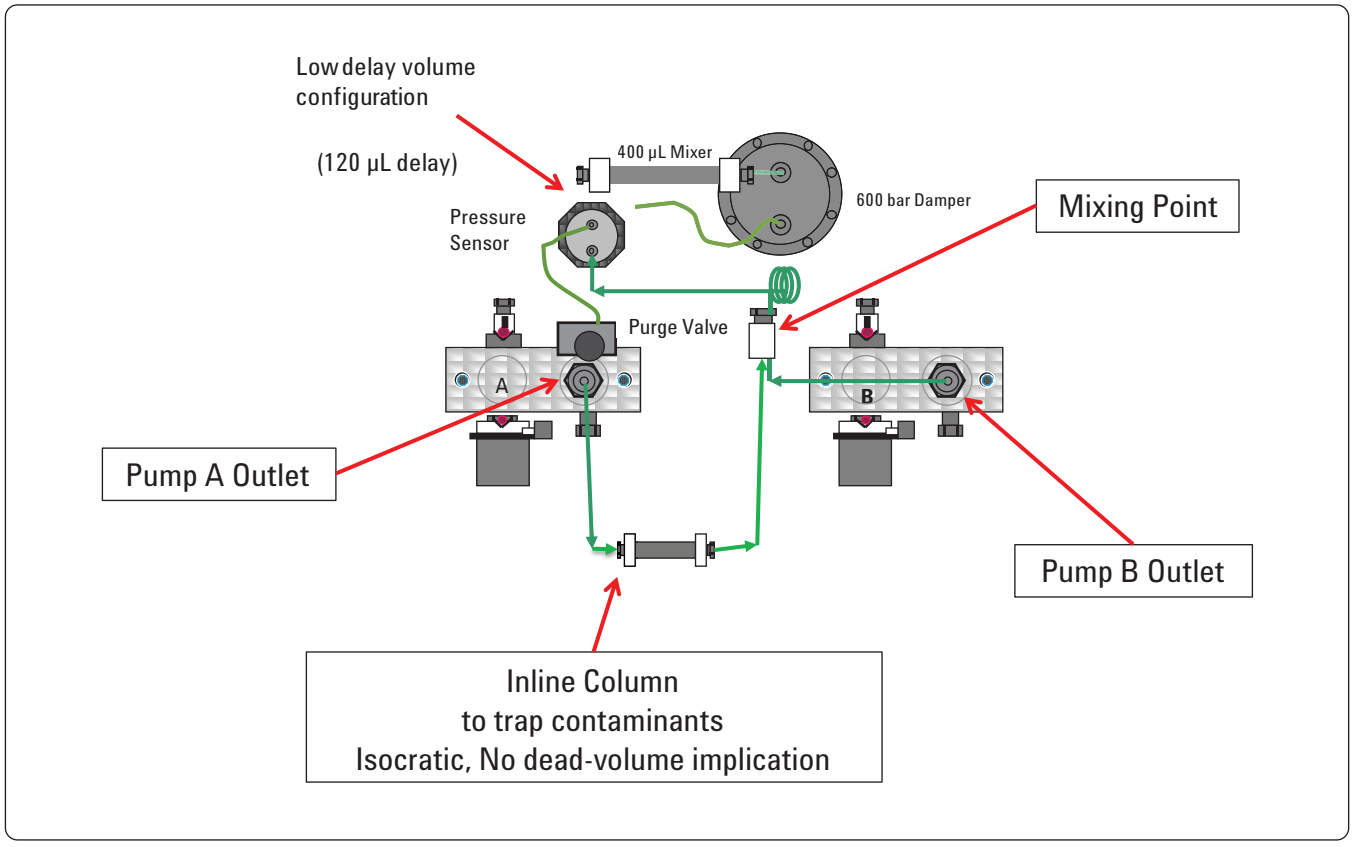

Figure 1. Inline contaminant trapping schematic.

A typical low-level injection (10 fg on-column) featuring a PFHxS (transition 398.9→80 *m/z*) is illustrated in Figure 2 in triplicate, together with a blank injection prior to these analyses. The blank sample baseline was completely clear of residual PFHxS, as a result of the cleanliness of the HPLC system from the inline contaminant trap. This was also true for all other analytes in this study; due to space restrictions, only PFHxS is shown here. The complete set of data will be published in a future application note.

Figure 2 also indicates the outstanding high level of sensitivity of the Agilent 6460 triple quad MS for the negative polarity analysis of such perfluorinated analytes spiked into potable water matrix. In this study, LODs for PFHxS were the lowest for the suite, giving an average of 2.6 fg on-column over triplicate injections and defined as having a signal-to-noise (S/N) ratio of greater than 3. All other analytes in the PFC suite exhibited LODs of less than 75 fg on-column. Figure 3 illustrates an overlaid chromatogram for all PFC analytes at a level of 100 fg on-column.

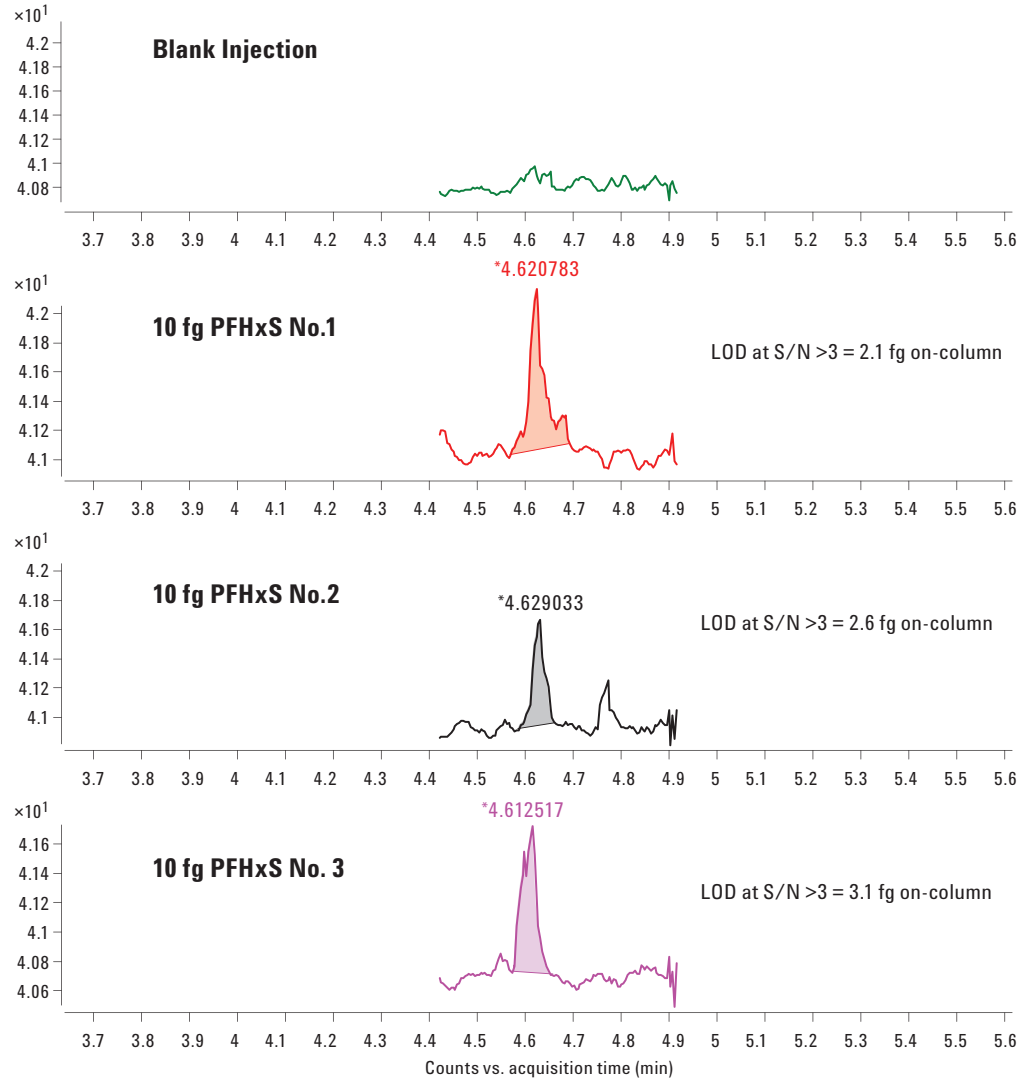

Figure 2. Blank injection with 3x replicates of PFHxS Standard, 10 fg on-column, spiked potable water.

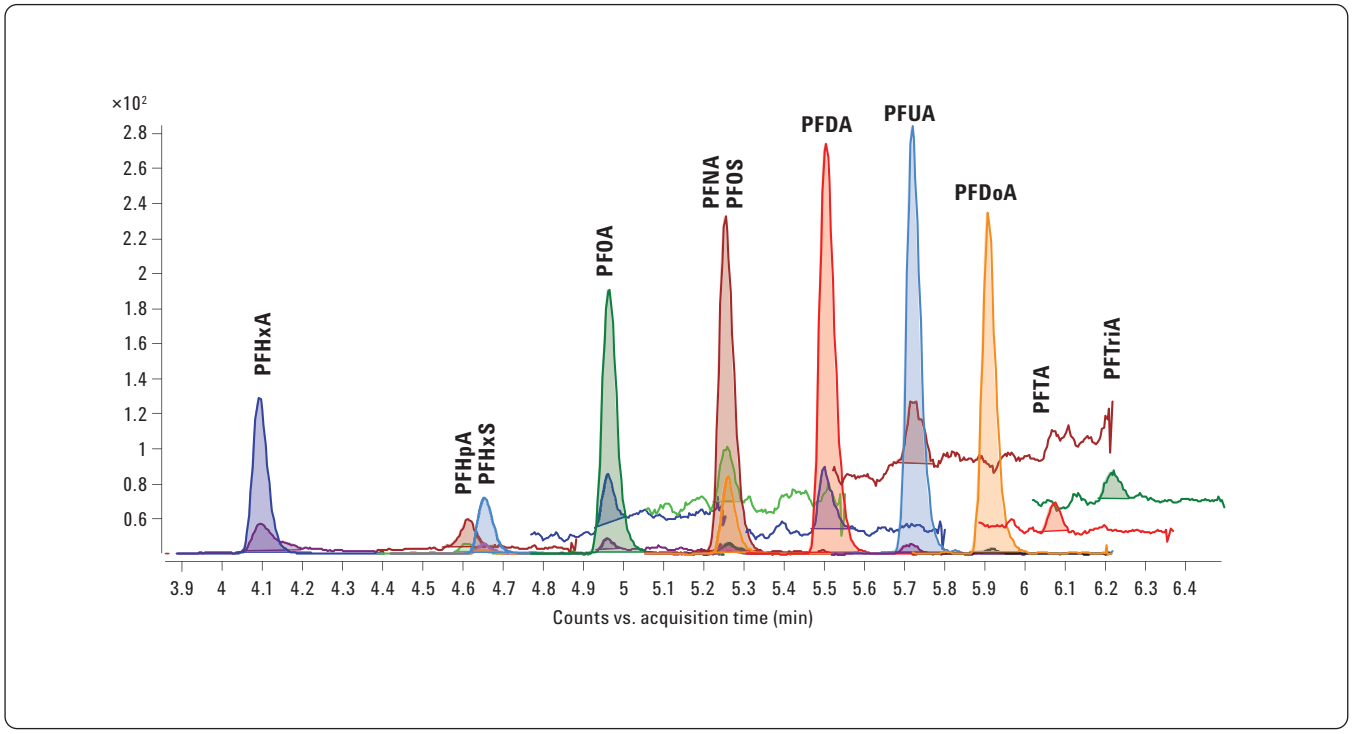

Figure 3. PFCA/PFSA Suite dynamic MRM chromatogram (overlaid) at 100 fg with quantifier and qualifier ions (spiked potable water).

Limits Of Detection (Potable Water Spiked Samples)

Table 3 outlines the limit of detection (LOD) values observed for the suite of PFCs undertaken in this study and spiked into untreated potable water matrix. All PFCA/PFSA LODs in this evaluation were below a value of 75 fg on-column. They were achieved with no background carryover at extremely high sensitivity by careful optimization of fragmentation and collision energy parameters and careful fine-tuning of Agilent Jet Stream and ion source parameters.

A typical ISTD-corrected calibration curve for one of the analytes in the suite (PFOS) is outlined in Figure 4. The linearity R^2 value was found to be 0.99957820 for triplicate injections for more than five orders of magnitude.

Table 3. LOD Results for Spiked Potable Water Samples

Compounds	LOD (fg on column, S/N >3)
PFBS	5
PFHxA	8.4
PFHpA	12.2
PFHxS	2.6
PFOA	43.7
PFNA	75
PFOS	5.7
PFDA	36.3
PFUA	44
PFDS	3.2
PFDoA	55.9
PFTriA	74.2
PFTA	21.7

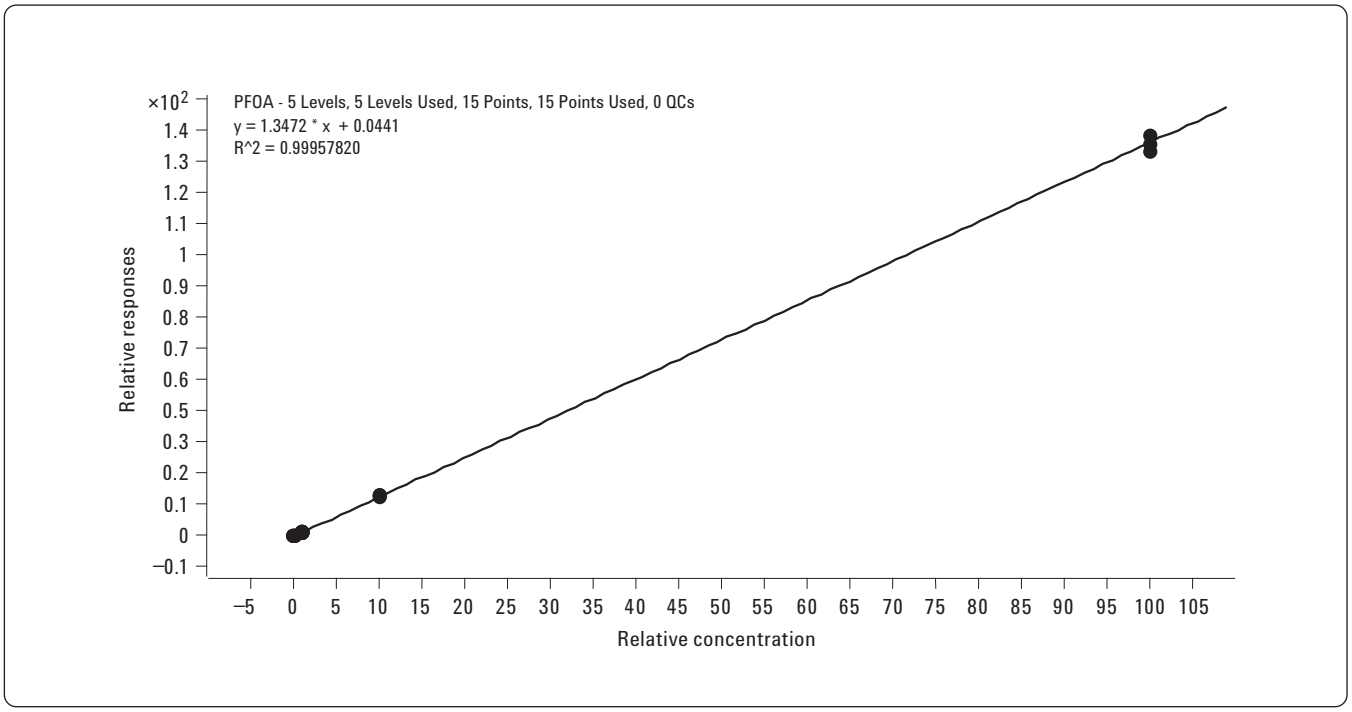

Figure 4. PFOA linearity over five orders of magnitude in potable water (10 fg – 100 pg on-column. [N = 3]).

Method Detection Limits (Spiked Pork Liver Samples)

Table 4 summarizes the method detection limits (MDL) which were observed for each individual PFCA or PFSA analyte when applied to spiked liver extracts.

More than half of the compounds showed a precision value significantly less than 10% RSD (based on peak area) at this challenging MDL concentration.

MDL values in spiked pork liver extracts ranged between 600 fg and 45 fg on-column for this reported methodology.

Table 4. PFCA/PFSA Method Detection Limits

Compounds (spiked pork liver extract)	Method detection limit (fg on column, S/N >10)
PFBS	97.7
PFHxA	110.5
PFHpA	249
PFHxS	44.62
PFOA	291.5
PFNA	421.3
PFOS	58.3
PFDA	275.3
PFUA	303.9
PFDS	54.9
PFDoA	594.5
PFTriA	494.5
PFTA	503.2

Conclusions

A highly sensitive low-femtogram dynamic MRM Agilent 6460 triple quad LC/MS method has been presented for the analysis of a suite of PFCAs and PFSAs analytes that illustrates excellent precision at low-femtogram on-column levels in a complex food matrix and potable water.

Background PFCA and PFCS interferences normally associated with low-level analyses of such perfluorinated suites were eliminated by careful preparation of samples, sample handling and an inline flow contaminant trapping cartridge set-up within the HPLC flow path.

Complete ion source optimization was undertaken for each of the analytes in the suite. This effectively increased the analytical sensitivity by at least a factor of 14x compared with standard ESI source settings.

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1. "New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Analyses," Agilent Technologies publication 5990-3595EN.
2. "Agilent Jet Stream Thermal Gradient Focussing Technology," Agilent Technologies publication 5990-3494EN.

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Printed in the USA
February 22, 2010
5990-5313EN

Agilent Technologies

Analysis of Polycyclic Aromatic Hydrocarbons in Fish with Agilent SampliQ QuEChERS AOAC Kit and HPLC-FLD

Application Note

Food

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Abstract

An HPLC-Florescence Detection (FLD) method was developed and validated for the determination of sixteen polycyclic aromatic hydrocarbons (PAHs) in fish fillets. The analyzed compounds included naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fln), pyrene (Pyr), 1,2-benza[a]anthracene (BaA), chrysene (Chr), benzo[e]pyrene (BeP), benzo[e]acenaphthylene (BeA), benzo[k]fluoranthene (BkF), dibenzo[a,h]anthracene (DahA), benzo[g,h,i]perylene (Bghi)P and indeno[1,2,3-cd]pyrene (InP). The method employs a quick, easy, cheap, effective, rugged and safe (QuEChERS) multiresidue sample preparation procedure adopted from the Association of Analytical Communities (AOAC) Official method 2007.01 for extraction and cleanup. The analytes were separated on an Agilent ZORBAX Eclipse PAH HPLC column (4.6 mm × 50 mm, 1.8 µm) by gradient elution with a binary system of acetonitrile - water and subsequent fluorescence detection set at appropriate excitation and emission wavelengths. The analyte recoveries ranged from 83.4% to 101% with relative standard deviations ranging from 0.6 to 1.9% at three different fortification levels. The limits of detection and quantification ranged from 0.04 to 0.84 and 0.1 to 2.80 ng/g, respectively.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds included in the European Union and US Environmental Protection Agency (US EPA) priority pollutant list because of their mutagenic and carcinogenic properties [1]. Excluding smokers and occupationally vulnerable populations, most individuals are exposed to PAHs predominantly from dietary sources [2]. In the marine environment, PAHs are bioavailable to marine species via the food chain, as waterborne compounds, and contaminated sediments. As lipophilic compounds they can easily cross lipid membranes and have the potential to bioaccumulate in aquatic organisms. Although for most people, fish and seafood represents only a small part of the total diet, the contribution of this food group to the daily intake of PAHs in some individuals may be comparatively important [3].

The AOAC QuEChERS method has been widely applied in the analysis of pesticides in food since it was introduced by USDA scientists [4-5]. In general, there are two major steps: extraction and dispersive SPE cleanup. The method uses a single step buffered acetonitrile extraction while simultaneously salting out water from the aqueous sample using anhydrous magnesium sulfate ($MgSO_4$) to induce liquid-liquid partitioning. After removing an aliquot from an organic layer, for further cleanup, a dispersive solid phase extraction (dSPE) step is conducted using a combination of primary secondary amine (PSA) sorbent to remove organic acids from other components and anhydrous $MgSO_4$ to reduce the remaining water in the extract. Other sorbents, such as graphitized carbon black (GCB), may be added to remove pigments and sterol, or C18 to remove lipids and waxes.

This application note presents a method for the analysis of PAHs at trace levels in fish tissue with HPLC-FLD. The HPLC methods are useful for PAH analysis since UV and fluorescence detection offer enhanced selectivity over other techniques such as GC with flame ionization detection [6]. The method includes sample preparation with SampliQ AOAC Buffered Extraction kit (p/n 5982-5755) and SampliQ AOAC Fatty Dispersive SPE 15 mL kit (p/n 5982-5158). Chemical structures of the PAHs in this study are shown in Figure 1.

Experimental

Reagents and Chemicals

All reagents were analytical or HPLC grade. Acetonitrile (CH_3CN) and PAHs were purchased from Sigma-Aldrich (St. Louis, MO, USA). The water used was from a MilliQ system (Milford, Mass, USA). The mobile phase was filtered through a Whatman membrane filter (47 mm diameter and 2 µm pore size).

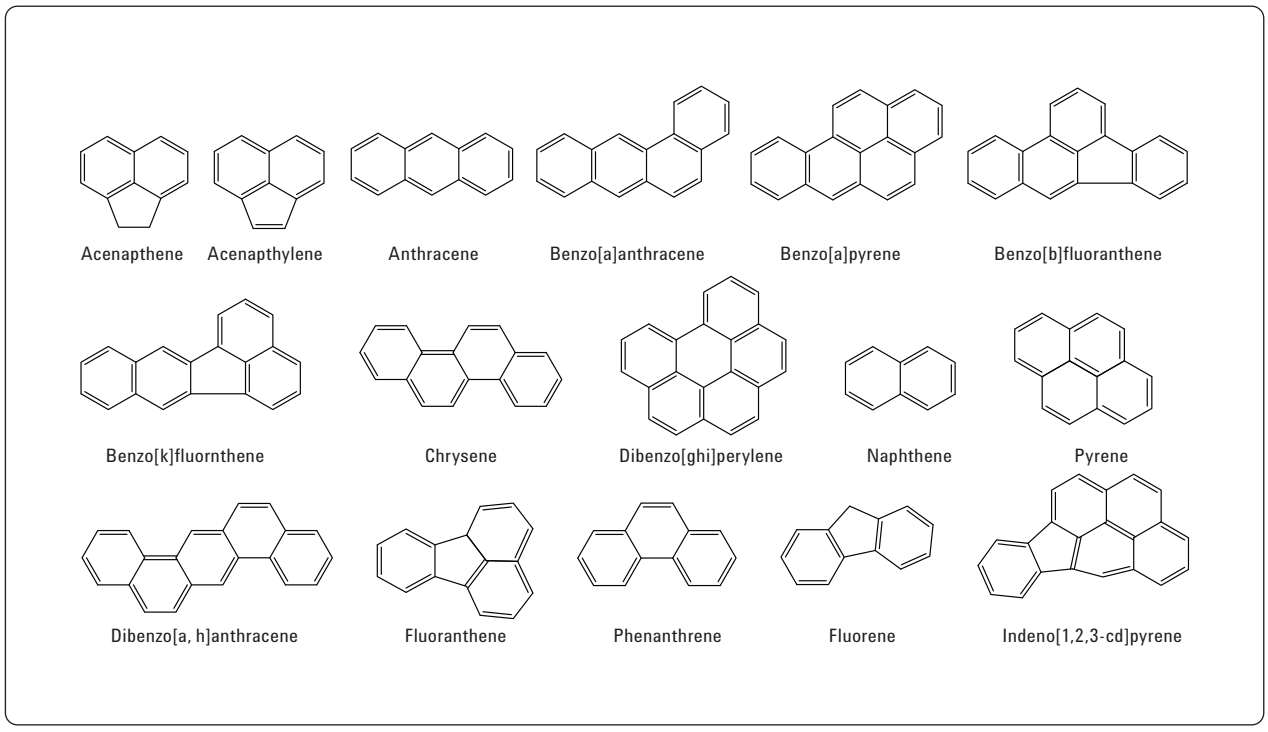

Figure 1. Chemical structures for the polycyclic aromatic hydrocarbons used in the study.

Standard Solutions

Standard stock solutions (1 mg/mL) were prepared by dissolving 10 mg of the desired PAH in 10 ml CH_3CN and stored at –20 °C. All working solutions were prepared fresh daily by serial dilution with CH_3CN.

Equipment and Material

The analysis was performed on an Agilent 1200 Series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump and a fluorescence detector (FLD) set at varying excitation and emission wavelengths (Table 1). The selection of the excitation and emission wavelengths for detection was based on the optimum responses for the various PAHs. Separation of the compounds was achieved on an Agilent ZORBAX Eclipse PAH column (4.6 mm × 50 mm, 1.8 µm), p/n 959941-918. The data was processed by HPLC 2D Chemstation software.

Extraction and cleanup were achieved with Agilent SampliQ Buffered QuEChERS AOAC Extraction kit, p/n 5982-5755 and SampliQ QuEChERS AOAC Dispersive SPE kit, p/n 5982-5158, (Agilent Technologies).

A Kenwood Grinder (obtained from a local appliance store) was employed for homogenizing the fish sample.

Instrument conditions

HPLC conditions

Table 1. HPLC Conditions used for Separation of PAHs

Column	Agilent ZORBAX Eclipse PAH C18 4.6 × 50 mm, 1.8 µm	
Flow rate	0.8 mL/min	
Column temperature	18 °C	
Injection volume	5 µL	
Mobile phase	A = Deionized H_2O	B = CH_3CN
Gradient	T (min)	% B
	0	60
	1.5	60
	7	90
	13	100
Detection	UV at 230 nm (Acy) and varying fluorescence excitation (Ex) and emission (Em) wavelengths	
Wavelengths:		
Time (min)	**Ex/Em wavelengths (nm)**	**PAH detected**
0 – 5 (dark blue)	260/352	Nap, Ace, Flu, Phe, Chr
0 – 14 (red)	260/420	Ant, Pyr, BeP, DahA, BghiP
0 – 14 (light blue)	260/460	Fln, 1,2-BaA,BeA, BkF, InP

Sample preparation

The fish fillets were purchased from a local food store, minced, and deep frozen until analysis.

Extraction

A 5.0 g sample of fish homogenate was placed into a 50 mL centrifuge tube from the SampliQ QuEChERS AOAC Extraction kit and the tube was centrifuged for 20 s. Samples were then spiked with appropriate spiking solutions to yield appropriate working solutions for recoveries and reproducibility studies. A 2000 µL volume of spiking solution was added to all samples except the blank, and the tubes were shaken vigorously for 1 min. Next, 8 mL of CH_3CN, then an Agilent SampliQ QuEChERS AOAC extraction salt packet (p/n 5082-5755) containing 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous NaOAc were added to the tubes. The sample tubes were hand shaken vigorously for 1 min, then further centrifuged at 4000 rpm for 5 min.

Dispersive SPE Cleanup

A 6.0 mL aliquot of the upper CH_3CN layer was transferred into a SampliQ QuEChERS AOAC Dispersive SPE 15 mL tube. This SPE tube contained 400 mg of PSA, 400 mg of C18EC, and 1200 mg of anhydrous $MgSO_4$. After one minute of shaking, the tubes were centrifuged at 4000 rpm for 5 min. A 4 mL aliquot of the extract was filtered through a 0.45 µm PVDF syringe filter, then 1000 µL of the extract was placed in an autosampler vial for HPLC-FLD analysis.

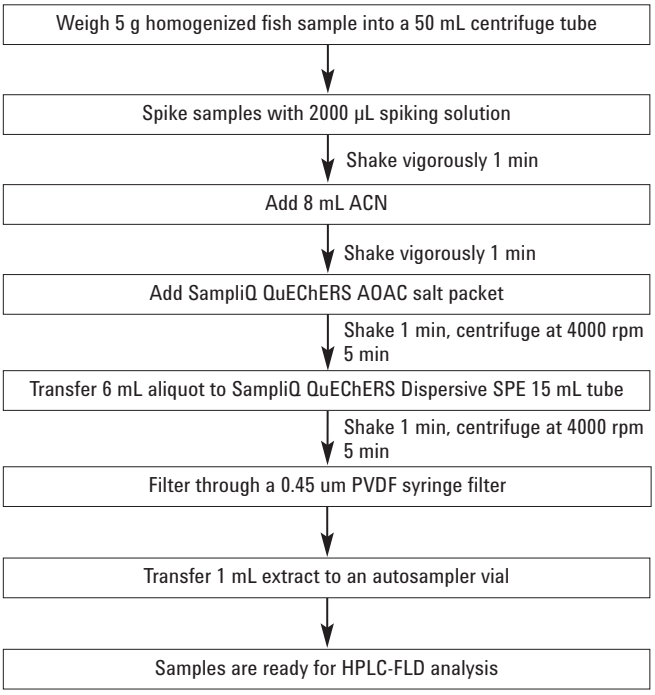

Figure 2. Flow chart of QuEChERS AOAC sample preparation procedure.

Results and Discussion

Chromatographic results

Figure 3 shows an overlay of color-coded chromatograms at various fluorescence conditions (Table 1) of the standard mixture of the 16 PAHs. A chromatogram of the blank fish extract is presented in Figure 4. Overlay chromatograms of the spiked fish sample at spiking level 1 are shown in Figure 5.

QuEChERS extraction

The use of CH_3CN as an extracting solvent in a salting-out condition, without the need to add co-solvents, attained high extraction yields as shown by the recoveries in Table 4. The CH_3CN solvent is compatible with the HPLC – FLD procedure in this application note. Therefore no evaporation or reconstitution solvent was required. This is particularly important for the PAHs since some of these compounds (naphthalene, acenaphthene and fluorene) are extremely volatile and may be lost during an evaporation step [1].

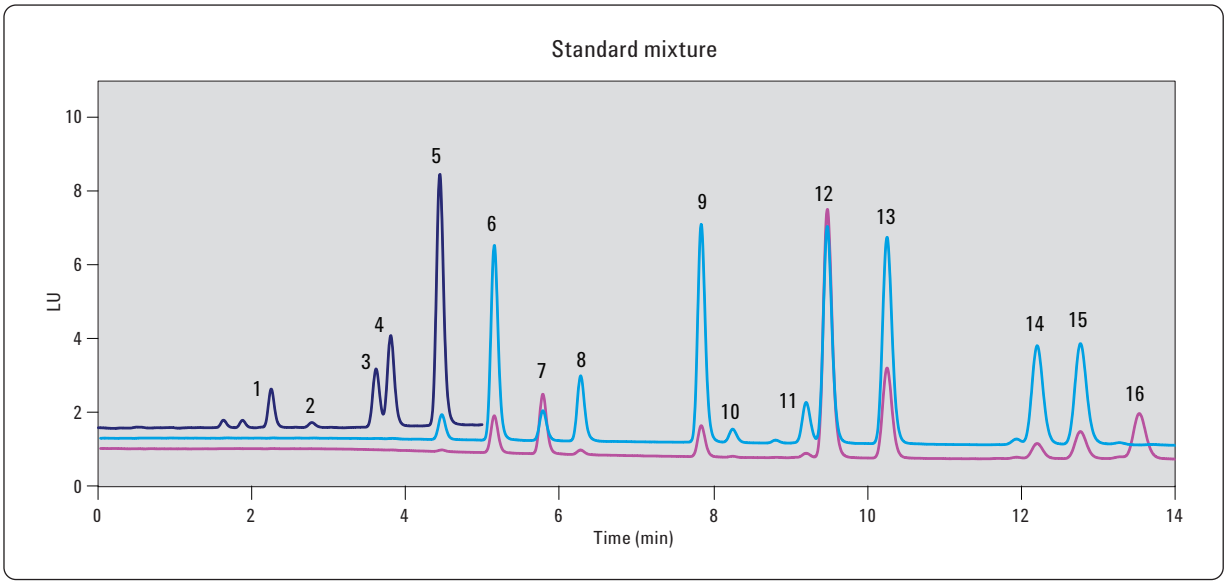

Figure 3. Overlay HPLC – FLD chromatograms of the standard mixture containing: 1. Nap 2. Acy 3. Ace 4. Flu 5. Phe 6. Ant 7. Fln 8. Pyr 9. BaA 10. Chr 11. BeP 12. BeA 13. BkF 14. DahA 15. BghiP 16. InP . The concentration of the PAHs was 1 mg/mL. The blue portion of the chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm; the red portion 260-nm/420-nm; the light blue-portion: 260-nm/440-nm. For acenaphthylene, UV detection at 230-nm was used. Chromatographic conditions are shown in Table 1.

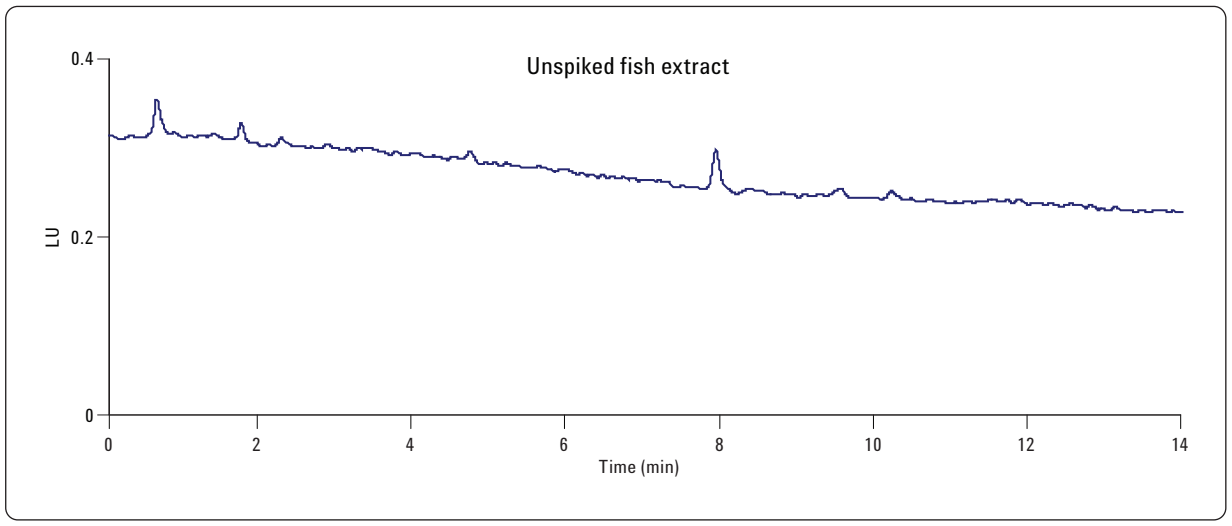

Figure 4. Chromatogram of the blank fish extract. Chromatographic conditions are shown in Table 1. The baseline chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm. The other excitation/emission conditions showed no other interferences.

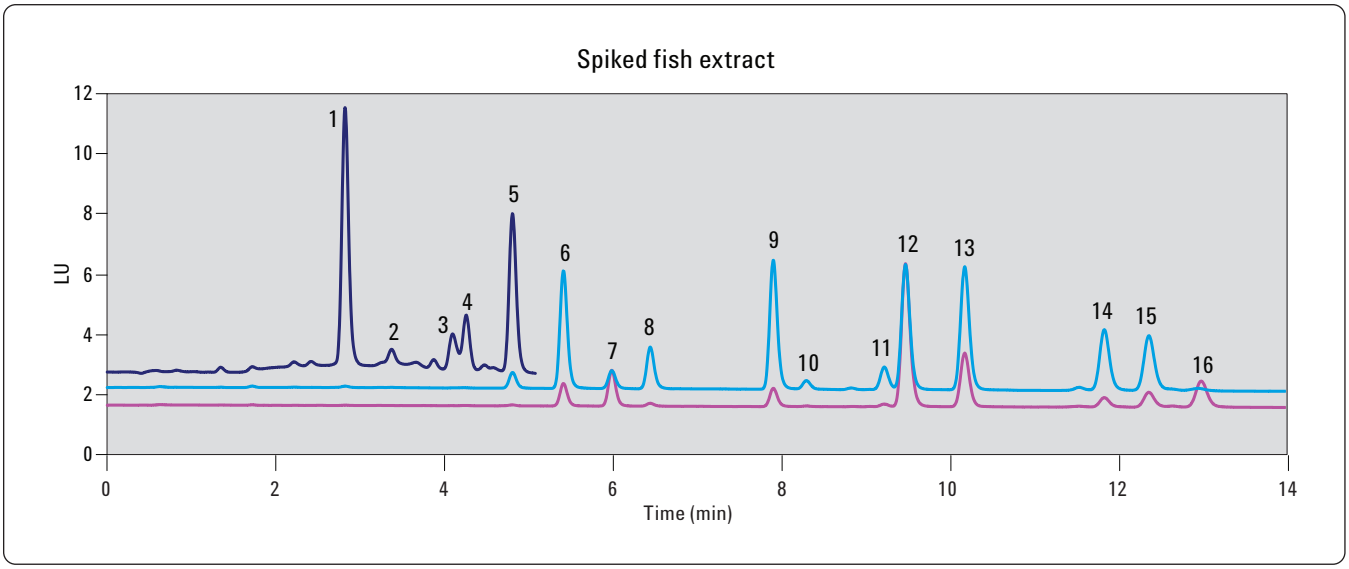

Figure 5. Overlay HPLC – FLD chromatograms of the spiked fish sample containing: 1. Nap 2. Acy 3. Ace 4. Flu 5. Phe 6. Ant 7. Fln 8. Pyr 9. BaA 10. Chr 11. BeP 12. BeA 13. BkF 14. DahA 15. BghiP 16. InP. The spiking level for this sample was level 1 (see Table 3). The blue portion of the chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm; the red portion 260-nm/420-nm; the light blue portion: 260-nm/440-nm. For acenaphthylene, UV detection at 230-nm was used. Chromatographic conditions are shown in Table 1.

Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Linearity

The linear calibration curves were obtained by plotting the peak area for each analyte versus its concentration. Curves were generated by spiking the sample blanks at a concentration range of 0 – 300 ng/g.

Limits of Detection and Quantification

The limits of detection and quantification were estimated from the concentration of sulfonamides required to give a signal-to-noise ratio of 3 and 10 respectively. Table 2 shows the regression equation, correlation coefficients, and very acceptable limits of detection and quantification.

Table 2. Linearity, LOD and LOQ for the Sixteen Polycyclic Aromatic Hydrocarbons

PAH	Regression equation	R^2	LOD	LOQ
Naphthalene	Y = 0.0222x + 0.1366	0.9991	0.62	2.07
*Acenaphthylene	Y = 0.0544x – 0.0130	0.9993	0.25	0.83
Acenaphthene	Y = 0.0184 x – 0.0204	0.9998	0.56	1.87
Fluorene	Y = 0.0323x – 0.1717	0.9990	0.12	0.40
Phenanthrene	Y = 0.0950x + 0.0086	0.9995	0.18	0.60
Anthracene	Y = 0.0838x – 0.1265	0.9991	0.24	0.80
Fluoranthene	Y = 0.0247x – 0.0237	0.9994	0.04	0.16
Pyrene	Y = 0.0218x – 0.0432	0.9998	0.09	0.30
1,2-Benzanthracene	Y = 0.0120x – 0.0103	0.9994	0.03	0.10
Chrysene	Y = 0.0052x + 0.0086	0.9990	0.28	0.93
Benzo[e]pyrene	Y = 0.0144x – 0.0037	0.9997	0.04	0.16
Benz[e]acenaphthylene	Y = 0.1186x – 0.032	0.9995	0.07	0.23
Benzo[k]fluoranthene	Y = 0.0464x + 0.0969	0.9997	0.05	0.16
Dibenzo[a,h]anthracene	Y = 0.0531x + 0.0001	0.9990	0.84	2.80
Benzo[g,h,i]perylene	Y = 0.0440x + 0.0722	0.9993	0.11	0.36
Indeno[1,2,3-cd]pyrene	Y = 0.0324x – 0.0912	0.9993	0.05	0.18

* UV detection at 230 nm

Recovery and Reproducibility

The recovery and reproducibility (RSD) were evaluated on spiked samples at three different levels (Table 3). The analysis was performed in replicates of six (n = 6) at each level. Table 4 shows the very good to excellent recoveries, and excellent RSD values for the sixteen polycyclic aromatic hydrocarbons.

Conclusions

A simple and fast mulitiresidue method based on SampliQ QuEChERS AOAC and HPLC-FLD has been developed for the simultaneous determination of sixteen polycyclic aromatic hydrocarbons at parts-per-billion (ppb) levels in fish tissue. High recoveries with excellent RSD were attained, therefore the method should be applied for quality control of PAHs in real samples.

Table 3. PAHs Spiking Levels

PAH	Spiking level (ng/g)		
	1	2	3
Naphthalene	20	100	200
*Acenaphthylene	20	100	200
Acenaphthene	10	50	100
Fluorene	10	50	100
Phenanthrene	10	50	100
Anthracene	10	50	100
Fluoranthene	10	50	100
Pyrene	10	50	100
1,2-Benzanthracene	5	20	50
Chrysene	10	50	100
Benzo[e]pyrene	5	20	50
Benz[e]acenaphthylene	5	20	50
Benzo[k]fluoranthene	5	20	50
Dibenzo[a,h]anthracene	5	20	50
Benzo[g,h,i]perylene	5	20	50
Indeno[1,2,3-cd]pyrene	5	20	50

* UV detection at 230 nm

Table 4. Recoveries and RSDs for the Sixteen Polycyclic Aromatic Hydrocarbons in Fish Sample (n = 6)

PAH	Level of spiking (ng/g) (n = 6)					
	1		2		3	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Naphthalene	94.7	1.4	97.9	1.1	93.8	1.4
*Acenaphthylene	87.8	1.7	96.3	1.2	85.6	0.8
Acenaphthene	92.1	1.5	93.0	1.8	96.7	0.8
Fluorene	98.1	1.5	89.9	1.0	97.2	0.9
Phenanthrene	90.6	0.9	93.8	0.8	83.1	1.7
Anthracene	96.7	1.0	87.6	0.8	92.1	0.6
Fluoranthene	83.4	1.3	93.9	1.5	95.9	1.2
Pyrene	93.5	1.8	86.1	1.3	95.0	1.4
1,2-Benzanthracene	94.5	1.3	89.6	1.6	94.9	1.0
Chrysene	101.0	1.4	97.8	1.7	87.2	1.6
Benzo[e]pyrene	88.8	1.5	85.2	1.9	95.0	1.4
Benz[e]acenaphthylene	95.5	0.7	92.7	0.7	89.2	0.9
Benzo[k]fluoranthene	93.5	0.8	94.6	0.9	98.9	0.8
Dibenzo[a,h]anthracene	88.2	0.9	97.3	1.1	97.1	0.6
Benzo[g,h,i]perylene	98.4	0.8	95.5	1.6	98.2	0.7
Indeno[1,2,3-cd]pyrene	91.5	1.5	97.9	0.9	94.3	0.7

* UV detection at 230 nm

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Printed in the USA
July 14, 2010
5990-5441EN

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Determination of Acrylamide in Cooking Oil by Agilent SampliQ QuEChERS Acrylamide Kit and HPLC-DAD

Application Note

Food Safety

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Abstract

This application note describes a method based on a quick, easy, cheap, effective, rugged and safe (QuEChERS) multiresidue sample preparation procedure. The QuEChERS method presents an extraction and cleanup protocol for the determination of acrylamide employing methacrylamide as the internal standard. The analyte and internal standard were separated on an Agilent ZORBAX HILIC Plus column (4.6 mm × 50 mm, 3.5 µm) by isocratic elution employing 3% 5 mM CH_3COOH and 97% CH_3CN with subsequent diode array detection at 210 nm. The acrylamide recoveries ranged from 84 to 93.8% with relative standard deviation of less than 4%. The limits of detection and quantification were 32.4 and 108 ng/mL respectively.

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Introduction

Acrylamide (Figure 1) is an organic compound used to manufacture plastic materials, paper, dyes, cosmetics and polyacrylamide, which is a water stabilizer for sewage water treatment and gel electrophoresis [1]. Acrylamide also occurs naturally as a byproduct of the cooking process and its presence in food was first confirmed by Swedish researchers in 2002 [2]. The Swedish findings about high levels of acrylamide in heat treated foods were quickly confirmed by the UK Food Standards Agency through its official website notification on 17 May 2002 and US Environmental Protection Agency (USEPA) found the limit for acrylamide in drinking water to be extremely low (0.5 µg/kg) [3]. Carbohydrate-rich foods such as french fries processed at high temperatures and under low moist conditions are of concern because high concentrations of acrylamides are produced in this process [4]. Acrylamide, at high concentrations, has adverse effects as a human neurotoxin and has also been classified as a probable carcinogen and genotoxicant [5]. Acrylamide vapors irritate the eyes and the skin and can cause paralysis of the cerebrospinal system [6].

The QuEChERS method, which was initially designed for the analysis of pesticides in food has since been adapted to include the extraction of a variety of analytes such as acrylamide [5]. In general, there are two major steps: extraction and dispersive SPE cleanup. The method uses a single step buffered acetonitrile extraction while simultaneously salting out water from the sample using anhydrous magnesium sulfate ($MgSO_4$) to induce liquid-liquid partitioning. After removing an aliquot from an organic layer for further cleanup, dispersive solid phase extraction (dSPE) is conducted using a combination of primary secondary amine (PSA) to remove fatty acids, and anhydrous $MgSO_4$ to reduce the remaining water in the extract.

This application note presents a method for the analysis of acrylamide in cooking oil with HPLC-DAD. The method includes sample preparation with SampliQ QuEChERS Extraction kit for acrylamides (p/n 5982-5850) and SampliQ EN Fruits and Vegetables with Fats and Waxes Dispersive SPE kit (p/n 5982-5156).

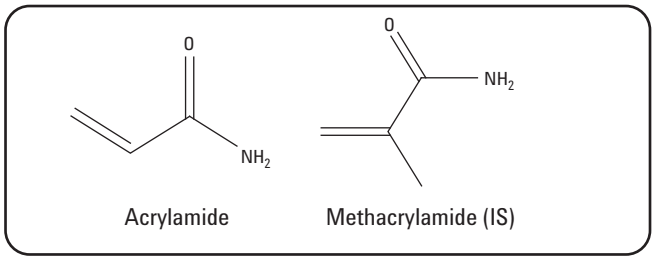

Figure 1. Chemical structures for acrylamide and methacrylamide (IS).

Experimental

Reagents and Chemicals

All reagents were analytical or HPLC grade. Acetonitrile (CH_3CN), n-hexane, acrylamide and methacrylamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). The water used was from a MilliQ system from Millipore (Milford, Mass, USA). The mobile phase was filtered through a Whatman membrane filter (47 mm diameter and 2 µm pore size).

Standard Solutions

Standard stock solutions (1 mg/mL) were prepared by dissolving 10 mg of the acrylamide/methacrylamide in 10 mL MilliQ water and stored at 4 °C. All working solutions were prepared daily by serial dilution also in MilliQ water.

Equipment and Material

The analysis was performed on an Agilent 1200 Series HPLC, Agilent Technologies Inc. (Santa Rosa, CA, USA) equipped with a binary pump and a diode array detector (DAD) set at 210 nm. Separation of the compounds was achieved on an Agilent ZORBAX HILIC Plus column (4.6 mm × 50 mm, 3.5 µm, p/n 959943-901). The data was processed by Agilent ChemStation for LC/MS 2D system software.

Extraction and cleanup were achieved with Agilent SampliQ QuEChERS Extraction kit for acrylamides, p/n 5982-5850 and SampliQ QuEChERS EN Dispersive SPE kit, p/n 5982-5156.

Instrument conditions

HPLC conditions

Table 1. HPLC Conditions used for Separation of Acrylamide and Methacrylamide

Column	Agilent ZORBAX HILIC Plus 4.6 × 50 mm, 3.5 µm
Flow rate	0.2 mL/min
Column temperature	30 °C
Injection volume	5 µL
Mobile phase	Isocratic elution: A = 3% 5 mM acetic acid B = 97% acetonitrile
Run time	10 min
Post time	3 min
Detection	DAD @ 210 nm

Sample preparation

The cooking oil (sunflower-based) was purchased from a local store.

Extraction

Figure 2 outlines the extraction procedure. A 1-g sample of cooking oil was placed into a 50-mL centrifuge tube from the SampliQ QuEChERS Extraction kit. Samples were spiked appropriately to yield working solutions for recoveries and reproducibility studies. Samples, with exception of the blank, were fortified with 1000 µL spiking solution and mixed with 9 mL of water. After shaking vigorously for 1 min, 10 mL of CH_3CN were added, followed by an addition of Agilent SampliQ QuEChERS extraction salt mixture for acrylamides (p/n 5082-5850). The QuEChERS extraction packet contained 4 g of anhydrous $MgSO_4$ and 0.5 g NaCl. Due to the high concentration of long chain fatty acids in the cooking oil, 5 mL of hexane were added to the extraction mixture. The sample tubes were hand-shaken vigorously for 1 min and then centrifuged at 4000 rpm for 5 min. Note that in the centrifuge tube, the hexane forms a third layer [hexane (top layer): acetonitrile (middle layer): water + salts (bottom layer). The hexane layer was discarded prior to the dSPE cleanup.

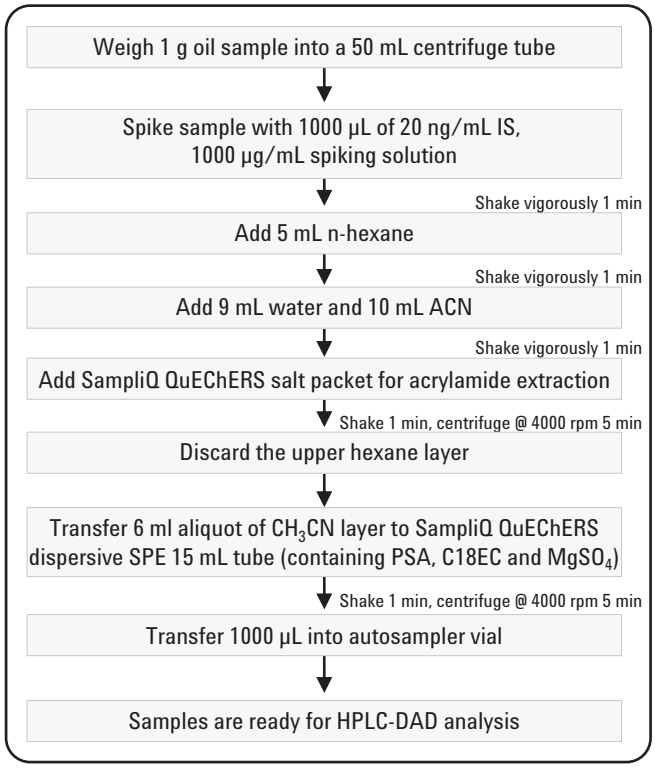

Figure 2. Flow chart for the QuEChERS sample preparation procedure.

Dispersive SPE cleanup

A 6-mL aliquot of the CH_3CN layer (now the top layer after hexane removal) was transferred into a SampliQ QuEChERS EN Dispersive SPE 15 mL tube. The SPE tube contained 150 mg PSA, 150 mg C18EC and 900 mg $MgSO_4$. The tubes were then further centrifuged at 4000 rpm for 5 min. A 1000-µL amount of extract was placed in an autosampler vial for an HPLC-DAD analysis.

Results and Discussion

Chromatographic analysis

The separation of acrylamide and methacrylamide (internal standard) was achieved on an Agilent ZORBAX HILIC Plus column (4.6 mm × 50 mm, 3.5 µm, p/n 959943-901) using isocratic elution, with 3% 5 mM acetic acid and 97% acetonitrile as the mobile phase. The column temperature was set at 30 °C while the flow rate was set at 0.2 mL/min. Figure 3 shows a typical chromatogram for the injection of the standard mixture. Different mobile phase polarity compositions, from 100% water to 100% acetonitrile, were evaluated. The best retention with a short run time was obtained with 97% acetonitrile and 3% acetic acid.

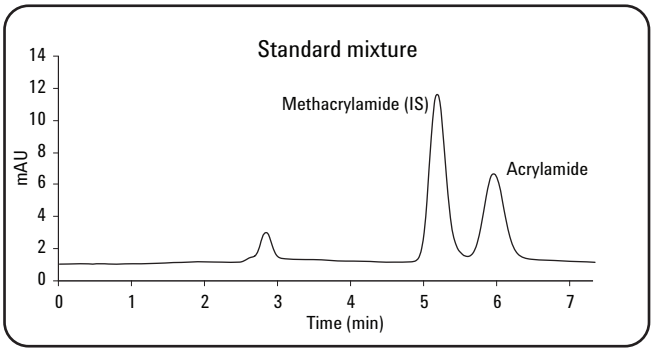

Figure 3. Chromatogram of the standard mixture of acrylamide and methacrylamide (IS).

QuEChERS method

QuEChERS salt packet (p/n 5982–5850) containing 4 g $MgSO_4$ and 0.5 g NaCl was used to extract acrylamide from 1 g of cooking oil sample. The addition of the salt induced the acetonitrile–water phase separation [5]. A 5-mL volume of n-hexane was added to the samples for defatting, which removed long chain fatty acids that could create challenges in chromatographic analysis by giving peaks overlapping with the analyte or clogging the column [6]. Dispersive SPE was employed for sample cleanup.

The QuEChERS protocol in this application note is simple and does not require evaporating the extracting solvent. This is beneficial because acrylamides are usually lost during this step, leading to low recoveries. [7].

Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Linearity

A linear calibration curve (Figure 4) was obtained by plotting the relative responses of analyte (peak area of analyte / peak area of IS) to the relative concentration of analyte (concentration of analyte / concentration of IS). The curve was generated by spiking the sample blanks at a concentration range of 0 - 1500 ng/mL. Good linearity was demonstrated with $r^2 = 0.9992$.

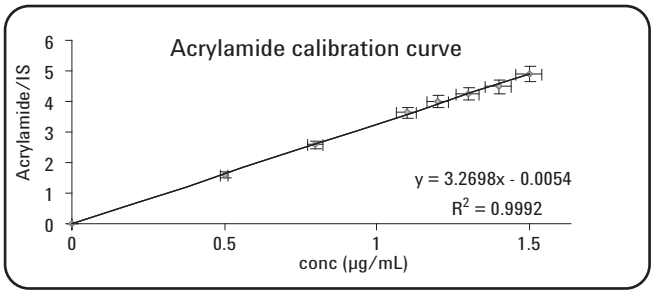

Figure 4. Acrylamide calibration curve.

Limits of Detection and Quantification

The limits of detection and quantification were evaluated from the concentration of acrylamide required to give a signal-to-noise ratio of 3 and 10 respectively. The limit of detection (LOD) was found to be 32.4 ng/mL while the limit of quantification (LOQ) was 108 ng/mL.

Recovery and Reproducibility

The recovery and reproducibility (RSD) were evaluated on spiked samples at three different fortification levels: 500, 1000, and 2000 ng/mL. The analysis was performed in replicates of six (n = 6) at each level. Table 2 shows the recoveries and RSD values for acrylamide.

Table 2. Recoveries and RSDs for the Acrylamide in Oil Sample (n = 6)

Concentration (ng/mL)	Recovery % (n = 6)	RSD % (n = 6)
500	84.0	3.2
1000	93.8	2.2
2000	92.2	1.5

The chromatograms of the oil blank and the spiked oil sample, after the QuEChERS extraction and cleanup, are shown in Figures 5 and 6 respectively. The blank oil extract did not show any detectable amounts of acrylamide. Similar results were reported on a study of acrylamide content in commercial frying oils [7]. The frying oils in that study did not contain any detectable amounts of acrylamide (detection limit of 0.02 µg/mL) prior to processing food samples.

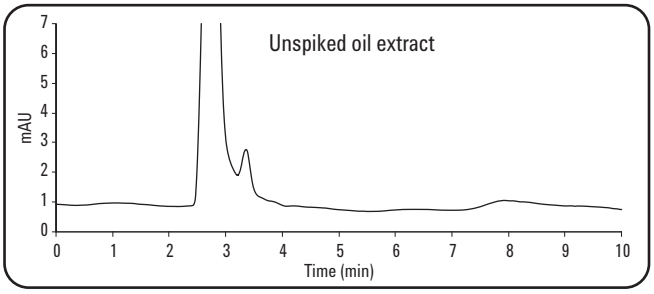

Figure 5. Chromatogram of the blank oil extract.

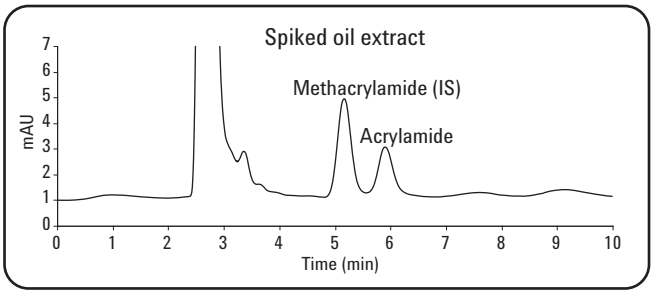

Figure 6. Chromatogram of the spiked oil extract.

Conclusions

A simple and fast multiresidue method using SampliQ QuEChERS for acrylamide extraction and cleanup with an HPLC-DAD analysis on a HILIC column has been developed. High extraction yields with excellent RSD, LOD (32.4 ng/mL), and LOQ (108 ng/mL) were obtained. Therefore, the method may be applied for quality control of acrylamide in real samples.

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Printed in the USA
July 6, 2010
5990-5988EN

Agilent Technologies

Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS AOAC Kits by GC/MS

Application Note

Food Safety

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Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) AOAC sample preparation approach for extraction and cleanup of 18 GC-amenable multiple pesticide class residues in spinach. The method employed involves initial extraction in a buffered aqueous/acetonitrile system, an extraction/partitioning step after the addition of salt, and a cleanup step using dispersive solid phase extraction (dispersive SPE). In order to address the significant loss of planar pesticides caused by graphitized carbon black (GCB) in dispersive SPE, a modified method with addition of toluene was employed for the planar pesticides. The target pesticides in the spinach extracts were then analyzed by gas chromatography/mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode. The method was validated in terms of recovery and reproducibility. The limit of quantitation (LOQ) for most pesticides is 10 ng/g; however folpet has an LOQ of 50 ng/g in spinach. This application, employing SampliQ QuEChERS kits, produced results well below the maximum residue limits (MRLs) for all pesticides screened. The spiked levels for the recovery experiments were 10, 50, and 200 ng/g.

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Introduction

The AOAC QuEChERS method has been widely applied for the analysis of pesticides in food since it was introduced by USDA scientists. [1-3] In summary, the method uses a single-step buffered acetonitrile (1% HAc) extraction while simultaneously salting out water from the sample using anhydrous magnesium sulfate ($MgSO_4$) to induce liquid-liquid partitioning. For cleanup, a dispersive SPE step is employed using a combination of primary secondary amine (PSA) to remove fatty acids as well as other components, and anhydrous $MgSO_4$ to reduce the remaining water in the extract. After mixing and centrifugation, the upper layer is ready for analysis. According to different food matrices, other ingredients may be added in this step, such as graphitized carbon black (GCB) to remove pigments and sterol, or C18 to remove more lipids and waxes.

Spinach is considered to be a highly pigmented matrix since it contains large amounts of chloryophyll. Therefore, the dispersive SPE kits with GCB were selected for further clean-up. GCB adsorbs planar molecules such as pigments and sterols; therefore it is very helpful in cleaning-up pigmented matrix like spinach. However, GCB also adsorbs pesticides with planar structure, such as carbendazim, chlorothalonil, and coumaphos. As a result, this kind of dispersive SPE kit is not recommended for the analysis of planar pesticides. Previously, we discussed the impact of toluene addition to the dispersive SPE tube on the analysis of pesticides in pigmented matrices [4]. It turned out that this modification can greatly increase the extraction efficiency of those problematic pesticides. With the combination of the original (w/o toluene) and modified (w/toluene) dispersive SPE, the performance of SampliQ AOAC Buffered Extraction Kits and SampliQ AOAC Dispersive SPE kits for pigmented produce was demonstrated to be excellent for the analysis of LC amenable pesticides in spinach. [5]

In this study, the performance of the SampliQ AOAC Buffered Extraction kit (p/n 5982-5755) and SampliQ AOAC Dispersive-SPE kits for Pigmented Fruits and Vegetables (p/n 5982-5222 and 5982-5258) was evaluated for the extraction of volatile and semi-volatile pesticides. Analysis was performed by GC/MS. Seventeen GC-amenable pesticides were selected which represent multiple classes, including non-polar organochlorine pesticides (OCs), certain organophosphorus pesticides (OPs) and organonitrogen pesticides (ONs). Table 1 shows the chemical and regulatory information for these pesticides in spinach.

Experimental

Reagents and Chemicals

All reagents and solvents were high-performance liquid chromatography (HPLC) or analytical grade. Methanol (MeOH) and toluene were from Honeywell (Muskegon, MI, USA), acetonitrile (ACN) and glacial acetic acid (HAc) were from Sigma-Aldrich (St Louis, MO, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards and internal standard (triphenyl phosphate, TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), Chem Service (West Chester, PA, USA), or Ultra Scientific (North Kingstown, RI, USA).

Solutions and Standards

A 1% acetic acid in ACN solution was prepared by adding 10 mL of HAc to 1 L of ACN.

Standard and internal standard (IS) stock solutions (2 mg/mL) were made in MeOH, respectively, and stored at –20 °C. Three QC spiking solutions of 1.5, 7.5 and 30 µg/mL were made fresh daily in 1:1 ACN/H2O containing 0.1% FA. A 2.5 µg/mL standard solution in ACN containing 0.1% FA was used to prepare the calibration curves in the matrix blank extract by appropriate dilution. A 15 µg/mL of TPP spiking solution in 1:1 ACN/H2O containing 0.1% FA was used as the internal spiking standard (IS).

Equipment and Material

Agilent Gas Chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA).

Agilent 5975C Mass Spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA).

SampliQ QuEChERS AOAC Extraction kits, p/n 5982-5755 (Agilent Technologies Inc., Wilmington, DE, USA).

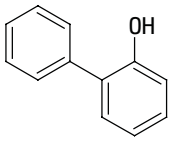
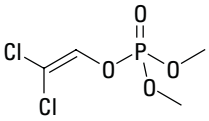
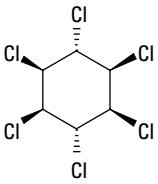
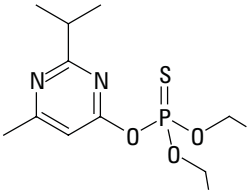
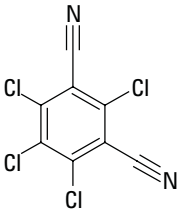
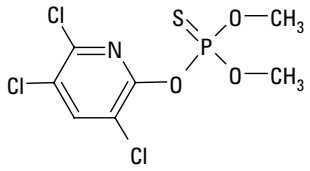
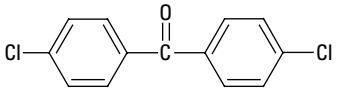
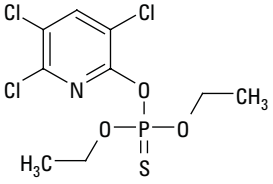
SampliQ QuEChERS AOAC dispersive SPE kits for Pigmented Fruits and Vegetables, p/n 5982-5222 and 5982-5258 (Agilent Technologies Inc., Wilmington, DE, USA).

CentraCL3R Centrifuge (Thermo IEC, MA, USA)

Bottle top dispenser (VWR, So Painfield, NJ, USA)

Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)

Table 1. Pesticides Chemical and Regulatory Information [6–9]

Name	Category	Log P	pKa	Structure	MRLs in spinach (ng/g)*
σ-Phenylphenol	Phenol	3.18	9.4		2000
Dichlorvos	Organophosphate	1.9	NA		10
Lindane	Organochlorine	3.69	NA		10
Diazinon	Organophosphate	3.69	2.6		100
Chlorothalonil	Chloronitrile	2.94	NA		4000
Chlorpyrifosmethyl	Organophosphate	4.00	NA		30
Dichlorobenzophenone	Organochlorine	4.44	NA		500
Chlorpyrifos	Organophosphate	4.7	NA		10

(Continued)

Table 1. Pesticides Chemical and Regulatory Information [6–9]

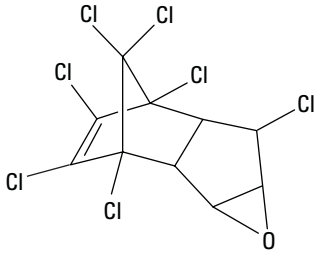
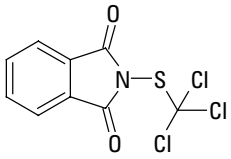
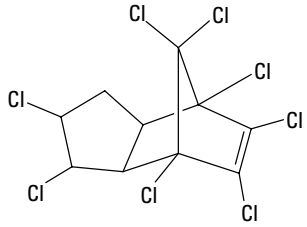
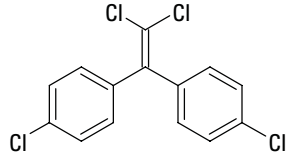
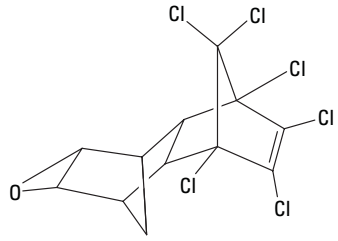
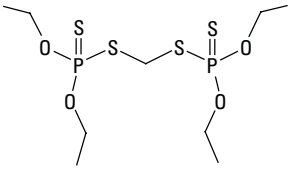
Name	Category	Log P	pKa	Structure	MRLs in spinach (ng/g)*
Heptachlor epoxide	Organochlorine	5.83	NA		30
Folpet	Phthalimide	3.02	NA		2000
Chlordane	Cyclodiene organochlorine	2.78	NA		20
DDE	Organochlorine	6.55	NA		50
Dieldrin	Chlorinated hydrocarbon	3.7	NA		10
Ethion	Organophosphate	5.07	NA		300

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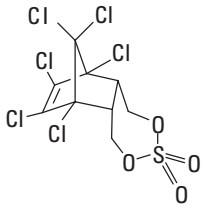
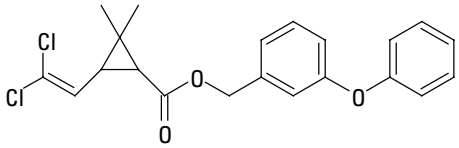
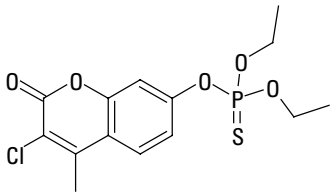
Table 1. Pesticides Chemical and Regulatory Information [6–9]

Name	Category	Log P	pKa	Structure	MRLs in spinach (ng/g)*
Endosulfan sulfate	Organochlorine	3.13	NA		50
Permethrins	Pyrethroid	6.1	NA		50
Coumaphos	Organothio phosphate	3.86	NA		100

*The MRLs numbers list in the table are for apple or lowest level in other fruit and vegetables. They could be higher in different commodities.

Instrument Condition

An Agilent GC/MS method for pesticides analysis was used for this study. [10]

GC conditions

Inlet:	Splitless
Inlet liner:	Helix double taper, deactivated (p/n 5188-5398)
Carrier gas:	Helium
Inlet pressure:	19.6 psi (constant pressure mode) during run 1.0 psi during backflush
Inlet temperature:	250 °C
Injection volume:	1.0 µL
Purge flow to split vent:	30 mL/min at 0.75 min
Oven temperature program:	70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C /min to 200 °C (0 min), 16 °C/min to 280 °C (6 min)
Post run:	3 min
Capillary flow technology:	Purged Ultimate Union (p/n G3186B) - used for backflushing the analytical column and inlet.
	Aux EPC gas: Helium plumbed to Purged Ultimate Union
Aux EPC pressure:	4.0 psi during run, 80.0 psi during backflush
Column:	Agilent J&W HP-5MS Ultra Inert 15 m × 0.25 mm, 0.25 µm (p/n 19091S-431UI)
Connections:	Between inlet and Purged Ultimate Union (p/n: G3186B)
Restrictor:	65 cm x 0.15 mm, 0.15 µm DB-5MS Ultra Inert
Connections:	Between the Purged Ultimate Union and the MSD

MS conditions

Tune file	Atune.u
Mode	SIM (refer to Table 2 for settings in detail)
Source, quad, transfer line temperature	230 °C, 150 °C and 280 °C respectively
Solvent delay	2.30 min
Multiplier voltage	Autotune voltage

Sample Preparation

The sample preparation procedure includes sample comminution, extraction and partitioning and dispersive SPE clean-up. This process has been described in detail in previous application notes. [8] The procedure used for spinach was similar with the exception of the dispersive SPE clean-up step which includes toluene addition.

The frozen chopped organic spinach was homogenized thoroughly. A 15 g (±0.1 g) amount of homogenized sample was placed into a 50 mL centrifuge tube. Samples were fortified with appropriate QC spiking solutions (100 µL) when necessary, then fortified with 100 µL of IS spiking solution (15 µg/mL of TPP). After vortexing the sample for 30s, 15 mL of 1% HAc in ACN was added to each tube using the dispenser. To each tube, an Agilent SampliQ QuEChERS AOAC extraction salt packet (p/n 5982-5755) was added directly. Sample tubes were capped tightly, and hand-shaken vigorously for 1 min. Tubes were centrifuged at 4000 rpm for 5 min.

Next, the ACN extracts were separated into two parts for both original and modified dispersive SPE methods. The modified dispersive SPE method has a different procedure; therefore, it is described below in detail. The volume of ACN extracts (about 14 mL) will be enough for simultaneously processing samples with original and modified dispersive SPE when using the 2 mL size dispersive SPE tube. If you are using the 15 mL size tube, then 14 mL of ACN extracts from the one sample will not be enough for processing dispersive SPE by the two methods (since 8 mL are required for each dispersive SPE method). Therefore, another sample must be extracted from the beginning.

Table 2. Instrument Acquisition Data Used for the Analysis of 18 Pesticides by GC/MS

Analyte	SIM	Collection window (min)	RT (min)
(1) Dichlorvos	184.9	2.3 – 4.0	2.88
(2) σ-Phenylphenol	170.1, 169.1	4.0 – 5.0	4.35
(3) Lindane	180.9, 182.9	5.0 – 6.9	6.67
(4) Diazinon	137.1, 179.1	6.9 – 7.7	7.19
(5) Chlorothalonil	265.8, 263.8	6.9 – 7.7	7.34
(6) Chlorpyrifos-methyl	285.9, 287.9	7.7 – 8.6	8.25
(7) Dichlorobenzophenone	250.0,139.0	8.6 – 10.0	9.55
(8) Chlorpyrifos	196.8, 198.8	8.6 – 10.0	9.57
(9) Heptachlor epoxide	352.8, 354.8	10.0 – 10.4	10.31
(10) Folpet	259.9, 261.9	10.4 – 10.85	10.75
(11) γ-Chlordane	372.8, 374.8	10.85 – 11.6	10.97
(12) DDE	245.9, 317.9	10.85 – 11.6	11.21
(13) α-Chlordane	372.8, 374.8	10.85 – 11.6	11.50
(14) Dieldrin	262.9, 264.9	11.0 – 12.3	11.89
(15) Ethion	230.9	12.3 – 13.6	12.97
(16) Endosulfan sulfate	273.8	12.3 – 13.6	13.35
TPP (IS)	325.1, 326.1	13.6 – 15.0	13.84
(17) Permethrin	183.1	15.0 – 23.0	15.69, 15.79
(18) Coumaphos	362.0, 225.9	15.0 – 23.0	15.83

A 1 mL aliquot of the upper ACN layer was transferred into an Agilent SampliQ QuEChERS dispersive SPE 2 mL tube (p/n 5982-5222); or an 8 mL aliquot was transferred into an Agilent SampliQ QuEChERS dispersive SPE 15 mL tube (p/n 5982-5258). The 2 mL tube contained 50 mg of PSA, 50 mg of GCB and 150 mg of anhydrous $MgSO_4$; while the 15 mL tube contained 400 mg of PSA, 400 mg of GCB and 1200 mg of anhydrous $MgSO_4$.

Next, 375 µL of toluene were added to the 2 mL tube, and 3 mL of toluene were added to the 15 mL tube. The tubes were tightly capped and vortexed for 1 min. We suggest vortexing the tubes for a few seconds before adding the sample, to prevent possible agglomerates. The 2 mL tubes were centrifuged with a micro-centrifuge at 13,000 rpm for 2 min, and the 15 mL tubes were centrifuged in a standard centrifuge at 4000 rpm for 5 min. An 825 µL amount of extract was then transferred into another tube, and dried by N_2 flow. Samples were reconstituted into 600 µL of ACN containing 0.1% FA. After vortexing and sonicating, the reconstituted samples were transferred directly into autosampler vials for GC/MS injection. The reconstituted blank samples were directly used to prepare the calibration curve.

Another aliquot of ACN extracts was processed following the original dispersive SPE clean-up procedure. Figure 1 shows the flow chart of the whole extraction procedure (original and modified dispersive SPE, 2 mL size) for spinach samples.

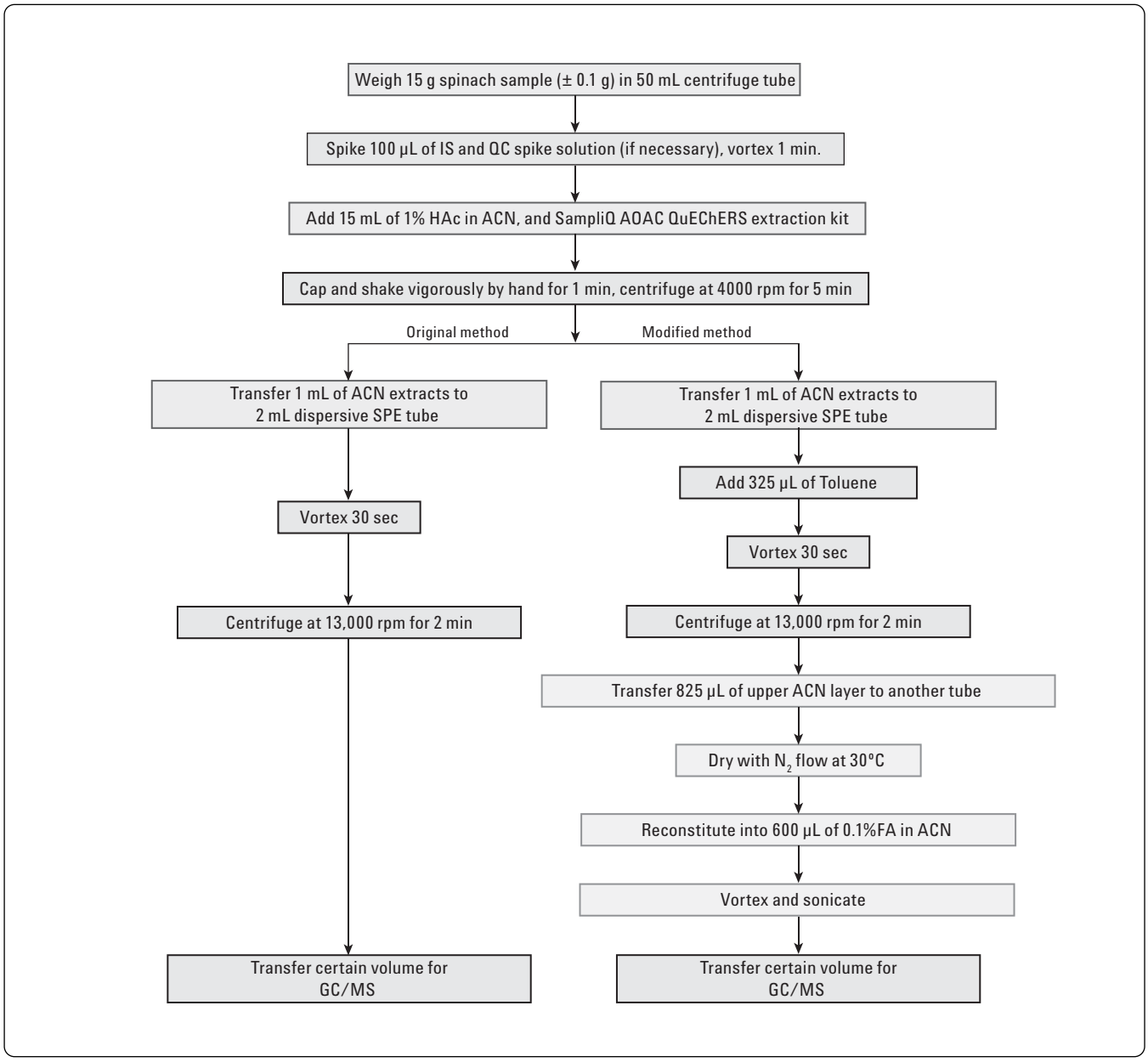


Figure 1. Flow chart of the QuEChERS AOAC extraction procedure (original and modified dispersive SPE, 2 mL size) for spinach sample.

Results and Discussion

The QuEChERS method for pesticide residues analysis provides high-quality results in a fast, easy, inexpensive approach. For the pigmented fruits and vegetables, the addition of GCB in the dispersive SPE tube can greatly remove pigments and sterols. In order to address the significant loss of planar pesticides, toluene was added to increase the extraction efficiency of those pesticides. Previously we discussed that the addition of toluene retained more matrix impurities in the final sample. [4] In the application using LC/MS/MS, there's no chromatographic differences between the samples processed by the original and modified methods due to the powerful selectivity of LC/MS/MS. The selectivity of GC/MS (SIM mode) is not as powerful as that of LC/MS/MS (MRM mode). In GC/MS there are interference peaks apparent in the blank chromatogram. Fortunately most of the pesticides tested are free of co-eluting interferences. There was also an interference eluting at a retention time very close to that of σ-phenylphenol, and this cannot be differentiated for quantitation. The response of this interferent within the blank was integrated to be less than 20% of the response of the σ-phenylphenol peak at the LOQ (10 ng/g) sample. Therefore, it was considered selectivity-acceptable for this compound. Finally, the GC/MS blank chromatograms showed minor differences from samples processed by the original and modified methods, but these differences did not affect the analysis of the target analytes. Figure 2 and Figure 3 show the GC/MS chromatograms of matrix blank (IS spiked) and 50 ng/g fortified spinach extract processed by the original and modified dispersive SPE methods.

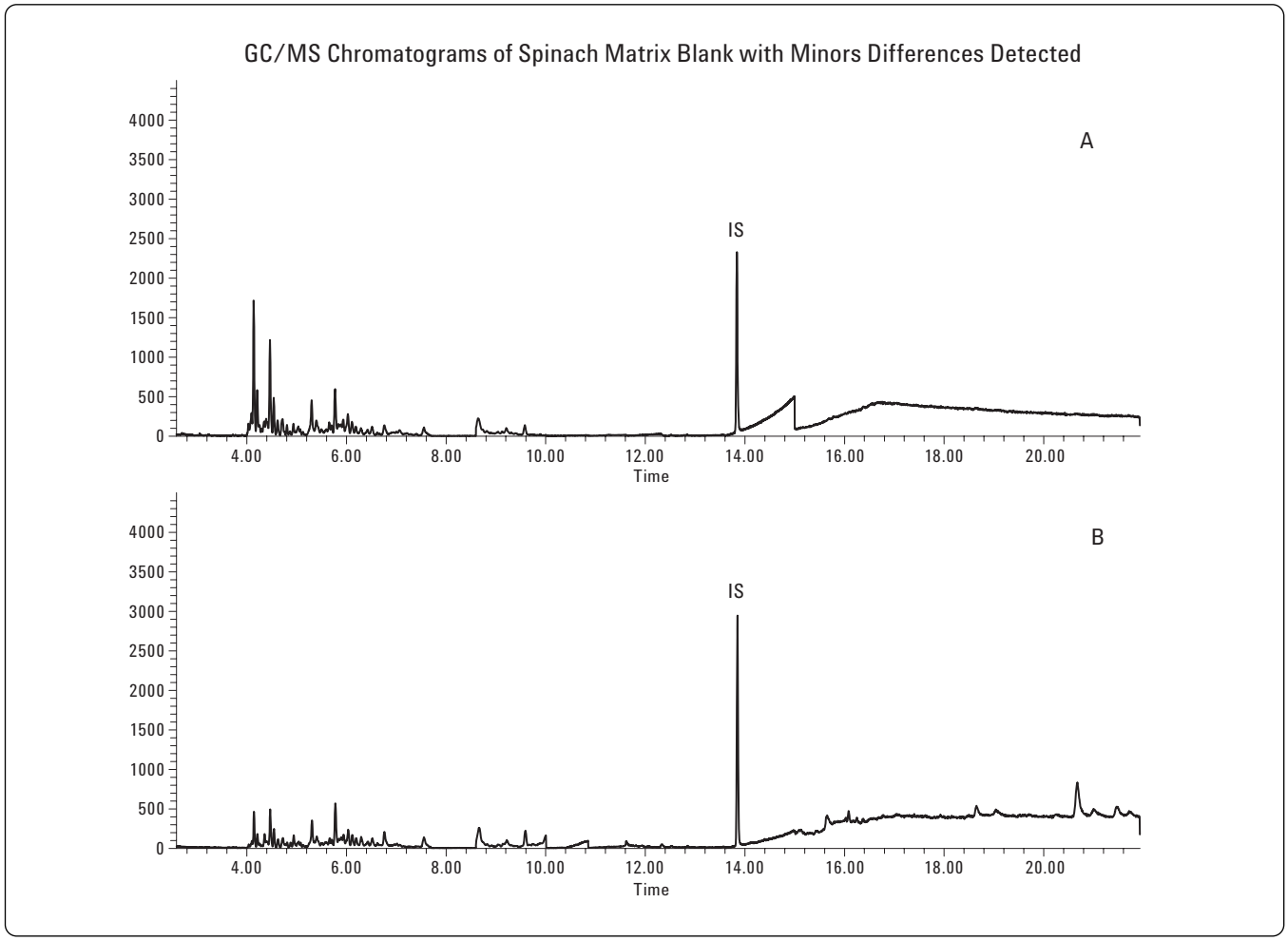


Figure 2. GC/MS chromatograms of spinach matrix blank processed by original dispersive SPE (A) and modified dispersive SPE (B). IS: Internal Standard TPP.

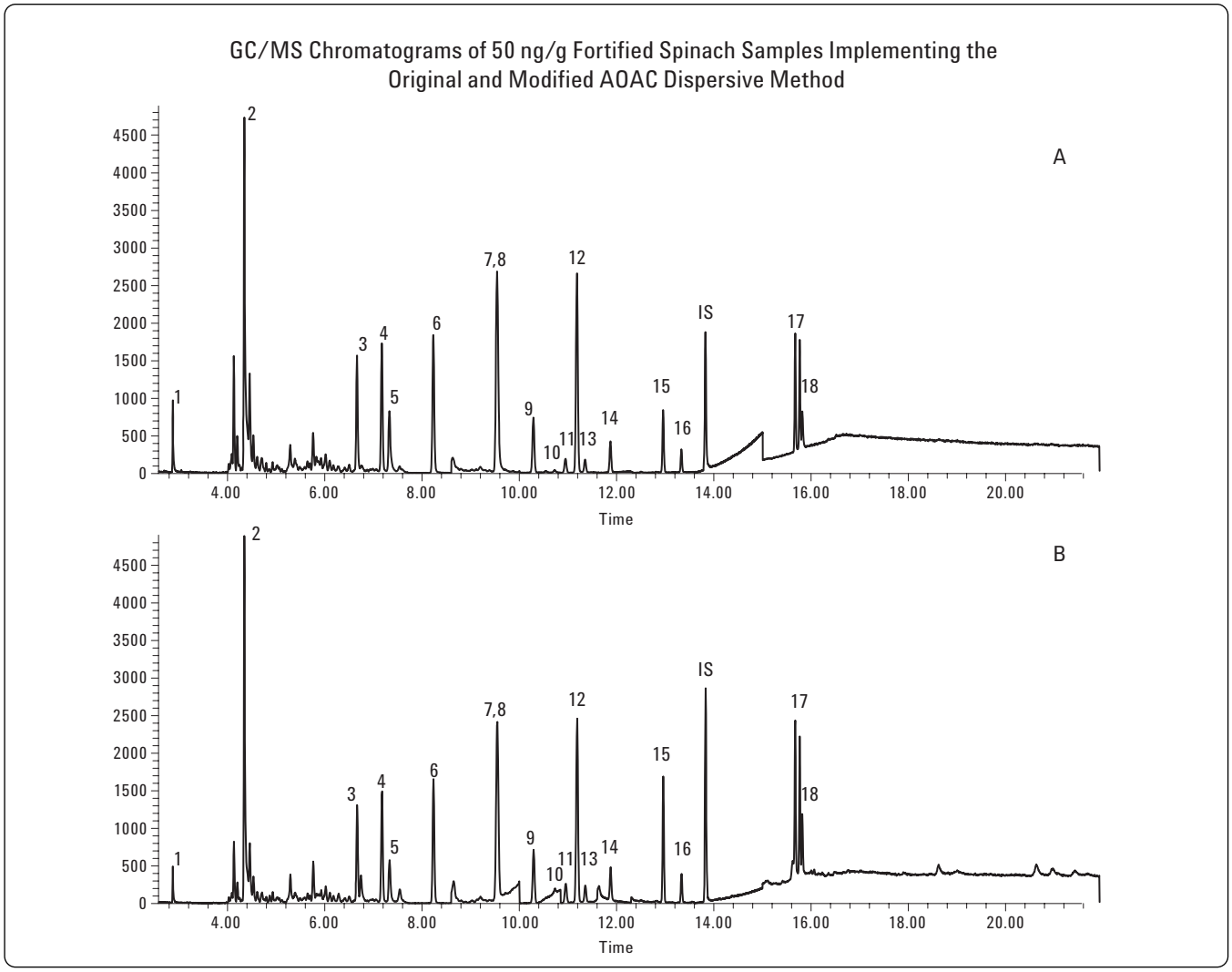


Figure 3. GC/MS chromatograms of 50 ng/g fortified spinach sample extracts processed by original dispersive SPE (A) and modified dispersive SPE (B). Peak identification: 1. Diachlorvos, 2. σ-Phenylphenol, 3. Lindane, 4. Diazinon, 5. Chlorothalonil 6. Chloropyrifos methyl 7. Dichlorobenzophenone, 8. Chlorpyrifos, 9. Heptachlor epoxide, 10. Folpet, 11. γ-Chlordane, 12. DDE, 13. α-Chlordane, 14. Dieldrin, 15. Ethion, 16. Endosulfan sulfate, 17. Permethrin, 18. Coumaphos. IS: Internal Standard, TPP.

Linearity and Limit of Quantification (LOQ)

The linear calibration range for all of the pesticides was 10–400 ng/g, except folpet, which was 50–400 ng/g. For the sample processed by original and modified methods, the corresponding matrix blank was used to prepare the calibration curves respectively. Calibration curves, spiked in matrix blanks, were made at levels of 10, 20, 50, 100, 250, and 400 ng/g. The TPP was used as an internal standard at 100 ng/g. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/ peak area of IS) to the relative concentration of analytes (concentration of analyte / concentration of IS). The 10 ng/g quantification limits LOQ (10 ppb) and 50 ng/g LOQ for folpet (50 ppb) established for the pesticides are substantially lower than many MRLs of those pesticides in fruit and vegetables. The regression fit used for the calibration curves was the average response factor. Table 3 shows the linear regression equation and correlation coefficient (R2) for both 1 mL and 8 mL dispersive SPE.

Table 3. Linearity of 17 Pesticides in Spinach Extract

	1 mL dispersive SPE		8 mL dispersive SPE	
Pesticide	**Linear Term**	**RF Rel Std Dev (%)**	**Linear Term**	**RF Rel Std Dev (%)**
Dichlorvos	5.55e-001	9.1	4.71e-001	6.8
σ-Phenylphenol	2.93e+000	7.9	2.30e+000	9.3
Lindane	8.34e-001	9.5	6.98e-001	8.1
Diazinon	1.03e+000	8.7	9.25e-001	11.2
Chlorothalonil *	7.67e-001	14.2	7.83e-001	13.7
Chlorpyrifos methyl	1.26e+000	12.5	1.20e+000	10.3
Dichlorobenzophenone *	3.03e+000	9.8	2.61e+000	12.2
Chlorpyrifos	6.46e-001	6.9	5.99e-001	11.5
Folpet *,**	4.36e-002	10.2	3.33e-002	11.4
γ-Chlordane	1.73e-001	6.2	1.38e-001	6.4
DDE	2.98e+000	7.4	2.49e+000	6.2
α-Chlordane	1.37e-001	8.1	1.07e-001	8.1
Dieldrin	3.41e-001	5.1	2.92e-001	10.6
Ethion	1.07e+000	15.6	1.42e+000	12.2
Endosulfan sulfate	3.06e-001	4.6	2.42e-001	4.2
Permethrin	1.14e+000	6.4	1.22e+000	9.7
Coumaphos *	3.45e-001	6.4	2.64e-001	11.3

* Results from modified dispersive SPE

** Calibration curve range: 50 – 400 ng/g.

Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticides standards in comminuted spinach sample at levels of 10, 50 and 200 ng/g. These QC samples were quantitated against the matrix spiked calibration curve. The analysis was performed in replicates of six (n = 6) at each level. The recovery and reproducibility (shown as % RSD) data for 1 mL and 8 mL dispersive SPE are shown in Table 4 and Table 5, respectively. Since it was demonstrated that the dispersive SPE size (1 mL and 8 mL) didn't affect the results, the 8 mL size modified dispersive SPE test was not performed due to sample volume limitation. In the 18 GC-amenable pesticides we screened, four pesticides, chlorothalonil, dichlorobenzophenone, folpet, and coumaphos, were found to be adversely affected by the GCB in the dispersive SPE step. With the addition of toluene, the recoveries of those pesticides were increased from 50% to 200% with better precision. However, the modified method also reduced the recovery of certain pesticides that had generated good results originally.

Therefore, the quantitation results shown here are the combination of 14 pesticides from original dispersive SPE and four pesticides from the modified method. It can be seen from the results that the 14 pesticides processed by the original method give out good recoveries (average of 88.8% for 1 mL and 86.3% for 8 mL) and precision (average of 5.4% RSD for 1 mL and 4.8% RSD for 8 mL). Although the four pesticides processed by the modified method give lower recovery (average of 75.3% for 1 mL) but great precision (average of 6.1% RSD for 1 mL), the results were much better than the results obtained by original methods (average recovery of 41.7% with 14.9% average RSD). Please refer to the previous application note [4] for discussions in more detail. Folpet was quantified, but the LOQ was found to be 50 ng/g due to poor sensitivity.

Table 4. Spinach AOAC Dispersive, 1 mL Sample Volume, 2 mL Tube, LC/MS/MS Results

Pesticide	Low QC (10 ng/g) Recovery	RSD	Mid QC (50 ng/g) Recovery	RSD	High QC (200 ng/g) Recovery	RSD
Dichlorvos	94.0	3.0	91.7	10.5	80.9	4.6
σ-Phenylphenol	95.0	2.2	92.0	7.9	78.7	3.8
Lindane	83.7	3.1	93.9	12.2	91.8	3.3
Diazinon	97.3	4.3	95.6	9.9	91.8	3.3
Chlorothalonil *	47.5	6.8	44.9	6.6	49.4	4.3
Chlorpyrifos methyl	74.1	4.6	71.7	4.5	72.2	5.8
Dichlorobenzo Phenone *	97.5	7.6	66.8	3.9	68.8	6.8
Chlorpyrifos	88.3	3.0	79.6	3.5	77.0	3.5
Heptachlor epoxide	74.9	1.9	81.6	11.7	78.2	3.9
Folpet *	NA	NA	98.8	6.0	77.7	6.7
γ-Chlordane	106.0	4.9	112.2	3.3	93.6	5.3
DDE	80.3	2.2	86.8	9.6	75.4	3.5
α-Chlordane	107.6	4.2	108.4	3.5	91.6	3.7
Dieldrin	99.7	2.6	93.7	9.6	78.9	3.4
Ethion	91.4	3.4	100.0	5.0	107.4	7.6
Endosulfan sulfate	93.7	4.8	97.3	8.8	89.8	4.3
Permethrin	84.7	5.7	74.8	9.9	84.6	6.0
Coumaphos *	98.4	5.5	84.2	9.5	81.2	3.2

* Results from modified dispersive SPE method.

Table 5. Spinach AOAC Dispersive 8 mL Volume, 15 mL Tube, Results by GC/MS

Pesticide	Low QC (10 ng/g) Recovery	RSD	Mid QC (50 ng/g) Recovery	RSD	High QC (200 ng/g) Recovery	RSD
Dichlorvos	93.7	2.6	92.5	4.2	86.2	5.9
σ-Phenylphenol	87.9	5.1	92.5	6.6	95.2	6.3
Lindane	83.1	5.1	85.4	2.9	84.5	5.2
Diazinon	85.8	6.9	85.2	2.9	87.3	5.5
Chlorothalonil*	21.1	49.7	23.6	14.3	23.2	14.0
Chlorpyrifos methyl	76.4	2.4	73.9	2.7	73.8	3.6
Dichlorobenzophenone*	93.3	4.1	56.6	2.0	61.4	4.5
Chlorpyrifos	77.8	3.6	70.2	4.6	69.0	3.1
Heptachlor epoxide	78.6	4.6	79.6	2.6	85.3	5.1
Folpet*	NA	NA	60.3	17.4	53.7	10.7
γ-Chlordane	106.8	5.7	110.7	3.5	100.4	4.9
DDE	80.8	4.2	81.8	2.6	81.3	4.9
α-Chlordane	104.2	6.2	103.6	3.3	95.8	5.3
Dieldrin	96.4	6.2	93.0	1.2	79.3	5.3
Ethion	83.8	4.0	82.8	2.3	85.3	4.9
Endosulfan sulfate	90.5	8.8	87.5	7.3	84.5	6.4
Permethrin	84.0	4.9	78.4	6.8	79.5	10.7
Coumaphos*	61.2	22.2	42.6	28.8	35.3	20.6

*Poor results caused by GCB added in dispersive SPE, can be improved by addition of toluene in the dispersive SPE.

Conclusions

Agilent SampliQ QuEChERS AOAC buffered extraction kits and dispersive SPE kits for pigmented fruits and vegetables provide a simple, fast and effective method for the purification of representative volatile to semi-volatile pesticides in spinach. The modified dispersive SPE method with the addition of toluene provides a very useful option to improve the loss of planar pesticides caused by GCB in dispersive SPE tubes. The recovery and reproducibility, based on matrix spiked standards, were acceptable for multiclass, multi-residue pesticide determination in spinach. The impurities and matrix effects from spinach did not interfere with the quantitation of target compounds. As the selected pesticides represented a broad variety of different classes and properties, the Agilent SampliQ QuEChERS AOAC Buffered Extraction and Dispersive kits for Pigmented Fruits and Vegetables can be used for other pesticides in similar pigmented matricies.

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Printed in the USA
July 16, 2009
5990-4305EN

Agilent Technologies

PAH Analyses with High Efficiency GC Columns: Column Selection and Best Practices

Food Quality and Environmental

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Abstract

The European Union (EU) regulates a series of PAHs found primarily in food matrices. This list is referred to as the EU 15 +1 list. The US Environmental Protection Agency (EPA) regulates a series of 16 PAHs historically addressed as environmental pollutants. Both lists contain unique analytes that present different separation challenges. However, there are eight analytes that are common to both lists. In this study, resolution of all 24 combined regulated PAHs is achieved, in under 28 minutes using an Agilent J&W DB-EUPAH 20 m x 0.18 mm, 0.14 µm High Efficiency GC column. Resolution of 23 of the 24 combined regulated PAHs is shown using an Agilent J&W DB-5ms 20 m x 0.18 mm, 0.18 µm High Efficiency GC column in under 22 minutes. Both the Agilent J&W DB-EUPAH and DB-5ms columns are excellent column choices for analysis of the regulated PAHs. The Agilent J&W DB-EUPAH is recommended when separation of benzo[b,j,k]fluoranthene isomers is required.

Agilent Technologies

Instruction

Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds containing two or more fused aromatic rings. PAHs often result from the incomplete combustion of organic substances such as wood, coal, and oil. The European Union PAH regulation has focused on these substances as potential contaminants in the food supply. A main source of potential human exposure to PAHs is through heat processing of meat and dairy products, such as grilling and smoking [1]. There are serious health concerns regarding PAHs since many are classified as carcinogenic or mutagenic [2].

In 2005, the European Commission recommended the monitoring of fifteen EU priority PAHs along with an additional PAH highlighted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [3]. The EU 15+1 priority PAHs along with the US-EPA-regulated PAHs are provided in Table 1 [4]. There are eight PAHs that are common to both the EU-15 +1 list and the US-EPA list.

A 5% phenyl methylpolysiloxane stationary phase column is the most commonly used GC column for PAH analysis. This nonpolar column yields good resolution for the 16 US-EPA PAHs [5,6], however, three critical pairs of the 15+1 EU PAHs co-elute and are difficult to resolve by mass spectrometry [7,8]. These challenging pairs are benz[a]anthracene-cyclopenta[c,d]pyrene- chrysene,benzo[b]fluoranthene-benzo[k]fluoranthene-benzo[j]fluoranthene, and indeno[1,2,3-cd] pyrene-dibenz[a,h]anthracene. Agilent J&W DB-EUPAH, a midpolar GC column, improves the resolution of these critical pairs allowing for more accurate detection and quantitation of the 15 +1 EU priority PAHs.

Another set of challenging analytes is the four dibenzopyrene isomers. Due to their high molecular weight (MW 302), these isomers are prone to discrimination and poor peak shape. Broad, tailing peaks make reliable quantitation difficult and decrease the signal-to-noise ratio, resulting in an increase in the limits of detection. Limiting analyte dwell time on the column and in the GC/MS interface can offset these deleterious chromatographic effects. Shorter columns with thinner film thickness and high operating temperatures are all factors that collectively can improve peak shapes for these analytes.

Table 1. EU and US-EPA Regulated PAH Compounds

Peak #	Component	CAS #	MW	EU 15+1	EPA
1	Naphthalene	91-20-3	128		x
2	Acenaphthylene	208-96-8	152		x
3	Acenaphthene	83-32-9	154		x
4	Fluorene	86-73-7	166		x
5	Phenanthrene	85-01-8	178		x
6	Anthracene	120-12-7	178		x
7	Fluoranthene	206-44-0	202		x
8	Pyrene	129-00-0	202		x
9	*Benzo[c]fluorene*	205-12-9	216	x	
10	**Benz[a]anthracene**	56-55-3	228	x	x
11	*Cyclopenta[c,d]pyrene*	27208-37-3	226	x	
12	**Chrysene**	218-01-9	228	x	x
13	*5-Methylchrysene*	3697-24-3	242	x	
14	**Benzo[b]fluoranthene**	205-99-2	252	x	x
15	**Benzo[k]fluoranthene**	207-08-9	252	x	x
16	*Benzo[j]fluoranthene*	205-82-3	252	x	
17	**Benzo[a]pyrene**	50-32-8	252	x	x
18	**Indeno[1,2,3-cd]pyrene**	193-39-5	276	x	x
19	**Dibenz[a,h]anthracene**	53-70-3	278	x	x
20	**Benzo[g,h,i]perylene**	191-24-2	276	x	x
21	*Dibenzo[a,l]pyrene*	191-30-0	302	x	
22	*Dibenzo[a,e]pyrene*	192-65-4	302	x	
23	*Dibenzo[a,i]pyrene*	189-55-9	302	x	
24	*Dibenzo[a,h]pyrene*	189-64-0	302	x	

Regulated PAH compounds shown in plain text are included only in the US-EPA set, compounds in Italic are included only in the EU 15+1 list, and the compounds in bold are included in both the US-EPA and EU 15+1 lists.

Experimental

GC EU PAH standard (Agilent p/n 5190-0487) and US-EPA mixture (Agilent p/n 8500-6035) were diluted separately to a concentration of 2 µg/ml using class A glassware and pipets. These solutions were then mixed 1:1 to for a final concentration of 1-2 µg/ml of all 24 regulated PAHs.

Table 2. Chromatographic Conditions DB-EUPAH Column

Sample:	0.5 µL 1-2 µg/mL EU + EPA PAH combined standards (EU PAH standard Agilent p/n 5190-0487 and EPA PAH standard Agilent p/n 8500-6035)
GC/MS:	Agilent 7890A GC System with an Agilent 5975C Series GC/MSD, TAD, and an Agilent 7873B automatic liquid sampler
Column:	Agilent J&W DB-EUPAH 20 m × 0.18 mm, 0.14 µm (Agilent p/n 121-9627)
Carrier:	Helium 60 cm/sec 1.8 ml/min constant flow
Oven:	70 °C (0.8 min), 70 °C/min to 180 °C, 7 °C/min to 230 °C (6 min), 40 °C/min to 280 °C (5 min) 25 °C/min to 335 °C (5 min)
Inlet:	300° C splitless, purge 100 mL/min at 0.25 min
Inlet liner:	Helix double taper deactivated (Agilent p/n 5188-5398)
MSD:	Sim/Scan mode 50-400 AMU, transfer line 340 °C, source 340 °C, quad 150 °C

Table 3. Chromatographic Conditions DB-5ms Column

Sample:	0.5 µL 1-2 µg/mL EU + EPA PAH combined standards (EU PAH standard Agilent part # 5190-0487 and EPA PAH standard Agilent part # 8500-6035)
GC/MS:	Agilent 7890A GC System with an Agilent 5975C Series GC/MSD, TAD, and an Agilent 7873B automatic liquid sampler
Column:	Agilent J&W DB-5 ms 20 m × 0.18 mm, 0.18 µm (Agilent p/n 121-5522)
Carrier:	Helium 60 cm/sec 1.8 ml/min constant flow
Oven:	55 °C (0.4 min), 25 °C/min to 200 °C, 8 °C/min to 280 °C, 10 °C/min to 320 °C (2 min) 25 °C/min to 335 °C (5 min)
Inlet:	300 °C splitless, purge 100 mL/min at 0.25 min
Inlet liner:	Helix double taper deactivated (Agilent part # 5188-5398)
MSD:	Sim/Scan mode 50-400 AMU, transfer line 340 °C, source 340 °C, quad 150 °C

Table 4. Flow Path Supplies

Vials:	Amber screw top glass vials (Agilent p/n 5183-2072)
Vial Caps:	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 5183-4729)
Septum:	Advanced green (Agilent p/n 5183-4759)
Inlet Seal:	Gold plated inlet seal (Agilent p/n 5188-5367)
Inlet liners:	Helix double taper deactivated (Agilent p/n 5188-5398)
Ferrules:	0.4 mm ID short; 85/15 vespel/graphite (Agilent p/n 5181-3323)
20 × magnifier :	20 × Magnifier loop (Agilent p/n 430-1020)

Discussion of Results

Figure 1 shows the separation of all 24 analytes included in the EU 15 + 1 and US-EPA PAH lists on a DB-EUPAH column. This separation was accomplished in under 28 minutes on an Agilent J&W DB-EUPAH 20 m x 0.18 mm, 0.14 µm column (Agilent p/n 121-9627). The selectivity of this midpolar column is necessary to resolve the benzo(b,j,k) fluoranthene isomers. High temperature stability is also required for elution of the high boiling point dibenzopyrenes.

The injection volume was reduced to 0.5 µL in order to scale the separation to the 0.18 or high efficiency GC (HEGC) format. This is often a necessary step when working with high efficiency columns because the higher efficiency of narrow bore columns is at the expense of sample loading capacity. Here the separation focused on achieving faster analysis. An example of a separation maximizing resolution with longer retention on this column was described previously [9].

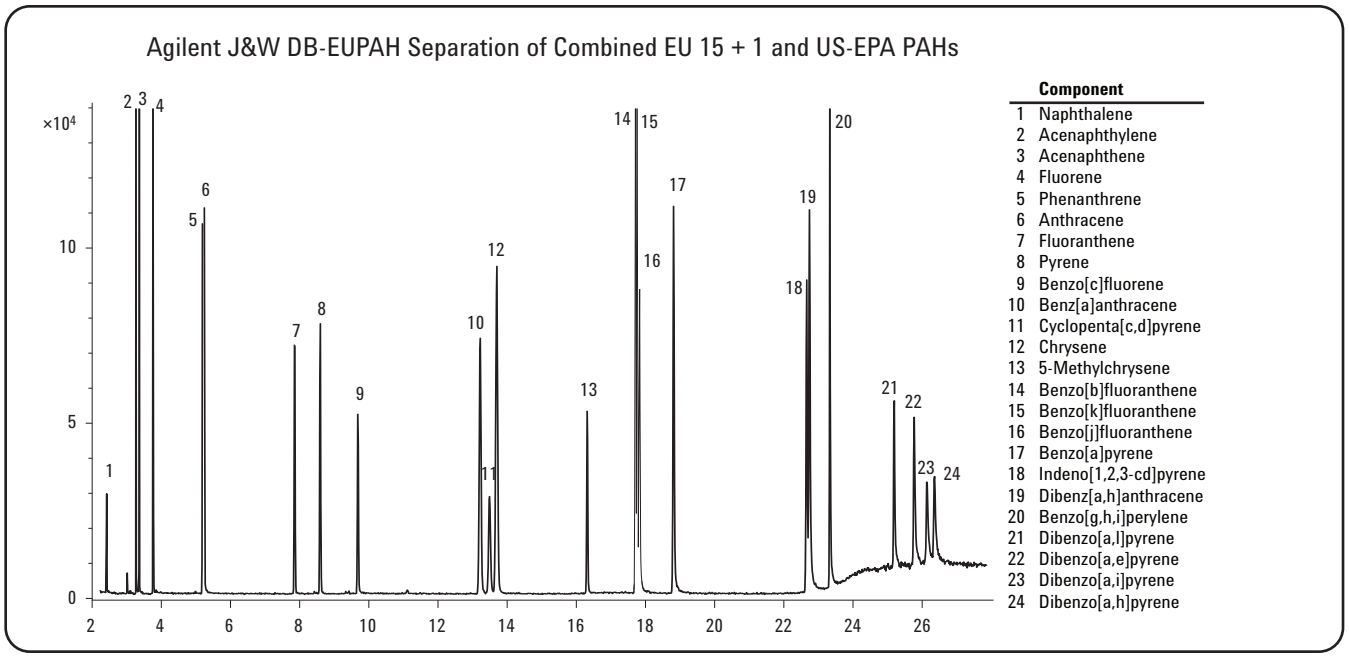

Figure 1. EU and US-EPA regulated PAH separation on an Agilent J&W DB-EUPAH 20 m × 0.18 mm, 0.14 µm column (Agilent p/n 121-9627). Chromatographic conditions as in Table 2 and flow path supplies as in Table 4.

Figure 2 shows the resolution of 23 of the 24 analytes included in the EU 15 + 1 and US-EPA PAH lists on an Agilent J&W DB-5ms column. Benzo[j] fluoranthene is not resolved from benzo[k]fluoranthene using this column. However, when it is sufficient to report the sum of the benzoflouranthene isomers, the Agilent J&W DB-5ms column is an excellent choice for the 24 regulated PAHs. This separation was accomplished in under 22 minutes on an Agilent J&W DB-5ms 20 m × 0.18 mm, 0.18 µm column (Agilent p/n 121-5522). The Agilent J&W DB-5ms separation offers a 27 % faster analysis time when compared to the DB-EUPAH separation shown in Figure 1.

There are a number of best practices to consider when optimizing a GC/MS system for PAH analysis. The use of retention gaps and/or inlet backflushing can reduce maintenance and cycle times. Close examination of injection parameters such as injection volume, inlet temperature, purge time activation, solvent focusing and holding the oven temperature stable during injection can all contribute to better results. Minimizing inlet and system dwell time by operating at high linear velocities can also improve results. Another best practice for PAHs is to keep heated zones well insulated and hot to reduce the potential for system cold spots and the resultant signal loss.

Conclusions

All 24 (EU 15 + 1 and US-EPA) regulated PAHs are resolved using an Agilent J&W DB-EUPAH 20 m × 0.18 mm, 0.14 µm column. The benzo[b,j,k]fluoranthene isomers were adequately separated for individual quantitation. This is the column of choice when resolution of benzo[j]fluoranthene is required. This separation was accomplished in under 28 minutes.

23 of 24 (EU 15 + 1 and US-EPA) regulated PAHs resolve using an Agilent J&W DB-5ms 20 m × 0.18 mm, 0.18 µm column. This column is an excellent choice when benzoflouranthene isomers are reported as a sum of the isomers and speed of analysis is critical. This separation was accomplished in under 22 minutes.

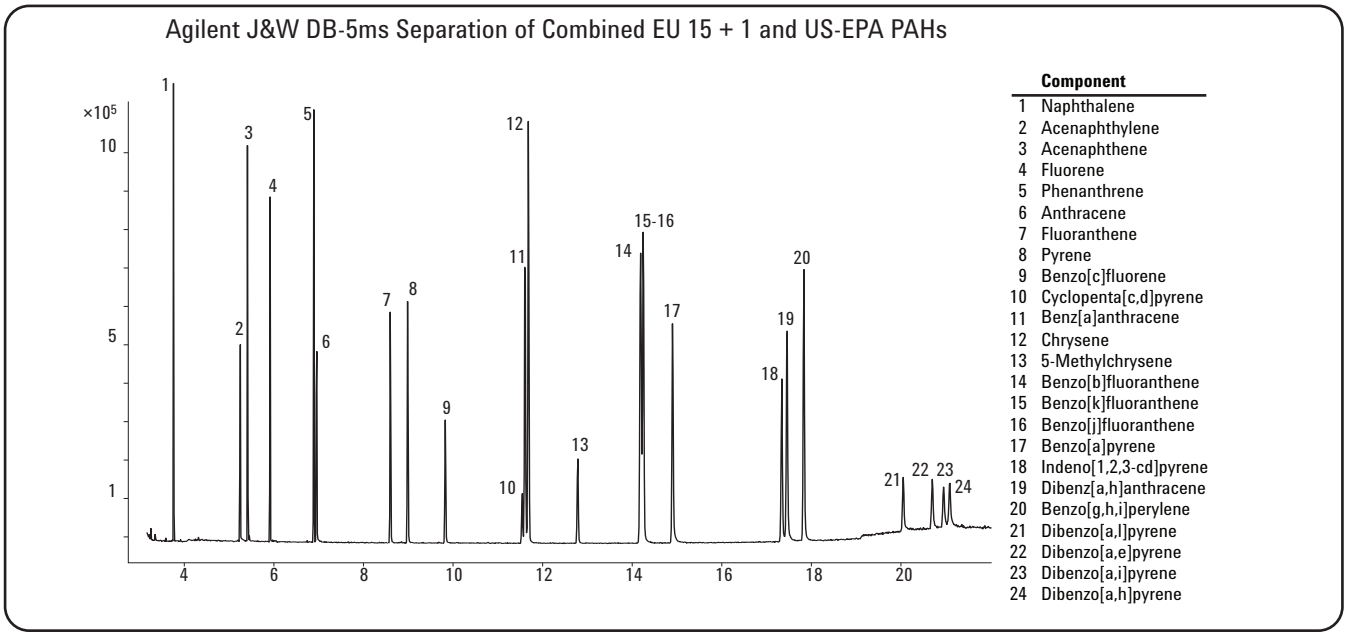

Figure 2. EU and US-EPA regulated PAH separation on an Agilent J&W DB-5 ms 20 × 0.18 mm, 0.18 µm (Agilent p/n 121-5522) column. Chromatographic conditions as in Table 3 and flow path supplies as in Table 4.

Factors to consider in optimizing EU 15 +1 and US-EPA PAH analyses

- Choose an Agilent J&W DB-EUPAH when the resolution of benzo[b,j,k]fluoranthene isomers (24 of 24 peaks in combined set) is required.
- Choose an Agilent J&W DB-5ms when benzo[b,j,k]fluoranthene isomers can be reported as a sum of the isomers. The Agilent J&W DB-5ms resolves 23 of 24 regulated PAHs in 27 % faster cycle time than the Agilent J&W DB-EUPAH column.
- Consider the use of retention gaps and inlet backflushing to reduce cycle time and maintenance.
- Achieve faster analysis times with no loss of resolution using 0.18 mm id high efficiency GC columns.
- Optimize injection volume, temperature, purge time, and solvent focusing for best results on your instrument.
- Minimize inlet and system dwell time with high linear velocities.
- Keep heated zones hot to avoid cold spots and signal loss.

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Printed in the USA
May 25, 2010
5990-5872EN

Agilent Technologies

Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS EN Kit by LC/MS/MS Detection

Application Note

Food Safety

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Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) EN sample preparation approach for extraction and cleanup of 13-pesticide residues representing various classes in spinach. Because spinach is considered a highly pigmented matrix, the EN dispersive SPE kit for highly pigmented fruits and vegetables is selected. Graphitized carbon black (GCB) in the amount of 7.5 mg/mL of ACN extract is added to the kit. The target pesticides in the spinach extracts are then determined by liquid chromatography coupled to an electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode. GCB is reported to have a significantly negative impact on the extraction of pesticides with planar structure. However, with the small amount of GCB addition in the EN dispersive SPE kit, our results show that the impact of GCB on planar pesticides is negligible and acceptable quantitation results are obtained. The 5 ng/g limit of quantitation (LOQ) for pesticides in spinach shown in this application is well below the maximum residue limits (MRLs). The spiking levels for the recovery experiments are 10, 50, and 200 ng/g. Mean recoveries range between 60 and 99% (85.4% on average), with an RSD below 11% (5.5% on average).

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Introduction

The EN QuEChERS method has been widely employed in the analysis of pesticides in food, especially in Europe. [1-2] The method uses acetonitrile extraction, followed by salting out water from the sample using anhydrous magnesium sulfate ($MgSO_4$), NaCl and buffering citrate salts to induce liquid-liquid partitioning. For cleanup, a dispersive solid phase extraction (dispersive SPE) is conducted using a combination of primary secondary amine (PSA) to remove fatty acids from among other components, and anhydrous $MgSO_4$ to reduce the remaining water in the extract. According to different food matrices, other ingredients may be added in this step, such as graphitized carbon black (GCB) to remove pigments and sterol, or C18 to remove more lipids and waxes.

Spinach is considered to be a highly pigmented vegetable since it contains high levels of chlorophyll. Therefore, the EN dispersive SPE kits for highly pigmented commodities were selected for this application. In these kits, besides 25 mg of PSA and 150 mg of $MgSO_4$, 7.5 mg of GCB is added per mL of ACN extracts. GCB adsorbs planar molecules like pigments and sterols; hence it is very helpful in cleaning up pigmented matrices like spinach. The efficiency of cleanup is dependent upon the amount of GCB used. The more GCB used, the more planar molecules are absorbed, and therefore, a cleaner sample matrix is obtained. The main difference between the EN method and AOAC method for cleaning up the highly pigmented matrix is the amount of GCB used in the dispersive SPE step. Instead of the relatively high amount of GCB used in AOAC method (50 mg of GCB per mL of ACN extracts), a much lower amount of GCB was used in the EN methods (2.5 mg of GCB per mL of ACN extracts for "pigmented" produce, or 7.5 mg of GCB per mL of ACN extracts for "highly pigmented" produce). The GCB impacted the extraction of planar pesticides differently, depending upon the method used. The AOAC method generated much cleaner final sample matrix but caused significant loss of planar pesticides; the EN method, on the contrary, caused little to no loss of planar pesticides but generated a more complicated sample matrix.

Previously, we described that a modified AOAC method with toluene addition in the dispersive SPE step greatly increased the extraction efficiency of planar pesticides in a pigmented matrix such as spinach. [3] Subsequently, we demonstrated the performance of SampliQ QuEChERS AOAC kit for the analysis of pesticides in spinach using combination of the modified (with toluene addition) and the original AOAC method (without toluene addition). [4, 5] In this study, 13 pesticides were used for evaluating the performance of the Agilent EN Buffered Extraction kit (p/n 5982-5650) and SampliQ QuEChERS EN Dispersive SPE kits for Highly Pigmented Fruits and Vegetables (p/n 5982-5321 and 5982-5356). The method was validated in terms of recovery and reproducibility. Table 1 shows the chemical and regulatory information for these pesticides in spinach.

Experimental

Reagents and Chemicals

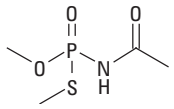
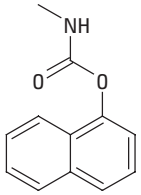
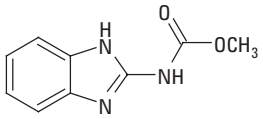
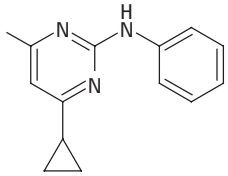
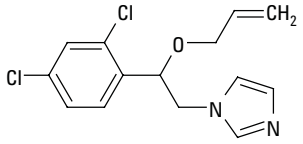
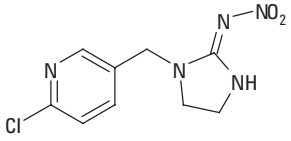
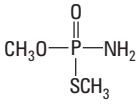
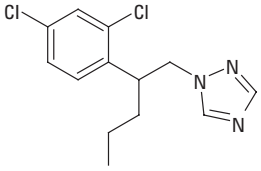
All reagents and solvents were HPLC or analytical grade. Methanol (MeOH), and toluene were from Honeywell (Muskegon, MI, USA). Acetonitrile (ACN), dimethyl sulfoxide (DMSO) and glacial acetic acid (HAc) were from Sigma-Aldrich (St Louis, MO, USA). Ammonium acetate (NH_4OAc) was from Fisher Chemicals (Fair Lawn, NJ, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards and internal standard (triphenyl phosphate, TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), ChemService (West Chester, PA, USA), Ultra (Kingstown, RI, USA), or AlfaAesar (Ward Hill, MA, USA).

Solutions and Standards

A 1 M ammonium acetate pH 5 stock solution was made by dissolving 19.27 g NH_4OAc powder in 250 mL Milli-Q water, pH adjusted to 5 with acetic acid monitored with a pH meter. The solution was stored at 4 °C. A 5 mM ammonium acetate in 20:80 MeOH/H_2O solution, pH 5, was made by combining 200 mL MeOH and 800 mL Milli-Q water, adding 5 mL of 1 M ammonium acetate pH 5 stock solution and mixing well. A 5 mM ammonium acetate in ACN was prepared by adding 5 mL of 1 M ammonium acetate pH 5 stock solution to 1 L ACN, mixing well and sonicating 5 min. A 1% formic acid in ACN solution was prepared by adding 1 mL of formic acid to 100 mL of ACN, and mixing well.

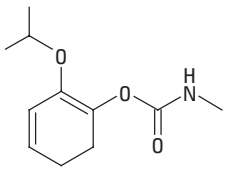
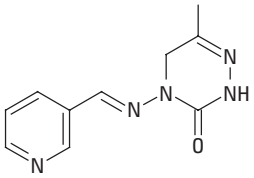
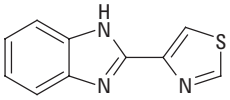
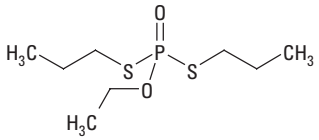
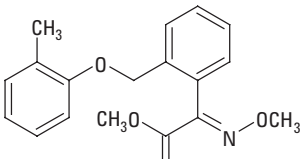
Standard and internal standard (IS) stock solutions (2.0 mg/mL for all except 0.5 mg/mL for carbendazim) were made in MeOH, 0.1% FA in ACN, or DMSO, respectively, and stored at -20 °C. Three QC spiking solutions of 1, 5, and 20 µg/mL, were made fresh daily in 1:1 ACN/H_2O with 0.1% FA. A 10 µg/mL standard spiking solution in 1:1 ACN/H_2O with 0.1% FA was made also for the preparation of a calibration curve in the matrix blank extract by appropriate dilution. A 15 µg/mL of TPP in 1:1 ACN/H_2O with 0.1% FA was made as an IS spiking solution.

Table 1. Pesticides Chemical and Regulatory Information [6–8]

Name	Class	Log P	pKa	Structure	MRLs in spinach (ng/g)*
Acephate	Organophosphate	–0.89	8.35		20
Carbaryl	Carbamate	2.36	10.4		50
Carbendazim	Benzimidazole	1.48	4.2		100
Cyprodinil	Anilinopyrimidine	4	4.44		500
Imazalil	Imidazole	3.82	6.53		20
Imidacloprid	Neonicotinoid	0.57	NA		1000
Methamidophos	Organophosphate	-0.79	NA		10
Penconazole	Triazole	3.72	1.51		50

(Continued)

Table 1. Pesticides Chemical and Regulatory Information [6–8]

Name	Class	Log P	pKa	Structure	MRLs in spinach (ng/g)*
Propoxur	Carbamate	0.14	NA		2000
Pymetrozine	Pyridine	–0.19	4.06		600
Thiabendazole	Benzimidazole	2.39	4.73 12.00 0		50
Ethoprophos	Organophosphate	2.99	NA		5
Kresoxim-methyl	Strobilurin	3.4	NA		50

*The MRLs numbers list in the table are for spinach or other vegetables. They could be higher in different commodities.

Equipment and Material

Agilent 1200 Series HPLC with Diode Array Detector (Agilent Technologies Inc., CA, USA).

Agilent 6410 triple quadrupole LC/MS system with Electrospray Ionization (Agilent Technologies Inc., CA, USA).

Agilent SampliQ QuEChERS EN Extraction kits, p/n 5982-5650, and SampliQ QuEChERS EN dispersive SPE kits for Highly Pigmented Fruits and Vegetables, p/n 5982-5321 and 5982-5356 (Agilent Technologies Inc., DE, USA).

CentraCL3R Centrifuge (Thermo IEC, MA, USA)

Bottle top dispenser (VWR, So. Painfield, NJ, USA)

Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)

Instrument Conditions

The previous LC/MS/MS method was used. [9]

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl, 3.0 x 150 mm, 3.5 µm (p/n 959963-312)
Flow rate:	0.3 mL/min
Column Temperature:	30 °C
Injection volume:	10 µL
Mobile Phase:	A, 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H_2O B, 5 mM ammonium acetate, pH 5.0 in ACN
Needle wash:	1:1:1:1 ACN/MeOH/IPA/H_2O w/0.2% FA.

Gradient:

Time	% Acetonitrile	Flow rate (mL/min)
0	20	0.3
0.5	20	0.3
8.0	100	0.3
10.0	100	0.3
13.0	STOP	

Post run:	4 min
Total cycle time:	17 min

MS conditions

Positive mode	
Gas temp.:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 Psi
Capillary:	4000 V

Other conditions relating to the analytes are listed in Table 2.

Sample Preparation

The sample preparation procedure includes sample comminution, extraction/partitioning and dispersive SPE cleanup. It was described in detail in the previous application notes. [9] The procedure used in spinach was similar to the one used in apple, except that the dispersive SPE kit was for highly pigmented produce rather than general fruits and vegetables.

Briefly, the frozen chopped organic spinach was homogenized thoroughly. A 10 g (±0.1g) of homogenized sample was placed into a 50 mL centrifuge tube. Samples were fortified with appropriate QC spiking solutions (100 µL) when necessary, and then 66.7 µL of IS spiking solution (15 µg/mL of TPP). After vortexing sample for 30 s, 10 mL of ACN was added to each tube using the dispenser. Tubes were then capped and shaken by hand for 1 min. To each tube, an Agilent SampliQ QuEChERS EN extraction salt packet (p/n 5982-5650), containing 4 g anhydrous $MgSO_4$, 1 g NaCl, 1 g Na_3Citrate, and 0.5 g Na_2HCitrate sesquihydrate, was added directly. Sample tubes were capped tightly, and hand-shaken vigorously for 1 min. Tubes were centrifuged at 4000 rpm for 5 min.

A 1 mL aliquot of upper ACN layer was transferred into Agilent SampliQ QuEChERS EN dispersive SPE 2 mL tube (p/n 5982-5321); or 6 mL aliquot into Agilent SampliQ QuEChERS EN dispersive SPE 15 mL tube (p/n 5982-5356). The 2 mL tube contains 25 mg of PSA, 150 mg of anhydrous $MgSO_4$ and 7.5 mg of GCB; while the 15 mL tube contains 150 mg of PSA, 900 mg of anhydrous $MgSO_4$ and 45 mg of GCB. The tubes were capped tightly and vortexed for 1 min. The 2 mL tubes were centrifuged with a micro-centrifuge at 13,000 rpm for 2 min, and the 15 mL tubes in a standard centrifuge at 4000 rpm for 5 min. A 200 µL aliquot of extract was transferred into an autosampler vial. An aliquot of 10 µL 1% FA in ACN was added immediately. Then 800 µL of water or appropriate standard solutions (prepared in water) were added. The samples were capped and vortexed thoroughly for LC/MS/MS analysis.

Table 2. Instrument Acquisition Data Used for the Analysis of 13 Pesticides by LC/MS/MS

Analyte	MRM channels (*m/z*)	Fragmentor (V)	CE (V)	RT (min)
Acephate	1) 184.0 > 94.9 2) 184.0 > 111.0	60	3 15	2.55
Methamidophos	1) 142.0 > 94.0 2) 142.0 > 124.9	60	8 8	2.54
Pymetrozine	1) 218.1 > 105.0 2) 218.1 > 78.0	115	20 50	2.97
Carbendazim	1) 192.1 > 160.0 2) 192.1 > 105.0	95	18 40	5.07
Imidacloprid	1) 256.1 > 209.1 2) 256.1 > 175.0	60	12 18	5.53
Thiabendazole	1) 202.1 > 175.0 2) 202.1 > 131.0	110	27 38	5.65
Propoxur	1) 210.1 > 111.0 2) 210.1 > 92.9	50	12 15	6.89
Carbaryl	1) 202.0 > 145.0 2) 202.0 > 115.0	50	3 40	7.30
Ethoprophos	1) 243.1 > 130.9 2) 243.1 > 172.9	80	15 15	8.50
Imazalil	1) 297.1 > 158.9 2) 297.1 > 200.9	80	22 15	8.52
Penconazole	1) 284.1 > 158.9 2) 284.1 > 172.9	80	32 32	8.95
Cyprodinil	1) 226.1 > 93.0 2) 226.1 > 108.0	120	35 35	9.23
Kresoxim methyl	1) 314.0 > 222.1 2) 314.0 > 235.0	70	10 10	9.44
TPP (IS)	1) 327.1 > 77.0 2) 327.1 > 151.9	70	45 45	9.49

1) Quantifier transition channel
2) Qualifier transition channel

Results and Discussion

The QuEChERS method for pesticide residue analysis provides high-quality results in a fast, easy, inexpensive approach. For the pigmented fruits and vegetables, the addition of GCB in the dispersive SPE tube can improve the removal of pigments and sterols. The cleaning efficiency of the method with GCB is related to the amount of GCB added. The more GCB used, the cleaner the matrix after treatment and less matrix interferences remaining in the final sample. Since GCB can also cause the removal of planar pesticides during the extraction procedure, smaller amounts of GCB used in the EN dispersive SPE step has less of an effect on the planar pesticides. Compared to the AOAC method, the EN method for pigmented produce uses much less GCB in the dispersive SPE step. For normal pigmented commodities like carrots and romaine lettuce, 2.5 mg of GCB can be used per mL of ACN extract; and for highly pigmented commodities like spinach or red sweet pepper, 7.5 mg of GCB can be used per mL of ACN extract. [1]

According to the recommendation, the EN dispersive SPE kit for highly pigmented products was used for spinach in our study. Given the highly pigmented kit, the amount of GCB used in the EN method is still much lower than that used in AOAC method, which is 50 mg of GCB per mL of ACN extract. Therefore, visually, the efficiency of matrix cleanup provided by the EN method was much weaker than that provided by AOAC method. The final sample processed by EN method still appeared dark green in color; while the previous final sample processed by AOAC method showed almost colorless transparency. The matrix blank differences are also shown in the UV chromatogram at λ = 254 nm shown in Figure 1. More interference peaks appear in the matrix blank processed by the EN method. Also more impurities may have accumulated

in the column or ionization source, which can have negative effects on the column and MS instrument. However, with the powerful selectivity provided by LC/MS/MS, the MRM chromatogram of matrix blank did not show any interference peaks to the target analytes. Figure 2 shows the LC/MS/MS chromatograms of matrix blank (IS spiked) and 50 ng/g fortified spinach extract processed by EN dispersive SPE method.

Four pesticides including Carbendazim, Thiabendazole, Cyprodinil, and Pymetrozine, with planar structure showed significant loss by the original AOAC dispersive SPE method. In addition, the modified method with toluene in the dispersive SPE step increased the extraction efficiency. [3,4] In order to investigate the impact of GCB on the planar pesticides, a comparison experiment with and without toluene addition in the dispersive SPE step for spinach samples fortified with the same level of pesticide standard (50 ng/g) was performed. The results showed little to no loss of planar pesticides caused by the small amount of GCB used in the EN method, and no significant improvement obtained by the addition of toluene. Therefore, the original EN method was employed for subsequent experiments. The method was validated in terms of recovery and reproducibility, and the quantitation results are discussed subsequently.

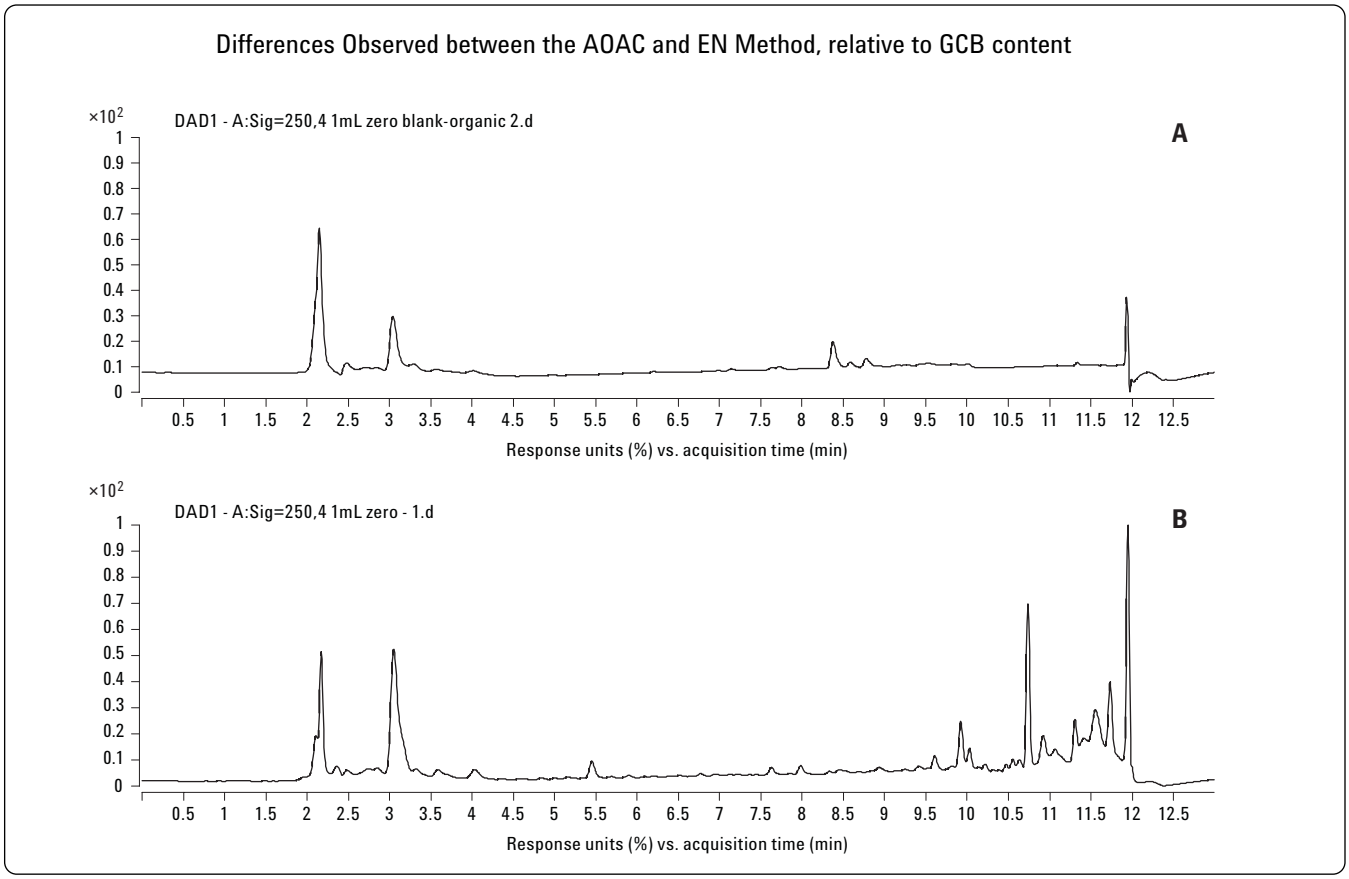


Figure 1. UV chromatogram (λ = 254nm) of spinach matrix blank processed by AOAC method (A) and EN method (B).

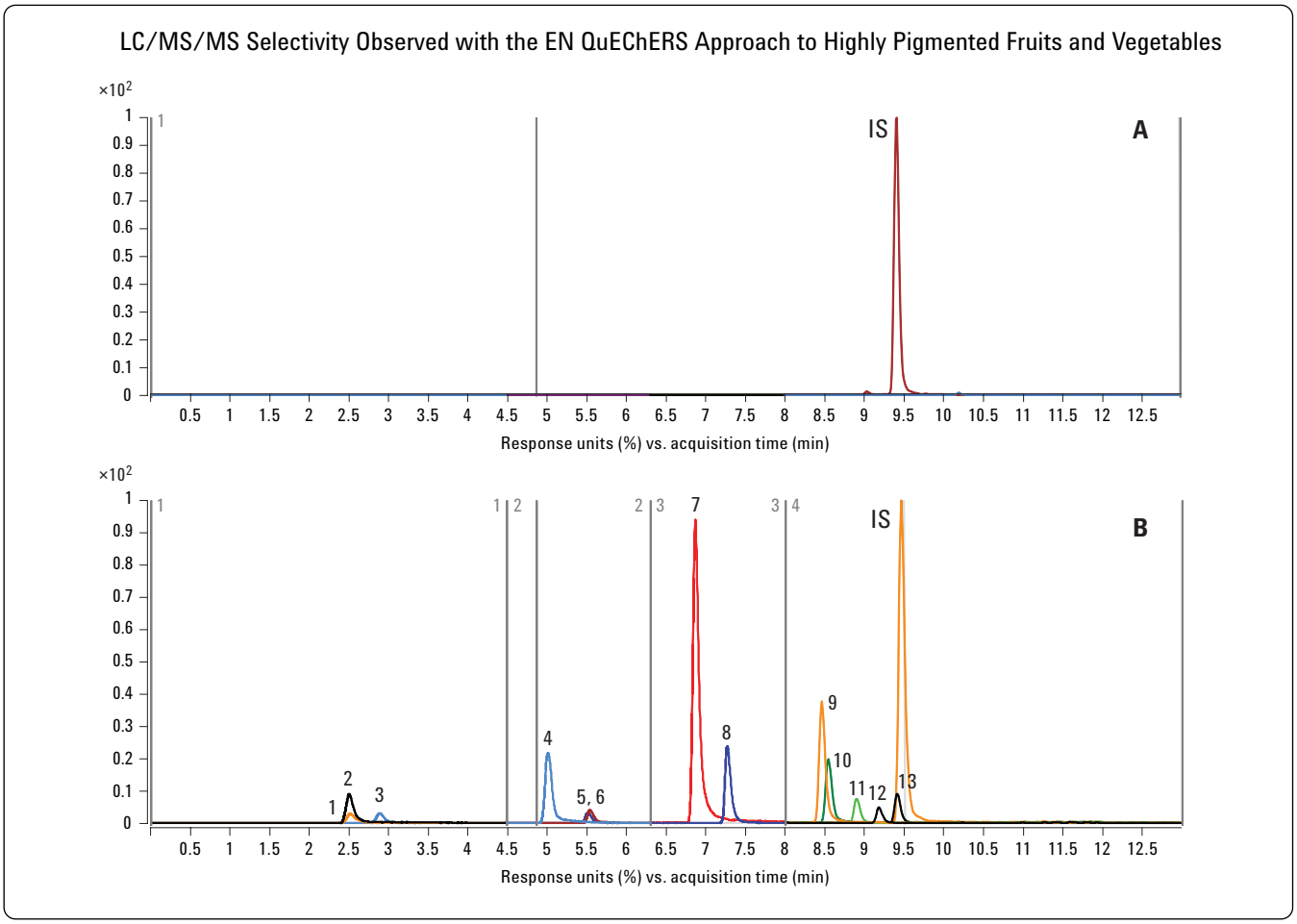


Figure 2. MRM chromatograms of spinach matrix blank (A) and 50 ng/g fortified sample (B) processed by EN method. Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid 6. Thiabendazole, 7. Propoxur, 8. Carbaryl, 9. Ethoprophos, 10. Imazalil, 11. Penconazole, 12. Cyprodinil, 13. Kresoxim methyl IS: Internal Standard, TPP.

Linearity and limit of quantification (LOQ)

The linear calibration range for all of the pesticides tested was 5–250 ng/g. Calibration curves, spiked in matrix blanks, were made at levels of 5, 10, 50, 100, 200, and 250 ng/g. The TPP was used as an internal standard at 100 ng/g. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). The 5 ng/g quantification limits LOQ (5 ppb) established for all of the pesticides is lower than the MRLs of these pesticides in fruits and vegetables. Table 3 shows the linear regression equation and correlation coefficient (R^2) for both 1 mL and 6 mL dispersive SPE.

Table 3. Linearity of Pesticides in Spinach Extract

Analytes	1 mL dispersive SPE Regression equation	R^2	6 mL dispersive SPE Regression equation	R^2
Methamidophos	Y = 0.2220X + 0.0005	0.9950	Y = 0.2244X + 0.0003	0.9893
Acephate	Y = 0.0814X + 0.0008	0.9972	Y = 0.0797X + 0.0005	0.9974
Pymetrozine	Y = 0.2063X + 0.0009	0.9559	Y = 0.1544X - 0.0006	0.9946
Carbendazim	Y = 0.9015X + 0.0164	0.9945	Y = 0.8526X + 0.0008	0.9917
Imidacloprid	Y = 0.0630X + 0.0001	0.9814	Y = 0.0682X - 0.0002	0.9952
Thiabendazole	Y = 0.3028X + 0.0059	0.9539	Y = 0.2315X + 0.0007	0.9968
Propoxur	Y = 1.3721X + 0.0018	0.9983	Y = 1.3304X + 0.0003	0.9981
Carbaryl	Y = 0.3459X + 0.0009	0.9968	Y = 0.3224X – 0.0003	0.9963
Ethoprophos	Y = 0.7588X - 0.0011	0.9979	Y = 0.7211X – 0.0023	0.9984
Imazalil	Y = 0.4644X + 0.0007	0.9889	Y = 0.4203X + 0.0002	0.9990
Penconazole	Y = 0.1647X – 0.0010	0.9937	Y = 0.1595X – 0.0008	0.9979
Cyprodinil	Y = 0.2575X + 0.0010	0.9884	Y = 0.2272X + 0.0007	0.9987
Kresoxim methyl	Y = 0.1175X – 0.0003	0.9976	Y = 0.1779X – 0.0008	0.9962

Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticides standards in comminuted spinach sample at levels of 10, 50 and 200 ng/g. These QC samples were quantitated against the matrix spiked calibration curve. The analysis was performed in replicates of six at each level. The recovery and reproducibility (shown as RSD) data of 1 mL and 6 mL dispersive SPE are shown in Tables 4 and Table 5, respectively. It can be seen from the results that the nine pesticides with non-planar structure give excellent recoveries (average of 90.4% for 1 mL and 94.3% for 6 mL) and precision (average of 4.7% RSD for 1 mL and 5.3% RSD for 6 mL). The four pesticides with planar structure give lower but still acceptable recovery (average of 71.8% for 1 mL and 79.8% for 6 mL) but good precision (average of 5.8% RSD for 1 mL and 4.8% RSD for 6 mL).

The impact of GCB on planar pesticides is visible and varies with different compounds. Cyprodinil gave excellent recovery and precision. Carbendazim gave excellent recovery and precision for low and mid level QCs, but poorer recovery for high level QC. Pymetrozine and thiabendazole gave lower recovery but still acceptable precision. The data in Table 6 show that the results of planar pesticides generated by EN method and AOAC modified method (with toluene addition) are comparable.

Table 4. Recovery and Reproducibility of Pesticides in Fortified Spinach with 1 mL Dispersive SPE Tube (p/n 5982-5321)

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Methamidophos	85.5	4.1	84.4	3.8	87.5	6.2
Acephate	83.7	8.3	84.6	5.9	91.6	5.8
Pymetrozine *	60.0	6.4	57.8	4.7	61.4	9.1
Carbendazim *	78.0	7.1	87.7	3.9	49.8	6.8
Imidacloprid	96.5	6.2	91.1	4.6	94.6	4.6
Thiabendazole *	64.3	7.0	71.5	6.5	71.5	5.8
Propoxur	93.7	4.7	92.0	4.1	86.7	4.3
Carbaryl	93.8	5.6	89.4	3.6	91.4	4.1
Ethoprophos	97.1	4.6	89.8	2.6	83.7	4.1
Imazalil	86.6	5.7	80.6	4.9	84.2	4.8
Penconazole	107.8	4.9	94.4	3.2	81.2	3.7
Cyprodinil *	89.6	4.4	88.6	4.5	80.8	3.9
Kresoxim methyl	101.5	3.8	94.6	1.4	92.8	3.8

* Pesticides with planar structure.

Table 5. Recovery and Reproducibility of Pesticides in Fortified Spinach with 6 mL Dispersive SPE Tube (p/n 5982-5356)

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Methamidophos	85.0	8.3	87.7	2.7	95.0	9.4
Acephate	88.6	5.1	84.6	3.1	94.6	9.3
Pymetrozine *	68.7	3.7	65.7	1.5	71.9	10.8
Carbendazim *	94.0	5.4	91.4	2.7	53.5	9.3
Imidacloprid	102.0	8.9	85.4	6.1	100.1	7.7
Thiabendazole *	77.2	4.4	77.6	2.4	79.2	9.7
Propoxur	98.2	5.7	96.3	1.8	93.9	7.2
Carbaryl	98.5	3.6	94.0	1.7	97.4	7.2
Ethoprophos	102.3	6.0	95.3	1.7	91.0	6.8
Imazalil	88.8	6.4	86.8	2.8	93.5	7.7
Penconazole	104.5	2.5	96.4	2.0	84.6	5.5
Cyprodinil *	101.5	4.2	92.2	2.4	86.8	7.6
Kresoxim methyl	99.7	6.1	97.4	1.6	95.3	6.9

* Pesticides with planar structure.

*Table 6. Results Comparison of Planar Pesticides Generated by EN Method and Modified AOAC Method (With Toluene Addition)**

Analytes	EN method for highly pigmented matrix		Modified AOAC method by toluene addition	
	Mean recovery (%)	Mean RSD (%)	Mean recovery (%)	Mean RSD (%)
Carbendazim	75.7	5.9	98.5	2.5
Cyprodinil	89.9	4.5	63.1	3.2
Pymetrozine	64.3	6.0	65.2	3.7
Thiabendazole	73.2	4.9	69.7	2.7

*The data can be found in reference [4].

Conclusions

Agilent SampliQ QuEChERS EN buffered extraction kits and dispersive SPE kits for highly pigmented fruits and vegetables provide a simple, fast and effective method for the purification of representative pesticides in spinach. The small amount of GCB used in dispersive SPE does not impact the extraction of planar pesticides significantly, which makes the extraction procedure in this highly pigmented matrix as simple as the one used in general fruit and vegetables. The recovery and reproducibility, based on matrix spiked standards, are acceptable for multiclass, multi-residue pesticide determination in spinach. However, the final extract matrix contains more impurities, which may result in more negative impacts on the column and MS instrument. The selected pesticides represent a broad variety of different classes and properties; therefore, the Agilent SampliQ QuEChERS EN Buffered Extraction and Dispersive kits for Highly Pigmented Fruits and Vegetables can be used for other pesticides in similar highly pigmented matrices.

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Printed in the USA
August 6, 2009
5990-4395EN

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Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS AOAC Kit by LC/MS/MS Detection

Application Note

Food Safety

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Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) AOAC sample preparation approach for the extraction and cleanup of 13 pesticide residues representing various pesticide classes in spinach. The original AOAC method employed involves initial extraction in a buffered aqueous/acetonitrile system, an extraction/partitioning step after the addition of salt, and a cleanup step using dispersive solid-phase extraction (dispersive SPE). In order to address the significant loss of planar pesticides caused by graphitized carbon black (GCB) in dispersive SPE, a modified method with the addition of toluene was employed. The presence of the target pesticides in the spinach extracts were then determined by liquid chromatography coupled to an electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode. With the combination of original and modified dispersive SPE, the method was validated in terms of recovery and reproducibility for all of the analytes of interest. The 5 ng/g limit of quantitation (LOQ) for pesticides in spinach shown in this application was well below the maximum residue limits (MRLs). The spiking levels for the recovery experiments were 10, 50, and 200 ng/g. Mean recoveries ranged between 64% and 108% (average of 91.9%), with RSD below 10% (average of 3.3%).

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Introduction

The AOAC QuEChERS method has been widely applied in the analysis of pesticides in food since it was introduced by USDA scientists. [1-3] In general, it contains two major steps: extraction and dispersive SPE cleanup. In the extraction step, the method uses a single step buffered 1% acetonitrile (ACN) extraction while simultaneously salting out water from the sample using anhydrous magnesium sulfate ($MgSO_4$) to induce liquid-liquid partitioning. For cleanup, a dispersive solid phase extraction (dispersive SPE) step is employed using a combination of primary secondary amine (PSA) to remove fatty acids as well as other components, and anhydrous $MgSO_4$ to reduce the remaining water in the extract. According to different food matrices, other ingredients may be added in this step, such as graphitized carbon black (GCB) to remove pigments and sterol, or C18 to remove more lipids and waxes.

Spinach is considered to be a highly pigmented vegetable since it contains high levels of chlorophyll. Therefore, the dispersive SPE kits with GCB were selected for further cleanup. In these kits, 50 mg of GCB per mL of ACN extracts are added to 50 mg of PSA and 150 mg of $MgSO_4$. GCB adsorbs planar molecules such as pigments and sterols. Therefore, it is helpful in the cleanup of pigmented matrices such as spinach. However, GCB also adsorbs pesticides with planar structure such as carbendazim, and thiabendazole. As a result, this type of dispersive SPE kit is not recommended for use with planar pesticides. This limitation will have a negative impact on the analysis of planar pesticides from pigmented matrices.

In the previous Application Note, [4] we discussed the impact of toluene addition to the dispersive SPE tube on the analysis of pesticides in pigmented matrix. This Application Note illustrated that this modification can greatly increase the extraction efficiency for problematic pesticides. GCB was employed for the analysis of planar pesticides in pigmented matrices such as spinach with the addition of toluene. In this study, 13 pesticides were used for evaluating the performance of the Agilent AOAC Buffered Extraction kit (p/n 5982-5755) and SampliQ QuEChERS AOAC Dispersive SPE kits for Pigmented Fruits and Vegetables (p/n 5982-5222 and 5982-5258). With the combination of original and modified dispersive SPE, the method was validated in terms of recovery and reproducibility. Table 1 shows the chemical and regulatory information for these pesticides in spinach.

Experimental

Reagents and Chemicals

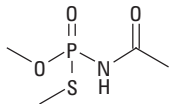
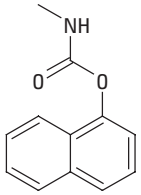
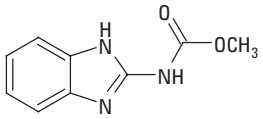
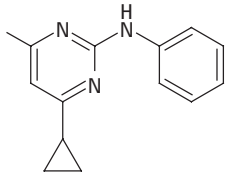
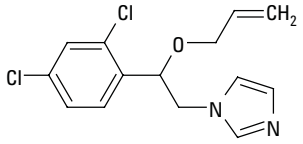
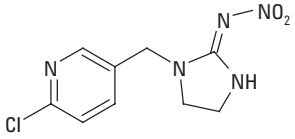
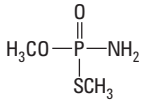
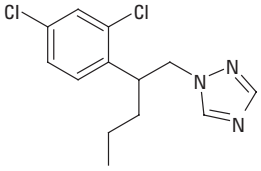
All reagents and solvents were HPLC or analytical grade. Methanol (MeOH), and toluene were from Honeywell (Muskegon, MI, USA). Acetonitrile (ACN), dimethyl sulfoxide (DMSO) and glacial acetic acid (HAc) were from Sigma-Aldrich (St Louis, MO, USA). Ammonium acetate (NH_4OAc) was from Fisher Chemicals (Fair Lawn, NJ, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards and internal standard, triphenyl phosphate, (TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), ChemService (West Chester, PA, USA), Ultra (Kingstown, RI, USA), or AlfaAesar (Ward Hill, MA, USA).

Solutions and Standards

The 1M NH_4OAc pH 5 stock solution was made by dissolving 19.27 g NH_4OAc powder in 250 mL Milli-Q water. The pH was adjusted to 5 with HAc monitored with a pH meter. The solution was stored at 4 °C. A 5 mM NH_4OAc solution in 20:80 MeOH/H_2O, pH 5, was made by combining 200 mL MeOH and 800 mL Milli-Q water, adding 5 mL of 1 M NH_4OAc pH 5 stock solution and mixing well. A 5 mM NH_4OAc in ACN solution was prepared by adding 5 mL of 1 M NH_4OAc pH 5 stock solution to 1 L ACN, mixing well and sonicating 5 min. A 1% HAc in ACN solution was prepared by adding 10 mL of HAc to 1 L of ACN, and mixing well.

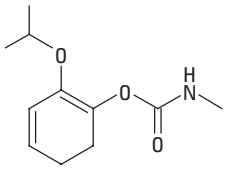
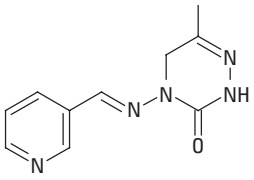
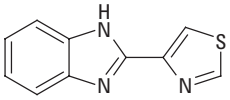
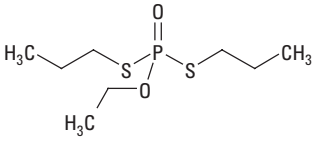
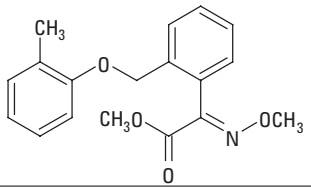
Standard and internal standard (IS) stock solutions (2.0 mg/mL for all, except 0.5 mg/mL for carbendazim) were made in MeOH, 0.1% FA in ACN, or DMSO, respectively, and stored at –20 °C. Three QC spiking solutions of 1.5, 7.5 and 30 µg/mL were made fresh daily in 1:1 ACN/H_2O containing 0.1% FA. A 10 µg/mL standard spiking solution in 1:1 ACN/H_2O containing 0.1% FA was made for the preparation of calibration curves in the matrix blank extract by appropriate dilution. A 15 µg/mL IS spiking standard of TPP was made in 1:1 ACN/H2O containing 0.1% FA.

Table 1. Pesticides Chemical and Regulatory Information [5–7]

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
Acephate	Organophosphate	–0.89	8.35		20
Carbaryl	Carbamate	2.36	10.4		50
Carbendazim	Benzimidazole	1.48	4.2		100
Cyprodinil	Anilinopyrimidine	4	4.44		500
Imazalil	Imidazole	3.82	6.53		20
Imidacloprid	Neonicotinoid	0.57	NA		1000
Methamidophos	Organophosphate	–0.79	NA		10
Penconazole	Triazole	3.72	1.51		50

(Continued)

Table 1. Pesticides Chemical and Regulatory Information [5–7]

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
Propoxur	Carbamate	0.14	NA		2000
Pymetrozine	Pyridine	–0.19	4.06		600
Thiabendazole	Benzimidazole	2.39	4.73 12.00 0		50
Ethoprophos	Organophosphate	2.99	NA		5
Kresoxim-methyl	Strobilurin	3.4	NA		50

*The MRLs numbers listed in the table are for spinach or other vegetables. They could be different in different commodities.

Equipment and Material

Agilent 1200 Series HPLC with Diode Array Detector (Agilent Technologies Inc., CA, USA).

Agilent 6410 triple quadrupole LC/MS/MS system with Electrospray Ionization (Agilent Technologies Inc., CA, USA).

Agilent SampliQ QuEChERS AOAC Extraction kits, p/n 5982-5755, and SampliQ QuEChERS AOAC dispersive SPE kits for Pigmented Fruits and Vegetables, p/n 5982-5222 and 5982-5258 (Agilent Technologies Inc., DE, USA).

CentraCL3R Centrifuge (Thermo IEC, MA, USA)

Bottle top dispenser (VWR, So. Painfield, NJ, USA)

Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)

Instrument Condition

The previous LC/MS/MS method was directly used. [8]

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl, 3.0 x 150 mm, 3.5 µm (p/n 959963-312)
Flow rate:	0.3 mL/min
Column Temperature:	30 °C
Injection volume:	10 µL
Mobile Phase:	A: 5 mM NH_4OAc, pH 5.0 in 20:80 $MeOH/H_2O$ B: 5 mM NH_4OAc, pH 5.0 in ACN
Needle wash:	1:1:1:1 ACN/MeOH/isopropyl alcohol (IPA)/H_2O w/0.2% FA.

Gradient:

Time	% B	Flow rate (mL/min)
0	20	0.3
0.5	20	0.3
8.0	100	0.3
10.0	100	0.3
10.01	20	0.5
13.0	STOP	

Post run:	4 min
Total cycle time:	17 min

MS conditions

Positive mode	
Gas temperature:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 psi
Capillary:	4000 V

Other conditions relating to the analytes are listed in Table 2.

Sample Preparation

The sample preparation procedure includes sample comminution, extraction/partitioning and dispersive SPE cleanup. It has been described in detail in previous Application Notes. [8] The procedure used in spinach was similar except for the addition of toluene to the dispersive SPE cleanup step.

Frozen chopped organic spinach was homogenized thoroughly. A 15 g (± 0.1g) amount of homogenized sample was placed into a 50 mL centrifuge tube. Samples were fortified with appropriate QC spiking solutions (100 µL) when necessary, and then 100 µL of IS spiking solution (15 µg/mL of TPP) were added. After vortexing sample for 30 s, 15 mL of 1% HOAc in ACN was added to each tube using the dispenser. To each tube, an Agilent SampliQ QuEChERS AOAC extraction salt packet (p/n 5982-5755) was added directly. Sample tubes were capped tightly, and hand-shaken vigorously for 1 min. Tubes were centrifuged at 4000 rpm for 5 min.

The ACN extracts were separated into two samples for both original and modified dispersive SPE methods. The modified dispersive SPE method follows a different procedure, therefore it is described below in detail. The volume of ACN extracts (~14 mL) was enough for simultaneously processing samples with original and modified dispersive SPE when using 2 mL size dispersive SPE tubes. When 15 mL size tubes were used, 14 mL of ACN extracts from one sample was not enough for processing dispersive SPE by two methods simultaneously. Therefore, another sample was extracted from the beginning.

A 1 mL aliquot of the upper ACN layer was transferred into an Agilent SampliQ QuEChERS dispersive SPE 2 mL tube (p/n 5982-5222); or 8 mL aliquot into an Agilent SampliQ QuEChERS dispersive SPE 15 mL tube (p/n 5982-5258). The 2 mL tube contained 50 mg of PSA, 50 mg of GCB and 150 mg of anhydrous $MgSO_4$; while the 15 mL tube contained 400 mg of PSA, 400 mg of GCB and 1200 mg of anhydrous $MgSO_4$. Next, 375 µL of toluene were added to the 2 mL tube, or 3 mL of toluene was added to 15 mL tube. The tubes were tightly capped and vortexed for 1 minute. The tubes were vortexed for a few seconds before sample addition, to prevent agglomerates. The 2 mL tubes were centrifuged with a microcentrifuge at 13,000 rpm for 2 min, and the 15 mL tubes in a standard centrifuge at 4000 rpm for 5 min. An 825 µL amount

Table 2. Instrument Acquisition Data for the Analysis of 13 Pesticides by LC/MS/MS

Analyte	MRM channels (*m/z*)	Fragmentor (V)	CE (V)	RT (min)
Acephate	1) 184.0 > 94.9 2) 184.0 > 111.0	60	3 15	2.55
Methamidophos	1) 142.0 > 94.0 2) 142.0 > 124.9	60	8 8	2.54
Pymetrozine	1) 218.1 > 105.0 2) 218.1 > 78.0	115	20 50	2.97
Carbendazim	1) 192.1 > 160.0 2) 192.1 > 105.0	95	18 40	5.07
Imidacloprid	1) 256.1 > 209.1 2) 256.1 > 175.0	60	12 18	5.53
Thiabendazole	1) 202.1 > 175.0 2) 202.1 > 131.0	110	27 38	5.65
Propoxur	1) 210.1 > 111.0 2) 210.1 > 92.9	50	12 15	6.89
Carbaryl	1) 202.0 > 145.0 2) 202.0 > 115.0	50	3 40	7.30
Ethoprophos	1) 243.1 > 130.9 2) 243.1 > 172.9	80	15 15	8.50
Imazalil	1) 297.1 > 158.9 2) 297.1 > 200.9	80	22 15	8.52
Penconazole	1) 284.1 > 158.9 2) 284.1 > 172.9	80	32 32	8.95
Cyprodinil	1) 226.1 > 93.0 2) 226.1 > 108.0	120	35 35	9.23
Kresoxim methyl	1) 314.0 > 222.1 2) 314.0 > 235.0	70	10 10	9.44
TPP (IS)	1) 327.1 > 77.0 2) 327.1 > 151.9	70	45 45	9.49

1) Quantifier transition channel
2) Qualifier transition channel

of extract was then transferred into another tube, and dried by N_2 flow. Samples were reconstituted into 600 µL of ACN containing 0.1%FA. After vortexing and sonicating, 200 µL of extract were transferred into an autosampler vial, and then 800 µL of water or other appropriate standard solution (prepared in water) were added. The samples were capped and vortexed thoroughly for LC/MS/MS analysis.

Another aliquot of ACN extracts was processed following the original dispersive SPE clean-up procedure. Figure 1 shows the flow chart of the whole extraction procedure (original and modified dispersive SPE) for a spinach sample.

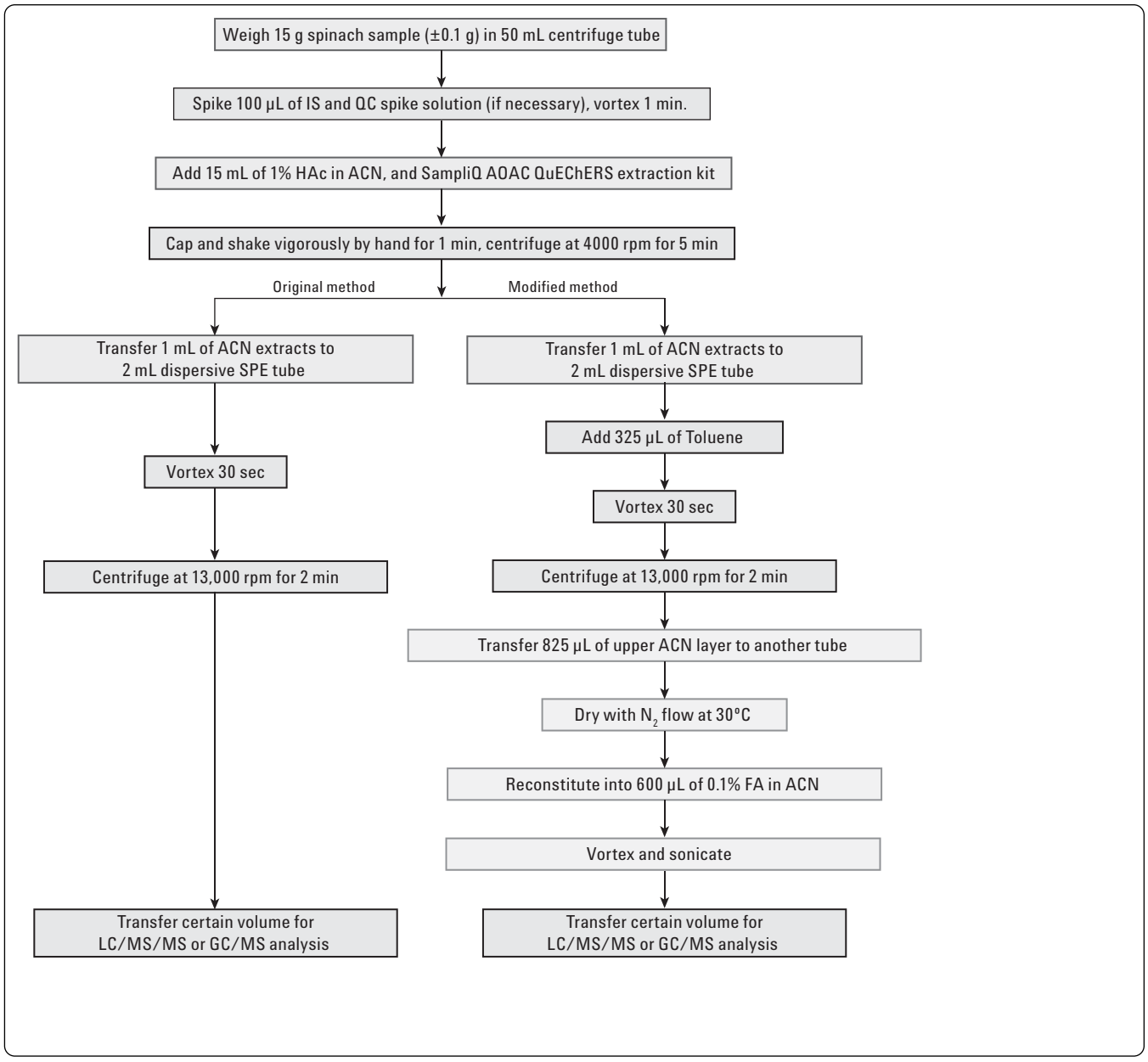


Figure 1. Flow chart of the QuEChERS AOAC extraction procedure (original and modified dispersive SPE, 2 mL size) for a spinach sample.

Results and Discussion

QuEChERS method for pesticide residues analysis provides high-quality results in a fast, easy, inexpensive approach. For the pigmented fruits and vegetables, the addition of GCB in the dispersive SPE tube can improve the removal of pigments and sterols. Toluene was added to increase the extraction efficiency of planar pesticides. Previously it was established that the addition of toluene produces a yellow final sample, indicating that matrix impurities are retained. [4] However, with the powerful selectivity provided by LC/MS/MS, there have not been any chromatographic differences found between the samples processed with the original and modified methods. Figures 2 and 3 show the LC/MS/MS chromatograms of matrix blank (IS spiked) and 50 ng/g fortified spinach extract processed by original and modified dispersive SPE method.

Four pesticides with planar structure showed significant loss by the original dispersive SPE method. The modified method with toluene addition increased the recovery of those four pesticides by two to three times, from 20% to 40% and 60% to 100%. In addition, the repeatability improved from >15% to <5% RSD. The addition of toluene had no affect on the quantitation results of other pesticides. Therefore, the results from the original method for high recovered pesticides were combined with the results from modified method for planar pesticides. The method was validated in terms of recovery and reproducibility, and the quantitation results are discussed.

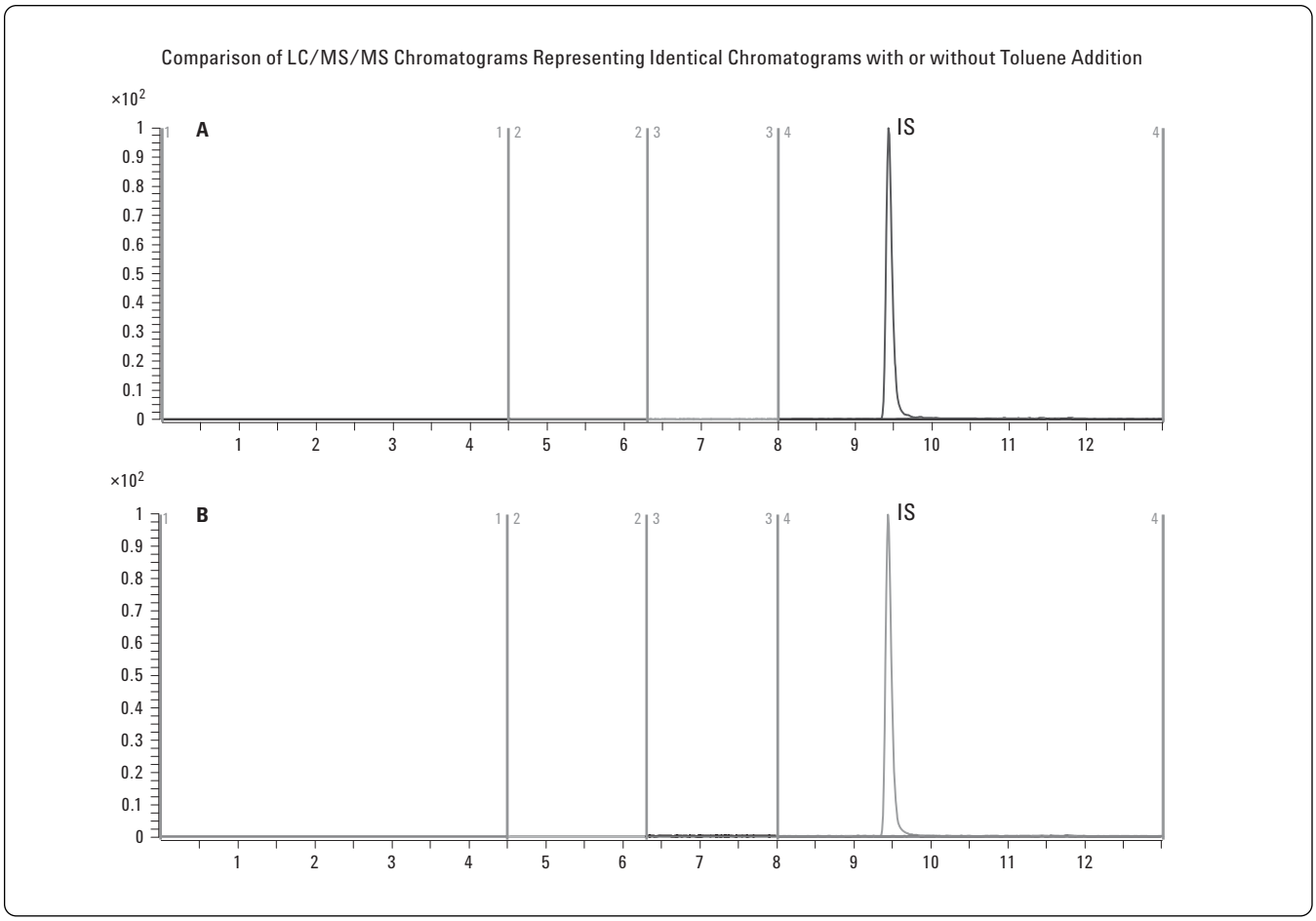


Figure 2. LC/MS/MS chromatograms of spinach matrix blank processed by original dispersive SPE (A) and modified dispersive SPE (B). IS: Internal Standard TPP.

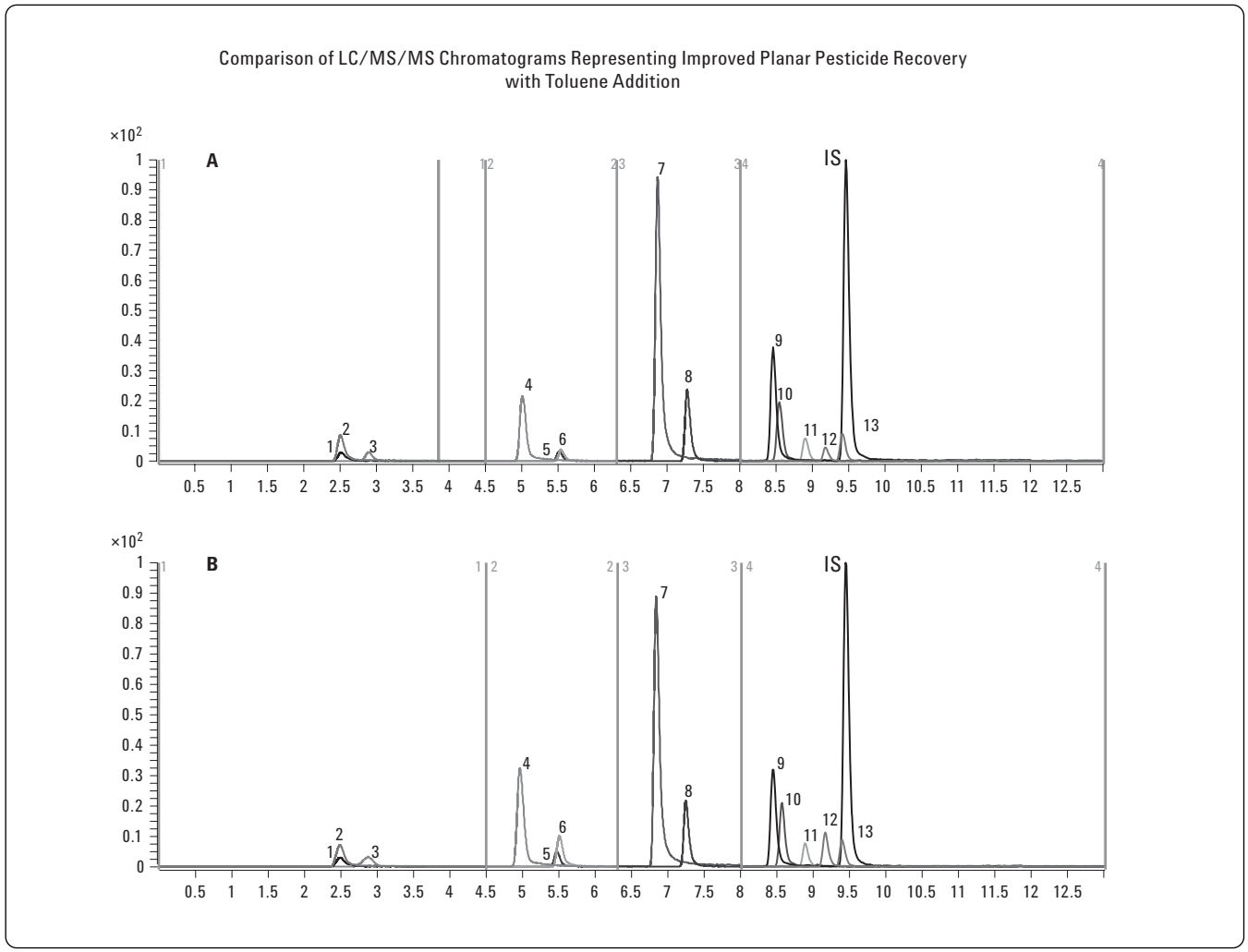


Figure 3. LC/MS/MS chromatograms of 50 ng/g fortified spinach sample extracts processed by original dispersive SPE (A) and modified dispersive SPE (B). Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid 6. Thiabendazole, 7. Propoxur, 8. Carbaryl, 9. Ethoprophos, 10. Imazalil, 11. Penconazole, 12. Cyprodinil, 13. Kresoxim methyl IS: Internal Standard, TPP.

Linearity and Limit of Quantification (LOQ)

The linear calibration range for all of the pesticides was 5–250 ng/g. For samples processed by original and modified methods, the corresponding matrix blanks were used to prepare the calibration curves respectively. Calibration curves, spiked in matrix blanks, were made at levels of 5, 10, 50, 100, 200, and 250 ng/g. The TPP was used as an internal standard at 100 ng/g. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte / peak area of IS) to the relative concentration of analytes (concentration of analyte / concentration of IS). The 5 ng/g quantification limits LOQ (5 ppb) established for all of the pesticides was lower than or equal to the MRLs of these pesticides in fruits and vegetables. Table 3 shows the linear regression equation and correlation coefficient (R^2) for both 1 mL and 8 mL dispersive SPE.

Table 3. Linearity of Pesticides in Spinach Extract

Analytes	1 mL dispersive-SPE Regression equation	R^2	8 mL dispersive-SPE Regression equation	R^2
Methamidophos	Y = 0.2358X – 0.0008	0.9976	Y = 0.2164X – 0.0014	0.9983
Acephate	Y = 0.0862X – 0.0003	0.9975	Y = 0.0804X – 0.0006	0.9942
Pymetrozine *	Y = 0.2073X – 0.0002	0.9995	Y = 0.2034X – 0.0013	0.9978
Carbendazim *	Y = 0.8375X + 0.0032	0.9915	Y = 0.8383X + 0.0002	0.9982
Imidacloprid	Y = 0.0652X – 0.0007	0.9905	Y = 0.0620X – 0.0011	0.9742
Thiabendazole *	Y = 0.4081X - 0.0008	0.9995	Y = 0.4102X – 0.0011	0.9975
Propoxur	Y = 1.9253X – 0.0042	0.9995	Y = 1.8253X – 0.0037	0.9996
Carbaryl	Y = 0.4243X – 0.0013	0.9979	Y = 0.3993X – 0.0019	0.9946
Ethoprophos	Y = 0.7859X – 0.0012	0.9983	Y = 0.7420X – 0.0012	0.9985
Imazalil	Y = 0.4586X + 0.0002	0.9954	Y = 0.4229X + 0.0005	0.9903
Penconazole	Y = 0.1643X – 0.0014	0.9923	Y = 0.1468X – 0.0003	0.9944
Cyprodinil *	Y = 0.3274X – 0.0024	0.9904	Y = 0.3067X – 0.0013	0.9978
Kresoxim methyl	Y = 0.1809X – 0.0015	0.9975	Y = 0.1659X – 0.0008	0.9928

* Results from modified dispersive SPE method.

Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticides standards in communited spinach sample at levels of 10, 50 and 200 ng/g. These QC samples were quantitated against the matrix spiked calibration curve. The analysis was performed six times at each level. The recovery and reproducibility (shown as RSD) data of 1 mL and 8 mL volume dispersive SPE are shown in Tables 4 and 5, respectively. The results show that the nine pesticides processed with the original method resulted in excellent recoveries (average of 97.8% for 1 mL and 103.4% for 8 mL) and precision (average of 3.6% RSD for 1 mL and 4.3% RSD for 8 mL). Although the four pesticides processed with the modified method resulted in lower recoveries (average of 78.5% for 1 mL and 69.7% for 8 mL) but high precision (average of 2.7% RSD for 1 mL and 3.3% RSD for 8 mL). The results from the modified method were much better than the results obtained by original methods. Please refer to previous Application Note [4] for a detailed discussion.

Table 4. Excellent Recovery and Reproducibility of Pesticides in Fortified Spinach with a 1 mL volume, 2 mL Dispersive SPE Tube (p/n 5982-5222)

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Methamidophos	91.8	4.2	93.3	3.7	93.8	5.7
Acephate	93.4	3.3	91.3	5.6	101.9	7.8
Pymetrozine *	74.0	2.9	71.1	3.2	70.3	2.9
Carbendazim *	105.3	4.0	109.1	2.5	88.9	1.7
Imidacloprid	98.2	4.5	100.4	3.7	100.0	2.7
Thiabendazole *	79.0	2.7	76.6	2.3	75.5	1.8
Propoxur	100.0	1.7	98.1	3.5	93.0	4.0
Carbaryl	110.8	3.2	108.1	1.0	105.1	3.2
Ethoprophos	98.8	1.6	98.2	3.3	95.1	3.1
Imazalil	84.0	3.8	89.6	2.5	89.8	1.7
Penconazole	103.1	5.4	98.4	3.5	97.2	1.9
Cyprodinil *	69.1	4.7	62.0	2.9	61.3	1.1
Kresoxim methyl	104.4	4.8	101.2	5.0	102.6	3.0

* Results from modified dispersive SPE method.

Table 5. Excellent Recovery and Reproducibility of Pesticides in Fortified Spinach with a 8 mL volume, 15 mL Dispersive SPE Tube (p/n 5982-5258)

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Methamidophos	98.6	3.8	94.2	7.1	97.8	2.9
Acephate	95.5	8.9	91.5	6.3	105.6	5.7
Pymetrozine *	62.4	4.3	53.9	3.4	59.3	5.4
Carbendazim *	95.7	1.6	98.6	1.9	93.3	2.9
Imidacloprid	112.7	4.2	107.6	7.7	110.4	3.7
Thiabendazole *	58.0	3.5	62.1	3.3	66.8	2.8
Propoxur	104.9	1.4	103.3	3.7	99.0	3.3
Carbaryl	116.9	2.2	114.6	2.4	110.8	2.1
Ethoprophos	105.3	2.5	105.7	2.8	103.0	2.3
Imazalil	86.3	3.9	94.9	4.3	93.9	3.4
Penconazole	103.5	10.4	106.9	3.6	99.2	6.4
Cyprodinil *	63.1	2.8	60.6	4.8	62.7	2.9
Kresoxim methyl	111.2	4.5	106.6	3.2	112.0	3.0

* Results from modified dispersive SPE method.

Figure 4 shows the recovery and precision results obtained by 1 mL and 8 mL volume dispersive SPE. To simplify the comparison, the average recovery and precision of three fortification concentrations were used for all pesticides. The results of two dispersive SPE cleanup approaches appeared to be independent of volume used. Apparently, both approaches provided efficient and similar sample cleanup, and thus generated relatively equivalent results. However, if 8 mL size dispersive SPE volume is used, two duplicated extractions must be performed initially to complete both original and modified dispersive SPE. If 1 mL size dispersive SPE is used, only one extraction is needed to provide enough volume to perform both original and modified dispersive SPE simultaneously. This is more cost effective saving time, sample amount, and labor. The extractions can be performed according to the user's requirements and regulations.

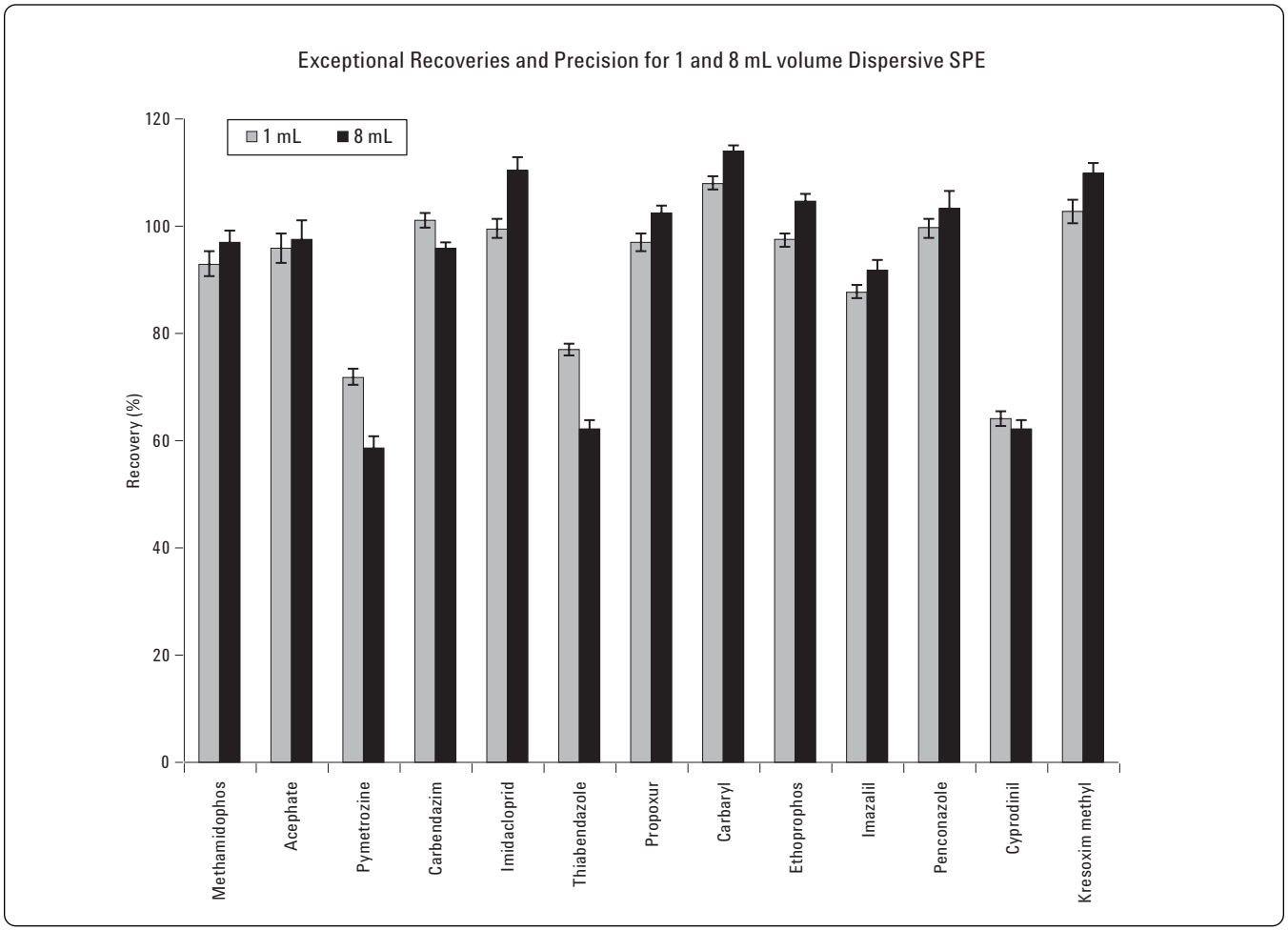


Figure 4. The recovery and precision results for 1 mL dispersive SPE and 8 mL dispersive SPE.

Conclusions

Agilent SampliQ QuEChERS AOAC buffered extraction kits and dispersive SPE kits for pigmented fruits and vegetables provide a simple, fast and effective method for the purification of representative pesticides in spinach. The modified dispersive SPE method with the addition of toluene provides a very useful way to limit the loss of planar pesticides caused by GCB in dispersive SPE. The recovery and reproducibility of this method, based on matrix spiked standards, were acceptable for multiclass, multi-residue pesticide determination in spinach. The impurities and matrix effects from spinach were minimal and did not interfere with the quantitation of any target compound. As the selected pesticides represented a broad variety of different classes and properties, the Agilent SampliQ QuEChERS AOAC Buffered Extraction and Dispersive kits for Pigmented Fruits and Vegetables can be used for other pesticides in other similar pigmented matrices.

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Printed in the USA
August 6, 2009
5990-4248EN

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Optimizing Recoveries of Planar Pesticides in Spinach Using Toluene and Agilent SampliQ AOAC QuEChERS Kits with Graphitized Carbon

Application Note

Food Safety

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Abstract

This application note describes the impact of toluene addition in the dispersive solid phase extraction (SPE) step on the analysis of pesticides in spinach using Agilent SampliQ QuEChERS AOAC kits for highly pigmented fruits and vegetables. Graphitized carbon black (GCB) is required in the dispersive SPE kits in order to remove high levels of pigments from the matrix. However, it also retains pesticides with planar structures resulting in poor recovery and precision. The eight problematic pesticides found in the original AOAC method, by either LC/MS/MS or GC/MS, generated poor results with about 20% to 60% recovery with >15% relative standard deviation (RSD). In the modified AOAC method, an aliquot of toluene was added to the dispersive SPE clean-up tube, in a ratio of 8:3 (acetonitrile (ACN) extracts/toluene). It significantly improved the extraction efficiency of the problematic planar pesticides. With the modified AOAC method, the eight problematic pesticides generated substantially improved recoveries, 50% to 100%, and < 10% RSD. However, the addition of toluene also introduced more matrix impurities into the final sample, and caused problems for some pesticides which gave good results originally. Therefore, the modified AOAC method cannot be considered a "drop in" replacement for the original AOAC method; but it can be a very useful alternative for the problematic pesticides affected by GCB in the pesticides analysis of highly pigmented matrix.

Agilent Technologies

Introduction

The AOAC quick, easy, cheap, effective, rugged, safe (QuEChERS) method has been widely applied in the analysis of pesticides in food since it was introduced by USDA scientists. [1-3] In general, it contains two major steps: extraction and dispersive SPE clean-up. In the extraction step, the method uses a single-step buffered acetonitrile (1% HAc) extraction while simultaneously salting out water from the sample using anhydrous magnesium sulfate ($MgSO_4$) to induce liquid-liquid partitioning. For cleanup, a dispersive solid phase extraction (dispersive SPE) step is employed using a combination of primary secondary amine (PSA) to remove polar organic acids as well as other components, and anhydrous $MgSO_4$ to reduce the remaining water in the extract. After mixing and centrifugation, the upper layer is ready for analysis.

Various food matrices require modifications to the dispersive SPE clean-up step. For general fruits and vegetables, 50 mg PSA and 150 mg $MgSO_4$, per mL of ACN extracts are used for clean-up to remove polar organic acids, some sugars and lipids, and excess water. Pigmented fruits and vegetables kits, besides PSA and $MgSO_4$, include 50 mg GCB per mL of ACN extracts to remove pigments like chlorophyll and carotinoides. For fruits and vegetables with fats and waxes, 50 mg C18 per mL of ACN extracts is added with PSA and $MgSO_4$ removing lipids and sterols. Therefore, according to the food matrix, analysts need to select a suitable dispersive SPE kit in order to analyze pesticides of interest.

Previously, we demonstrated the excellent performance of SampliQ QuEChERS AOAC buffered AOAC extraction kits and dispersive SPE kits for general fruits and vegetables on a representative group of pesticides in apple by LC/MS/MS and GC/MS. [4, 5] For the SampliQ QuEChERS AOAC kits for pigmented fruits and vegetables, spinach was selected as the matrix in order to evaluate the extraction and performance of the dispersive kit. GCB was added to the dispersive SPE kit to remove the high level of pigments, such as chlorophyl and carotinoides, which can cause more matrix effect and introduce more interferences. Conversely, GCB can cause a significant loss of planar pesticides, for example, thiobendazole, chlorothalonil, coumaphos, cyprodinil. [3, 6] Therefore, the use of GCB is recommended when planar pesticides are not being analyzed; greatly limiting the usefulness of GCB to the clean-up of pigmented matrix. In previous GCB SPE column extractions [7], solvent mixtures containing toluene were commonly used to elute pesticides through GCB columns. ACN/toluene (3:1) mixtures have been used for the multiclass multiresidue method (MRM) elution of pesticides through tandem GCB-NH_2 [8], GCB-PSA [9], and GCB SAX-PSA. [10] In this study, toluene was added into the ACN extracts in the second step of QuEChERS, the dispersive SPE clean-up. We determined that the ratio of 8:3 (ACN extract toluene) generated higher recoveries (50% to 300% higher), and substantially better precision (< 10% RSD) for the eight GCB retained pesticides. However, it was noted that the addition of toluene caused adverse affects, such as additional matrix impurities in the final extracted samples, lower recovery and higher imprecision for certain pesticides which originally produced good results without the addition of toluene.

Experimental

Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. Methanol (MeOH), and toluene were from Honeywell (Muskegon, MI, USA). Acetonitrile (ACN), dimethyl sulfoxide (DMSO) and acetic acid, glacial (HAc) were from Sigma-Aldrich (St Louis, MO, USA). Ammonium acetate (NH_4OAc) was from Fisher Chemicals (Fair Lawn, NJ, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards and internal standard triphenyl phosphate, (TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), ChemService (West Chester, PA, USA), Ultra (North Kingstown, RI, USA), or AlfaAesar (Ward Hill, MA, USA).

Solutions and Standards

The 1 M ammonium acetate pH 5 stock solution was made by dissolving 19.27 g NH_4OAc powder in 250 mL Milli-Q water, and the pH adjusted to 5 with glacial acetic acid. The solution was stored at 4 °C. Methanol/H_2O (20:80) containing 5 mM ammonium acetate pH 5 was made by combining 200 mL MeOH and 800 mL Milli-Q water, adding 5 mL of 1M ammonium acetate pH 5 stock solution and mixing well. A 5 mM ammonium acetate in ACN solution was prepared by adding 5 mL of 1 M ammonium acetate pH 5 stock solution to 1 L ACN, mixing well and sonicating 5 min. 1% HAc in ACN was prepared by adding 10 mL of glacial acetic acid to 1 L of ACN, and mixing well.

Standard and internal standard (IS) stock solutions (2.0 mg/mL for all, except 0.5 mg/mL for carbendazim) were made in MeOH, 0.1% FA in ACN, or DMSO, respectively, and stored at –20°C. Three QC spiking solutions of 1.5, 7.5, and 30 µg/mL were made fresh daily in 1:1 ACN/H_2O containing 0.1% FA. A 10 µg/mL standard spiking solution in 1:1 ACN/H_2O containing 0.1% FA was made for preparation of LC/MS/MS calibration curves in the matrix blank extract by appropriate dilution.

A 2.5 µg/mL standard solution in ACN containing 0.1% FA was used to prepare the GC/MS calibration curves in the matrix blank extract by appropriate dilution. A 15 µg/mL IS spiking standard of TPP in 1:1 ACN/H_2O containing 0.1% FA was made.

Equipment and material

Agilent 1200 Series HPLC with Diode Array Detector (Agilent Technologies Inc., CA, USA).

Agilent 6410 triple quadrupole MS/MS system with Electrospray Ionization (Agilent Technologies Inc., CA, USA).

Agilent Gas Chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA).

Agilent 5975C Mass Spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA).

Agilent SampliQ QuEChERS AOAC Extraction kits, p/n 5982-5755, and SampliQ QuEChERS AOAC dispersive SPE kits for Pigmented Fruits and Vegetables, p/n 5982-5222 and 5982-5258 (Agilent Technologies Inc., DE, USA).

CentraCL3R Centrifuge (Thermo IEC, MA, USA)

Bottle top dispenser (VWR, South Painfield, NJ, USA)

Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)

Instrument Conditions

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl, 3.0 × 150 mm, 3.5 µm (p/n: 959963-312)
Flow rate:	0.3 mL/min
Column temperature:	30°C
Injection volume:	10 µL
Mobile phase:	A: 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H2O; B: 5 mM ammonium acetate, pH 5.0 in ACN
Needle wash:	1:1:1:1 ACN/MeOH/IPA/H2O w/0.2% FA.

Gradient:

Time	% B	Flow rate (mL/min)
0	20	0.3
0.5	20	0.3
8.0	100	0.3
10.0	100	0.3
10.01	20	0.5
13.0	STOP	

Post run:	4 min
Total cycle time:	17 min.

GC conditions

Inlet:	Splitless
Inlet liner:	Helix double taper, deactivated (p/n: 5188-5398)
Carrier gas:	Helium
Inlet pressure:	19.6 psi (constant pressure mode) during run 1.0 psi during back flush
Inlet temperature:	250 °C
Injection volume:	1.0 µL
Purge flow to split vent:	30 mL/min at 0.75 min
Oven temperature program:	70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C /min to 200 °C (0 min), 16 °C/min to 280 °C (6 min)
Post run:	3 min
Capillary flow technology:	Purged Ultimate Union (p/n: G3186B) – used for backflushing the analytical column and inlet.
Aux EPC gas:	Helium plumbed to Purged Ultimate Union
Aux EPC pressure:	4.0 psi during run, 80.0 psi during backflush
Column:	Agilent J&W HP-5ms Ultra Inert 15 m × 0.25 mm, 0.25 µm (p/n: 19091S-431UI)
Connections:	Between inlet and Purged Ultimate Union (p/n: G3186B)
Restrictor:	65 cm x 0.15 mm, 0.15 µm DB-5 ms Ultra Inert.
Connections:	Between the Purged Ultimate Union and the MSD.

For the instrument acquisition data of MS/MS in LC/MS/MS and MS in GC/MS relating to the analytes, please refer to the acquisition data table in the previous Agilent publications. [4, 11]

Sample Preparation

The sample preparation procedure includes sample comminution, extraction/partitioning and dispersive SPE clean-up. The QuEChERS method employing spinach as the vegetable matrix is similar to the method described in detail in previous application notes [4,5], with the exception of the dispersive SPE step which includes a toluene addition.

The frozen chopped organic spinach was homogenized thoroughly. Fifteen grams (± 0.1g) of homogenized sample was placed into 50 mL centrifuge tubes. Samples were fortified with appropriate QC spiking solutions (100 µL) if necessary, and then 100 µL of IS spiking solution (15 µg/mL of TPP). After vortexing the samples for 30 s, 15 mL of 1% HAc in ACN was added to each tube. An Agilent SampliQ QuEChERS AOAC extraction salt packet (p/n 5982-5755) was added directly to each tube. Sample tubes were sealed tightly, and hand-shaken vigorously for 1 min. Tubes were centrifuged at 4,000 rpm for 5 min.

A 1 mL aliquot of the upper ACN layer was transferred into an Agilent SampliQ QuEChERS dispersive SPE 2 mL tube (p/n 5982-5222); or an 8 mL aliquot was transferred into an Agilent SampliQ QuEChERS dispersive SPE 15 mL tube (p/n 5982-5258). The 2 mL tube contained 50 mg of PSA, 50 mg of GCB and 150 mg of anhydrous $MgSO_4$, while the 15 mL tube contained 400 mg of PSA, 400 mg of GCB and 1200 mg of anhydrous $MgSO_4$. Subsequently, 375 µL of toluene were added to the 2 mL tubes, and 3 mL of toluene were added to the 15 mL tubes. The tubes were tightly capped and vortexed for 1 min. The 2 mL tubes were centrifuged with a micro-centrifuge at 13,000 rpm for 2 min, and the 15 mL tubes centrifuged in a standard centrifuge at 4,000 rpm for 5 min. An 825 µL amount of extract was transferred into a 15 mL centrifuge tube and dried by N_2 flow. Samples were reconstituted with 600 µL of ACN containing 0.1% FA, vortexed and sonicated. A 200 µL aliquot of the extract was transferred into an autosampler vial, and 800 µL of water or appropriate standard solutions (prepared in water) were added. The samples were capped and vortexed thoroughly prior to LC/MS/MS analysis. For samples analyzed by GC/MS, a 600 µL reconstituted sample was either transferred directly to an autosampler vial or used to prepare the calibration curves.

In order to determine toluene's affect on the dispersive SPE procedure, another aliquot of ACN extracts was processed following the original dispersive SPE clean-up procedure.

Figure 1 shows the dispersive SPE procedure scheme according to the original method (w/o toluene) and the modified method (w/ toluene).

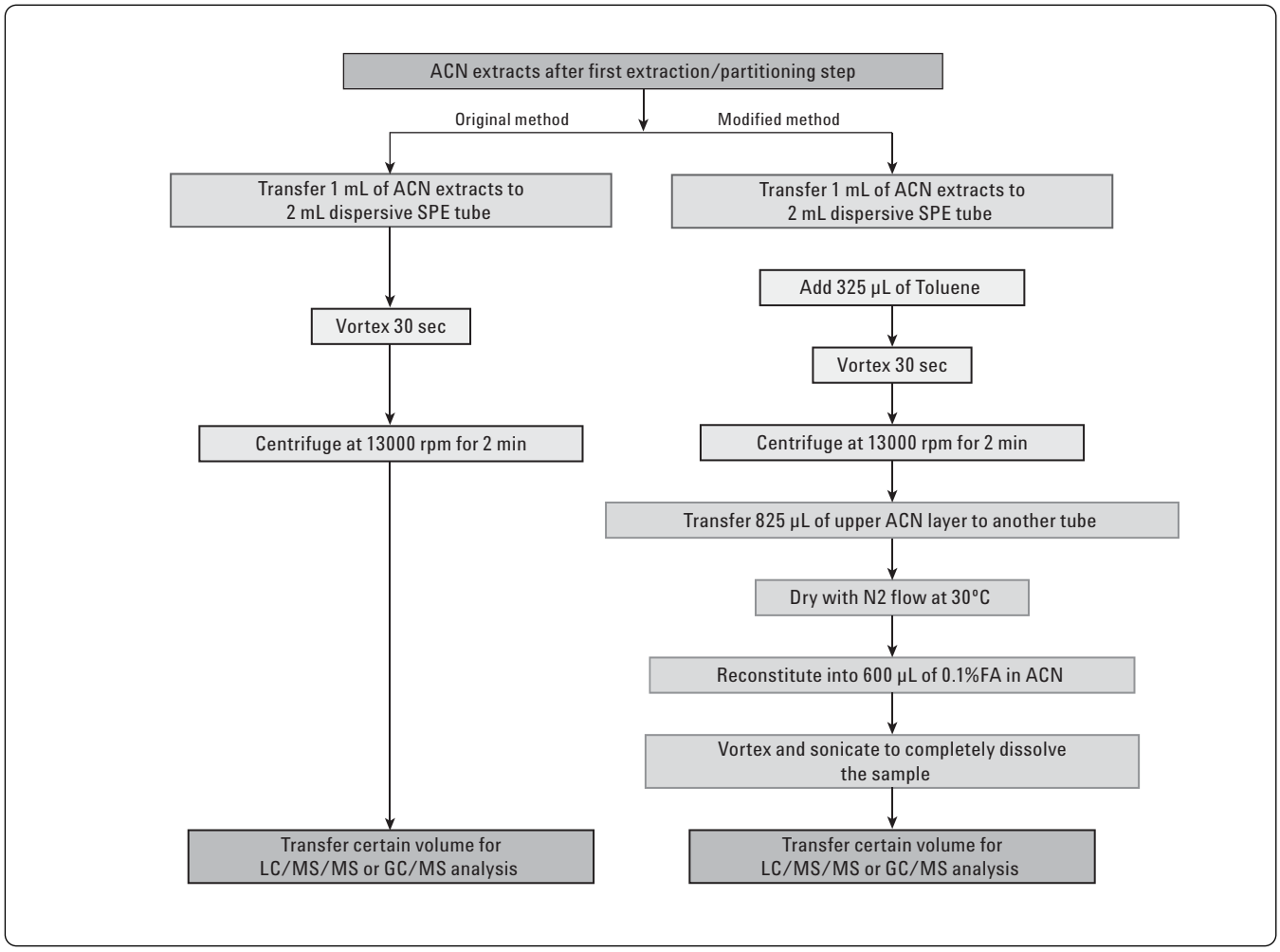

Figure 1. Dispersive SPE procedures of original method (w/o toluene) and modified method (w/toluene).

Results and Discussion

Impact on the Clean-up of Matrix

The QuEChERS methodology for pesticide residue analysis provided high-quality results with a fast, easy, inexpensive approach. For pigmented fruits and vegetables, the addition of GCB in the dispersive SPE tube can greatly remove pigments and sterols. This was clearly shown by the color of the extracts. The spinach ACN extract after the first salt extraction step was very dark green in color. When a dispersive SPE kit for pigmented produce (with GCB) was employed for dispersive SPE clean-up, the upper ACN extract layer became clear with an almost colorless to very light yellow color. On the contrary, when a dispersive SPE kit for general fruits and vegetables was used without GCB, the upper layer was still a dark green to black color. The dispersive SPE extracts modified by the addition of toluene gave a bright yellow color after vortexing and centrifuging. The increase of color for the extracts suggested that the addition of toluene either reduced the affinity of GCB for those pigment molecules, or back-extracted those molecules from the GCB. The addition of toluene resulted in more impurities in the final extracted sample which is demonstrated by the comparison of the UV chromatograms (λ = 254 nm) for the two matrix blanks as shown in Figure 2.

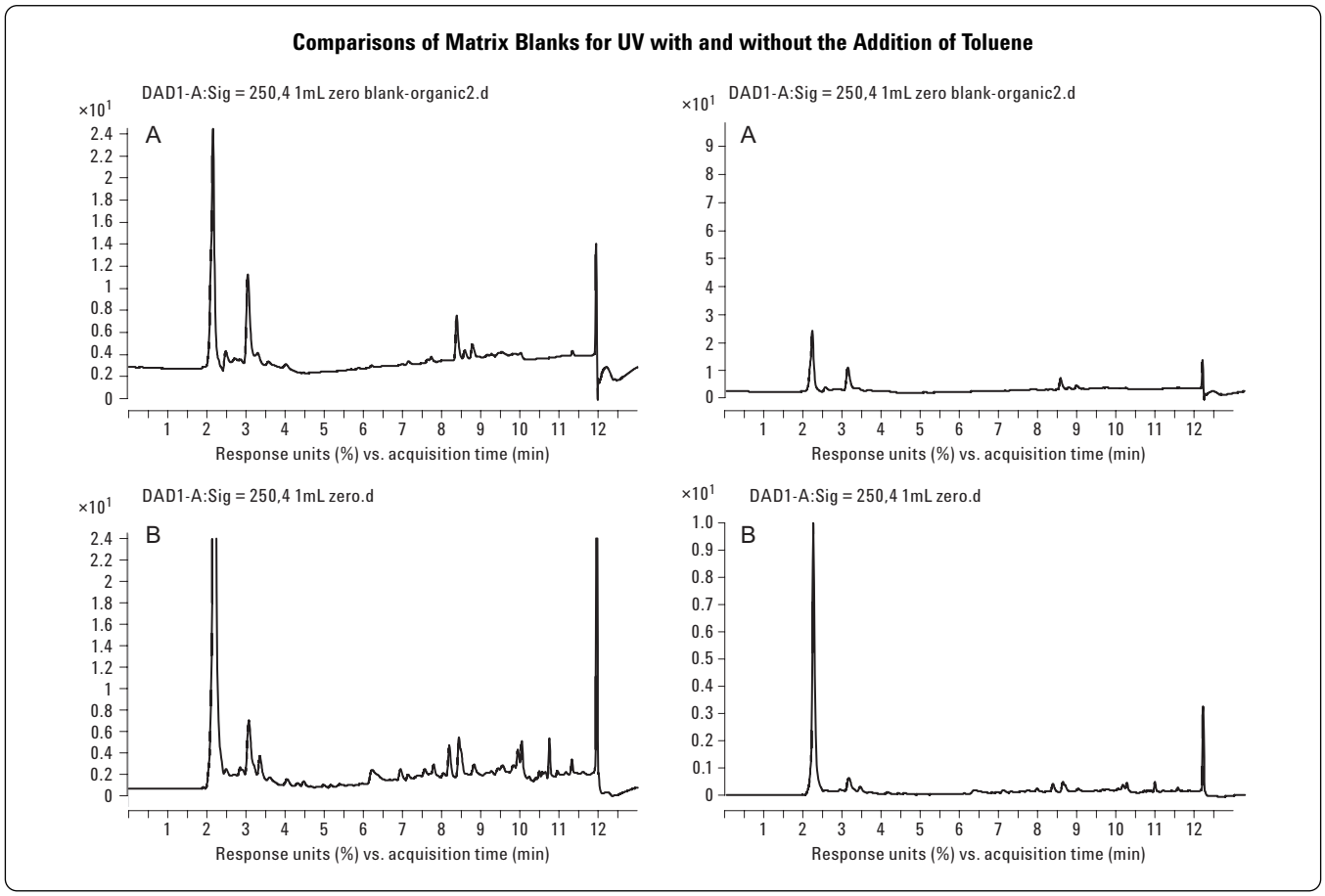

Figure 2. UV chromatogram (λ = 254 nm) comparison of matrix blank obtained with original method without toluene (A) and modified method with addition of toluene (B). Left chromatograms shown in small scale for detail comparison, and right chromatograms shown full scale for big interference peaks comparison. In both cases, the same scale was used for blank A and B chromatograms.

However, the increase in matrix impurities didn't affect the LC/MS/MS or GC/MS pesticide analysis. Figure 3 shows the spinach matrix blank LC/MS/MS chromatograms processed by the modified method with addition of toluene (A) and the original method without addition of toluene (B). With the enhanced selectivity of LC/MS/MS, the two blank samples (A and B) showed similarly clean chromatograms. Figure 4 shows the spinach matrix blank GC/MS chromatograms processed by the modified method with the addition of toluene (A) and the original method without the addition of toluene (B). The two blank chromatograms show some minor differences, but similarities are confirmed.

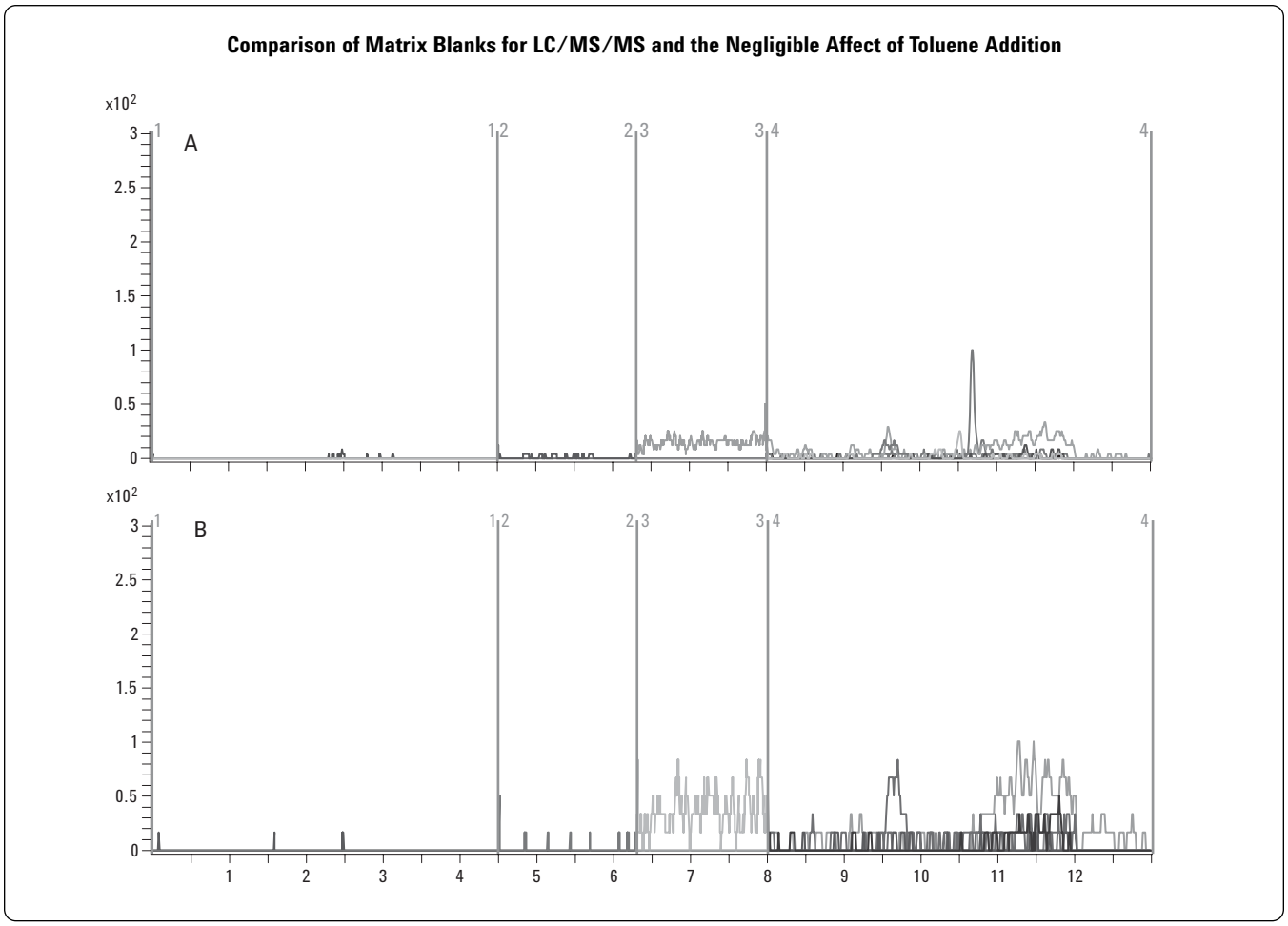

Figure 3. Spinach matrix blank LC/MS/MS chromatogram. A. Spinach matrix blank processed by modified method (w/toluene); B. Spinach matrix blank processed by original method (w/o toluene).

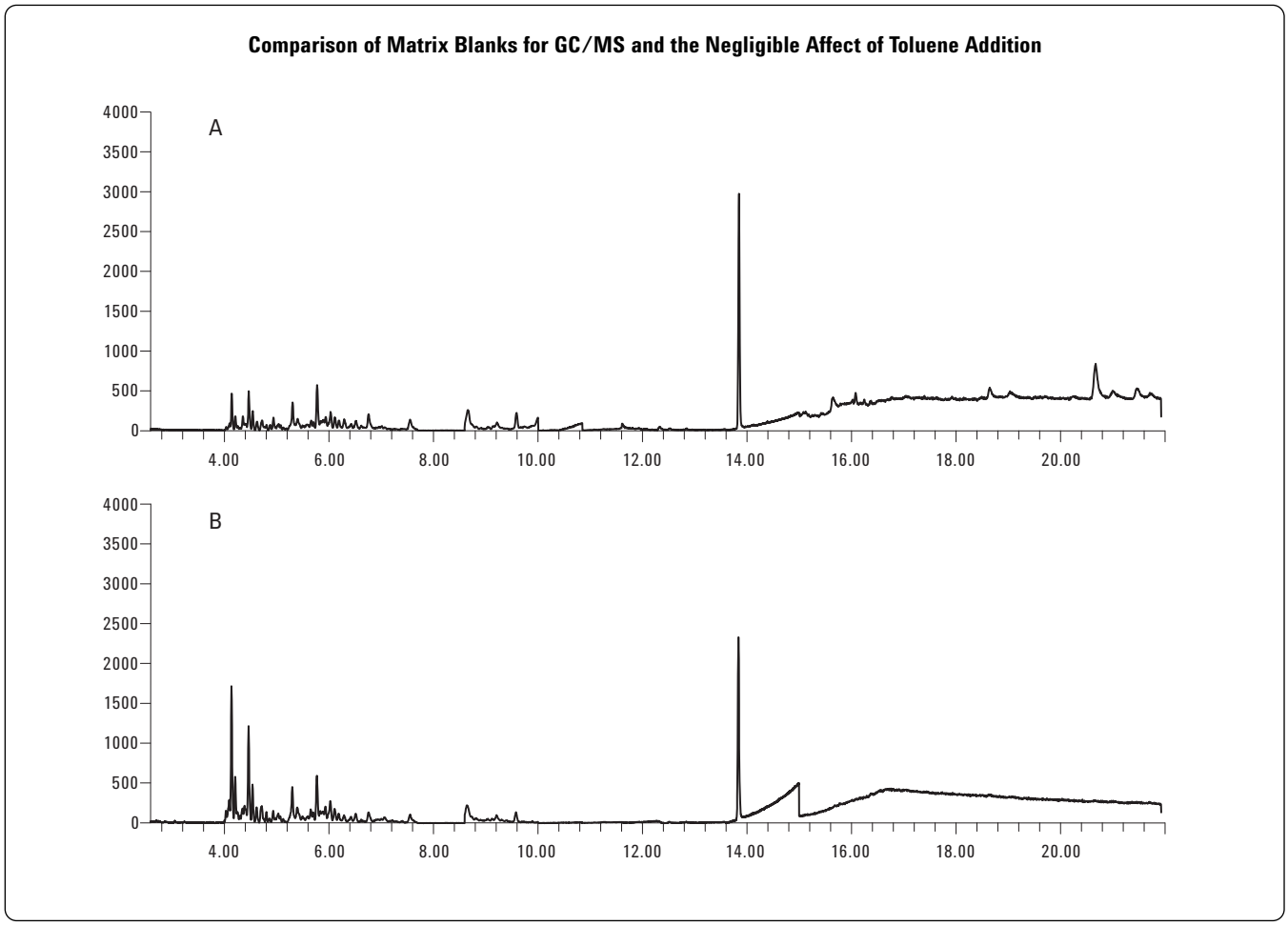

Figure 4. Spinach matrix blank GC/MS chromatograms. A. Spinach matrix blank processed by modified method (w/toluene); B. Spinach matrix blank processed by original method (w/o toluene).

Significant Improvement Made on Some Pesticides

The improvements made by the addition of toluene on certain pesticides was very significant (50% to 300% increase in recovery). Because GCB adsorbs planar compounds, the method produced very low recoveries (20% to 60%) of pesticides with planar compounds and poor precision (>14% RSD). These problematic pesticides determined by the original method included carbendazim, thiabendazole, pymetrozin, cyprodinil, chlorthalonil, coumaphous, dichlorobenzophenone, and folpet. The first four pesticides were analyzed by LC/MS/MS, and the second four pesticides by GC/MS.

The optimum volume of toluene addition was determined by parallel spinach samples spiked at the same concentration level and subjected to buffered salt extraction. An 8 mL aliquot of ACN extract was transferred into a 15 mL dispersive tube. Different volumes of toluene were added according to the following ratios: 8:1, 8:2 and 8:3 (ACN extracts/toluene, n = 3). Samples without the addition of toluene were also processed for comparison. The final samples were analyzed by LC/MS/MS, and an average of analyte responses (peak area of analyte/peak area of IS) were used for response comparison. As shown in Figure 5, the addition of toluene increased the extraction efficiency, as indicated by a 200% to 300% higher analyte response. In general, the more toluene added, the higher the responses obtained. Therefore, the addition of toluene at a ratio of 8:3 was selected for both the LC/MS/MS and GC/MS experiments. This ratio is comparable to the ratio of 3:1 ACN/toluene that Schenck recommended. [7]

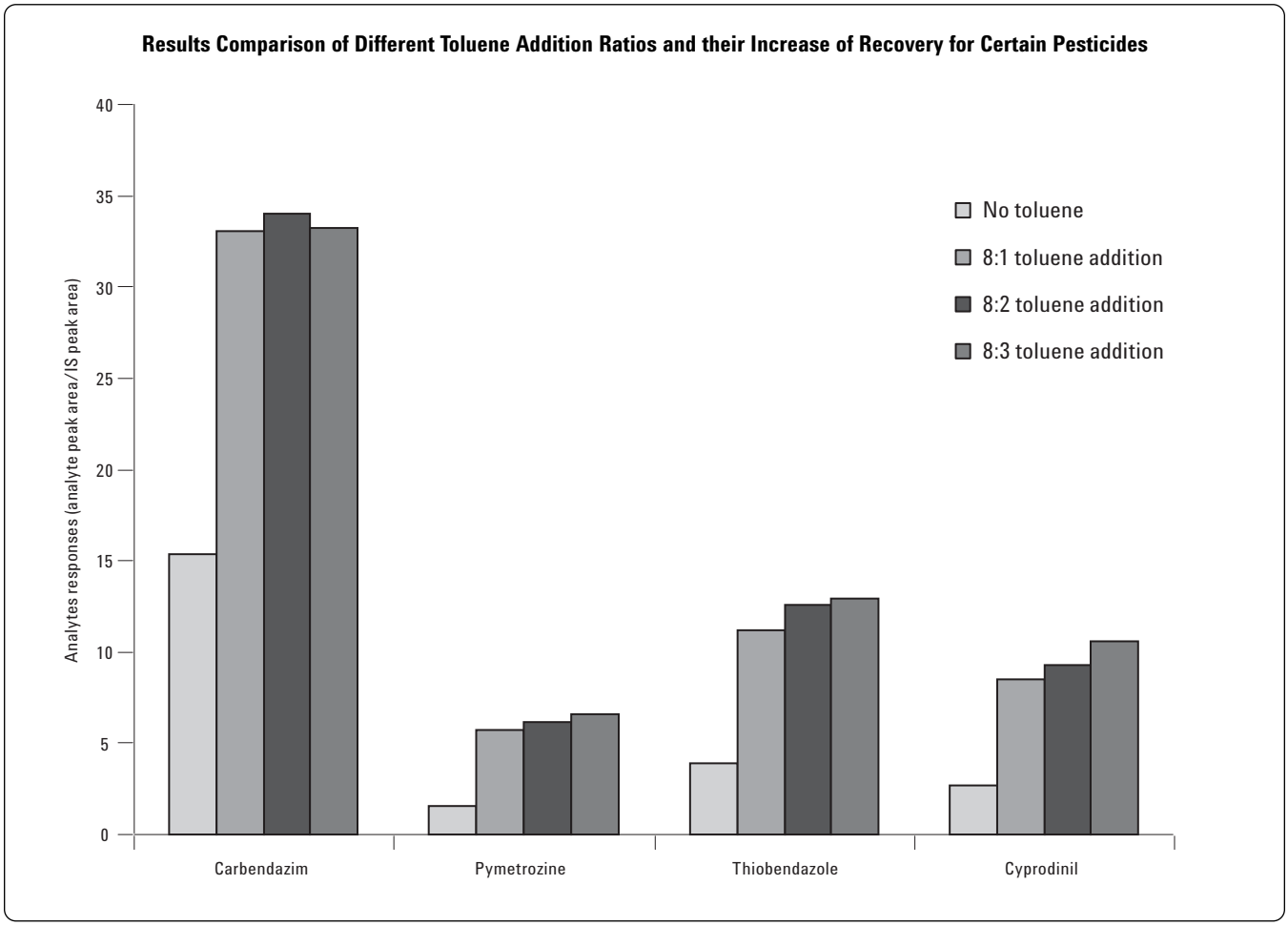

Figure 5. Results comparison of different toluene addition volumes. First column: results generated with no toluene addition; second column: results generated with toluene addition at ratio of 8:1 (ACN extracts/toluene); third column: results generated with toluene addition at ratio of 8:2; fourth column: results generated with toluene addition at ratio of 8:3.

The two different sizes of dispersive SPE (1 mL and 8 mL) were also compared for toluene addition. According to the ratio of 8:3, 3 mL of toluene were added to the 8 mL tubes; while 375 µL of toluene were added to the 1 mL tubes. The results obtained by the modified method were also compared to those from the original method. As shown in Figure 6, both dispersive SPE volumes incorporating the modified method significantly increased the recovery of the difficult pesticides by 200-300%, and gave a substantial improvement in precision. The 1 mL volume dispersive SPE provided slightly higher recovery compared to the 8 mL volume dispersive SPE, especially for pymetrozine and thiabendazole. Processing a single sample with the buffered salt extraction and partitioning step produced about 14 mL of ACN extract, which is enough to process dispersive SPE by both the original and modified methods at a 1 mL volume simultaneously. Additionally, a smaller amount of toluene was required. Therefore, the use of the1 mL volume dispersive SPE kits with the modified method is recommended for problematic pesticides. This eliminates the need for another buffered salt extraction, saving analyst time, labor and additional sample and solvents.

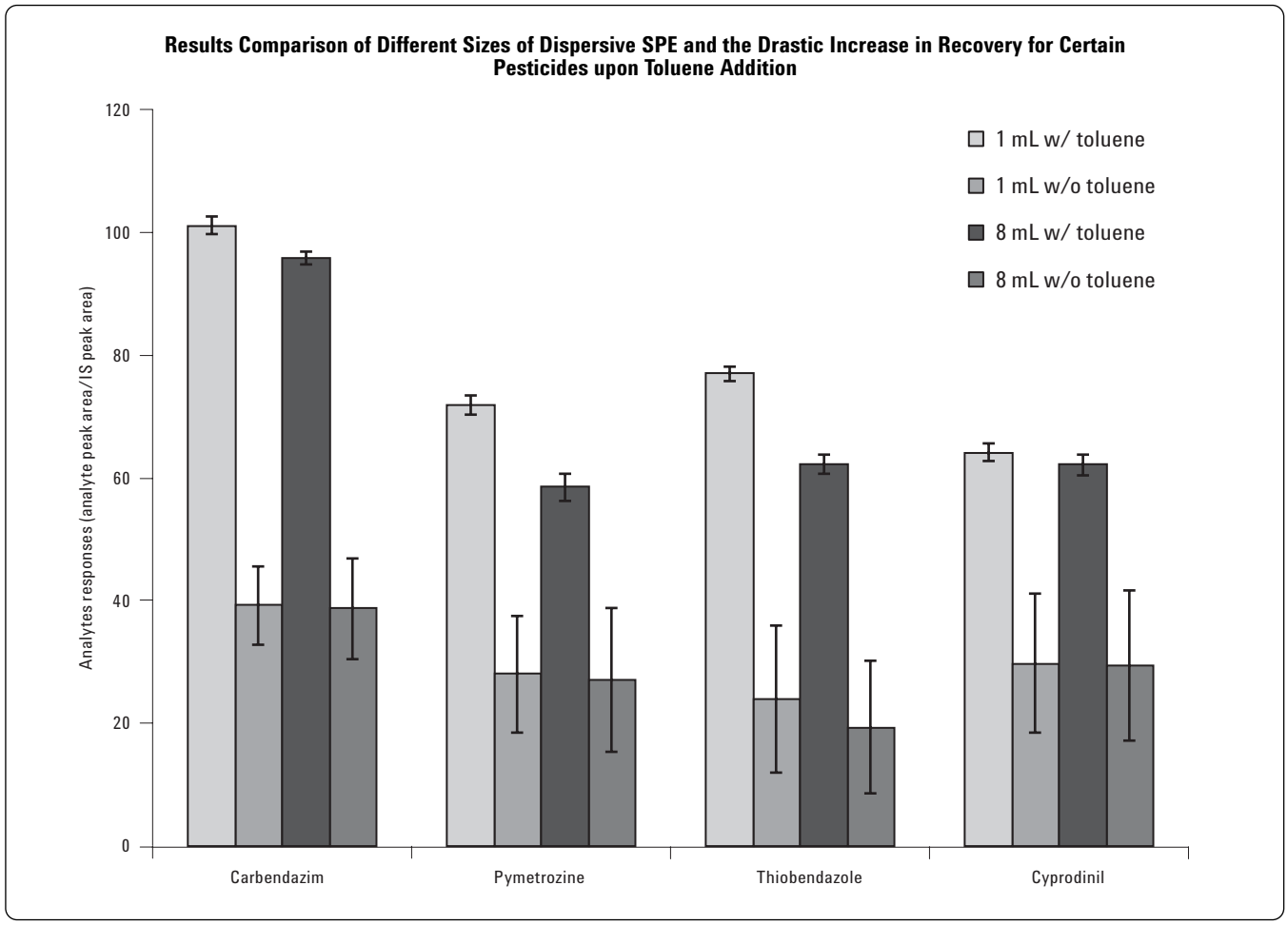

Figure 6. Results comparison of 1 mL and 8 mL dispersive SPE with the modified method (w/ toluene) and the original method (w/o toluene).

Impact on Other Pesticides

The impact of toluene addition on other pesticides was monitored and the results used to classify these pesticides into three groups. The first group of pesticides showed the same recovery and precision from both the original method and modified method. The second group of pesticides were those in which the addition of toluene generated about 10% to15% less recovery, but still showed acceptable precision. The third group included only one pesticide, dichlorvos, from the 34 pesticides screened by LC/MS/MS or GC/MS. For this pesticide, the addition of toluene adversely affected the analysis of dichlorvos producing much lower recovery and unacceptable precision. In general, these negative impacts were observed more on GC amenable pesticides than LC amenable pesticides, and may be linked to the additional drying step in the modified method.

Table 1 shows the impact the addition of toluene made on the modified dispersive SPE analysis of representative pesticides.

Table 1. The Impact on Certain Pesticides by the Modified Dispersive-SPE with Addition of Toluene

Analytes	Original method (w/o toluene)		Modified method (w/ toluene)		Impact with modified method	Detection method
	Recovery	RSD (n=6)	Recovery	RSD (n=6)		
Carbendazim	38.9	14.6	98.5	2.5	Positive	LC/MS/MS
Thiabendazole	21.8	19.7	69.7	2.7	Positive	LC/MS/MS
Pymetrozine	27.6	21.2	65.2	3.7	Positive	LC/MS/MS
Cyprodinil	29.6	23.4	63.1	3.2	Positive	LC/MS/MS
Chlorthalonil	21.1	16.4	47.3	5.9	Positive	GC/MS
Coumaphos	30.1	24.0	87.9	6.1	Positive	GC/MS
Dichlorobenzophenone	53.7	4.5	77.7	6.1	Positive	GC/MS
Folpet	62.0	14.6	88.2	6.3	Positive	GC/MS
Dichlorvos	88.8	6.0	20.4	89.8	Greatly negative	GC/MS
σ-Phenylphenol	88.6	4.6	73.7	7.4	Slightly negative	GC/MS
Diazinon	94.9	5.9	81.3	4.0	Slightly negative	GC/MS
Chlordane	103.9	4.5	101.3	4.5	None	GC/MS
Permethrin	81.4	7.2	83.3	5.1	None	GC/MS
Acephate	95.5	5.6	99.8	4.7	None	LC/MS/MS
Carbaryl	108.0	2.5	109.1	1.9	None	LC/MS/MS
Propoxur	97.0	3.1	96.7	2.5	None	LC/MS/MS

Conclusion

This application note discusses the impact of the addition of toluene on the AOAC QuEChERS method for the analysis of multiclass pesticide residues using Agilent SampliQ AOAC buffered extraction kits and SampliQ AOAC dispersive SPE kits for pigmented fruits and vegetables. The addition of toluene at a ratio of 8:3 (ACN extracts/toluene) to the dispersive SPE step can significantly increase the recovery of problematic pesticides with planar structure by 50% to 300% and improve precision. The addition of toluene can also generate some negative effects, by introducing more matrix impurities, and reducing the recovery of certain pesticides. Therefore, the modified method should not be considered a direct replacement for the original method. It does provide an option for problematic pesticides affected by GCB in the analysis of a highly pigmented matrix. The extraction will not have to be repeated from the beginning. The ACN extracts after the first buffered salt extraction step can be processed by both the original and modified AOAC methods simultaneously with Agilent SampliQ 2 mL dispersive SPE kits for pigmented matrix, saving the analyst additional sample preparation and solvent usage. By combining the results from the original and modified methods, analysts can obtain extremely impressive results and analyze a greater variety of multiclass pesticides in pigmented fruits and vegetables relative to the original method.

Reference

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Printed in the USA
August 6, 2009
5990-4247EN

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Analysis of Pesticide Residues in Apple Using Agilent SampliQ QuEChERS EN Kits by GC/MS

Application Note

Food Safety

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Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation approach described in the European Committee (EN) for extraction and cleanup of 17 GC-amenable multiple pesticide class residues in apple. The method involves initial extraction in an aqueous/acetonitrile system, an extraction/partitioning step after the addition of salt, and a cleanup step using dispersive solid phase extraction (dispersive SPE). The two different dispersive SPE cleanup approaches (1 mL and 6 mL aliquot volumes) were evaluated simultaneously after sample extraction. The target pesticides in the apple extracts were then analyzed by gas chromatography/mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode. The method was validated in terms of recovery and reproducibility. The limit of quantitation (LOQ) for pesticides in apple is 10 ng/g. This application employed Agilent's SampliQ QuEChERS kit and produced results well below the maximum residue limits (MRLs) for all the pesticides screened. The spiked levels for the recovery experiments were 10, 50, and 200 ng/g. Recoveries ranged between 68 and 112% (86.0% on average), with RSD below 15% (4.7% on average).

Agilent Technologies

Introduction

The QuEChERS method for pesticide analysis was first introduced by USDA scientists in 2003. [1] The method was modified to address some problematic pesticides by including a buffered extraction system. [2] The EN method 15662:2007 is a European variation to the QuEChERS method. [3, 4] The method uses acetonitrile extraction, followed by the salting out of water from the sample using anhydrous magnesium sulfate ($MgSO_4$), NaCl and buffering citrate salts to induce liquid-liquid partitioning. A dispersive solid phase extraction (dispersive SPE) is conducted for cleanup using a combination of primary secondary amine (PSA) to remove fatty acids among other components and anhydrous $MgSO_4$ to reduce the remaining water in the extract. After mixing and centrifugation, the upper layer is ready for analysis.

Although the EN and AOAC are similar methods, they do have several differences. First, the extraction buffered system in the EN method uses sodium chloride, sodium citrate and disodium citrate sesquihidrate instead of sodium acetate in the AOAC extraction step. Second, in the dispersive SPE step, the EN method uses 25 mg PSA per mL of extract rather than 50 mg PSA per mL of extract as stated in the AOAC method.

Gas Chromatography/Mass Spectrometry (GC/MS) has been widely used in pesticide analysis for many years. Many pesticides are volatile or semi-volatile, which makes them GC-amenable compounds. Previously, we evaluated the performance of Agilent's SampliQ EN buffered extraction kit and SampliQ EN dispersive SPE kits for the analysis of polar pesticides in apple using LC/MS/MS for detection and quantification. [5] In this study, the performance of the SampliQ EN Buffered Extraction kit (p/n 5982-5650) and SampliQ EN dispersive SPE kit for General Fruits and Vegetables (p/n 5982-5021 and 5982-5056) was evaluated for the extraction of volatile and semi-volatile pesticides. Analysis was performed by GC/MS. Seventeen GC-amenable pesticides were selected which represent multiple classes, including non-polar organochlorine pesticides (OCs), certain organophosphorus pesticides (OPs) and organonitrogen pesticides (ONs). The MRLs of these pesticides are a function of both the pesticide class and food matrix and have been set at 10 ng/g or higher. Table 1 shows the chemical and regulatory information for these pesticides in apple.

Experimental

Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN), methanol (MeOH) were from Honeywell (Muskegon, MI, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards and internal standard (triphenyl phosphate, TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), Chem Service (West Chester, PA, USA), or Ultra Scientific (North Kingstown, RI, USA).

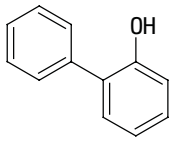
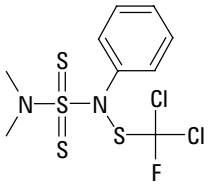
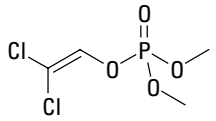
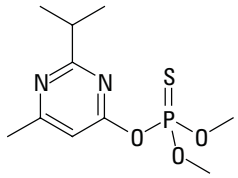
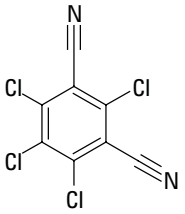
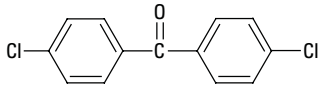
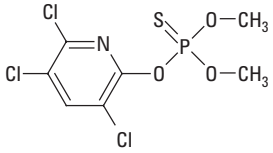
Solutions and Standards

Standards and internal standard (IS) stock solutions (2 mg/mL) were made in MeOH, respectively, and stored at –20 °C. Three QC spiking solutions of 1, 5 and 20 µg/mL were made fresh daily in 1:1 ACN/H2O (0.1% FA). A 2.5 µg/mL standard solution (17 pesticides) in ACN (0.1% FA) was used to prepare the calibration curves in the matrix blank extract by appropriate dilution. A 10 µg/mL amount of TPP spiking solution in 1:1 ACN/H_2O (0.1% FA) was used as the internal spiking standard (IS).

Equipment and Materials

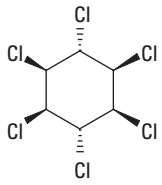
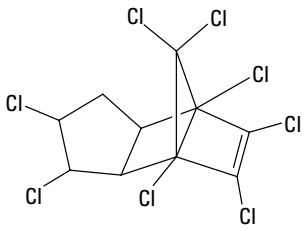
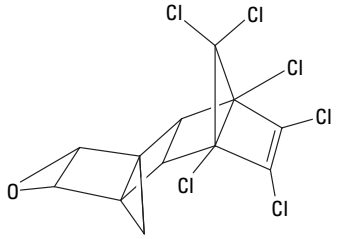
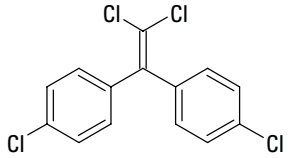
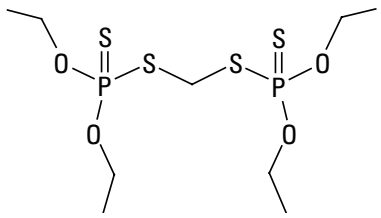
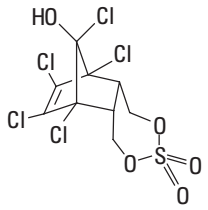
- Agilent Gas Chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA).
- Agilent 5975C Mass Spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA).
- SampliQ QuEChERS EN Extraction kit, p/n 5982-5650 (Agilent Technologies Inc., Wilmington, DE, USA).
- SampliQ QuEChERS EN dispersive SPE kits for General Fruits and Vegetables, p/n 5982-5021 and 5982-5056 (Agilent Technologies Inc., Wilmington, DE, USA).
- CentraCL3R Centrifuge (Thermo IEC, MA, USA)
- Bottle top dispenser (VWR, So Painfield, NJ, USA)
- Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)
- Grinder (St Joseph, MI USA)

Table 1. Pesticides Chemical and Regulatory Information [6–9]

Name	Category	Log P	pKa	Structure	MRLs in apple (ng/g)*
σ-Phenylphenol	Phenol	3.18	9.4		20
Dichlofluanid	Sulphamide	3.7	NA		5000
Dichlorvos	Organophosphate	1.9	NA		10
Diazinon	Organophosphate	3.69	2.6		100
Chlorothalonil	Chloronitrile	2.94	NA		10
Dichlorobenzophenone	Organochlorine	4.44	NA		500
Chlorpyrifosmethyl	Organophosphate	4.00	NA		500

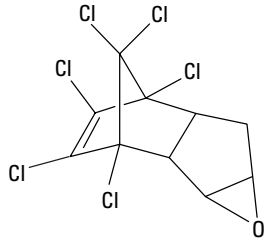
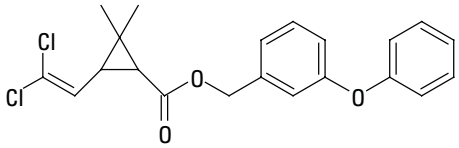
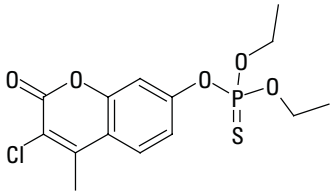
(Continued)

Table 1. Pesticides Chemical and Regulatory Information [6–9]

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
Lindane	Organochlorine	3.69	NA		10
Chlordane	Cyclodiene organochlorine	2.78	NA		20
Dieldrin	Chlorinated hydrocarbon	3.7	NA		10
DDE	Organochlorine	6.55	NA		50
Ethion	Organophosphate	5.07	NA		300
Endosulfan sulfate	Organochlorine	3.13	NA		50

(Continued)

Table 1. Pesticides Chemical and Regulatory Information [6–9]

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
Heptachlor epoxide	Organochlorine	5.83	NA		10
Permethrins	Pyrethroid	6.1	NA		50
Coumaphos	Organothiophosphate	3.86	NA		100

*The MRLs numbers list in the table are for apple or lowest level in other fruit and vegetables. They could be higher in different commodities.

Instrument Condition

An Agilent GC/MS method for pesticides analysis was used for this study. [10]

GC conditions

Inlet	Splitless
Inlet liner	Helix double taper, deactivated (p/n 5188-5398)
Carrier gas	Helium
Inlet pressure	20.18 psi (constant pressure mode) during run 1.0 psi during backflush
Inlet temperature	250 °C
Injection volume	1.0 µL
Purge flow to split vent	30 mL/min at 0.75 min
Oven temperature program	70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C /min to 200 °C (0 min), 16 °C/min to 280 °C (6 min)
Post run	3 min
Capillary flow technology	Purged Ultimate Union (p/n G3186B) – used for backflushing the analytical column and inlet.
Aux EPC gas	Helium plumbed to Purged Ultimate Union
Aux EPC pressure	4.0 psi during run, 80.0 psi during backflush
Column	Agilent J&W HP-5ms Ultra Inert 15 m × 0.25 mm × 0.25 µm (p/n 19091S-431UI)
Connections	Between inlet and Purged Ultimate Union (p/n G3186B)
Restrictor	65 cm × 0.15 mm × 0.15 µm DB-5MS Ultra Inert.
Connections	Between the Purged Ultimate Union and the MSD

MS conditions

Tune file	Atune.u
Mode	SIM (refer to Table 2 for settings in detail)
Source, quad, transfer line temperatures	230 °C, 150 °C and 280 °C respectively,
Solvent delay	2.30 min
Multiplier voltage	Autotune voltage

Table 2. Instrument Acquisition Data Used for the Analysis of 17 Pesticides by GC/MS.

Analyte	SIM	Collection window (min)	RT (min)
(1) Dichlorvos	184.9	2.3 – 4.0	2.88
(2) σ-Phenylphenol	170.1, 169.1	4.0 – 5.0	4.35
(3) Lindane	180.9, 182.9	5.0 – 6.9	6.67
(4) Diazinon	137.1, 179.1	6.9 – 7.7	7.19
(5) Chlorothalonil	265.9, 263.9	14.65 – 16.0	14.8
(6) Chlorpyrifos-methyl	285.9, 287.9	7.7 – 8.6	8.25
(7) Dichlofluanid	123, 167.0	8.6 – 9.35	9.16
(8) Dichlorobenzophenone	139, 249.9	18.8 – 20.5	19.2
(9) Heptachlor epoxide	352.8, 354.8	10.0 – 10.4	10.31
(10) γ-Chlordane	372.8, 374.8	10.85 – 11.6	10.97
(11) DDE	245.9, 317.9	10.85 – 11.6	11.21
(12) α-Chlordane	372.374.8	10.85 – 11.6	11.50
(13) Dieldrin	262.9, 264.9	11.0 – 12.3	11.89
(14) Ethion	230.9	12.3 – 13.6	12.97
(15) Endosulfan sulfate	273.8	12.3 – 13.6	13.35
TPP (IS)	325.1, 326.1	13.6 – 15.0	13.84
(16) Permethrin	183.1	15.0 – 23.0	15.69, 15.79
(17) Coumaphos	362.0, 225.9	15.0 – 23.0	15.83

Sample preparation

Sample comminution

Organically grown, pesticide free apples were purchased from a local grocery store. Approximately three pounds of apples were chopped into small, bean sized cubes. Skin was included, but the core was discarded. The chopped apple cubes were then placed into a clean plastic bag and frozen at –20 °C overnight. The bag was massaged occasionally to make sure the cubes remained separate. The following day, only the required amount of frozen apple cubes was removed and thoroughly blended. Dry ice was added while comminuting, when possible. Samples were comminuted thoroughly to get the best sample homogeneity. It was verified that no pieces of apple were visible in the final sample.

Extraction/Partitioning

A 10 g (± 0.1 g) amount of previously homogenized sample was placed into a 50 mL centrifuge tube. QC samples were fortified with 100 µL of appropriate QC spiking solution. 100 µL of IS spiking solution (10 µg/mL of TPP) was added to all the samples except the control blank to yield a 100 ng/g concentration in the samples. Tubes were capped and vortexed for 1 min. A 10 mL aliquot of ACN was added to each tube using the dispenser. Tubes were capped and shaken by hand for 1 min. An Agilent SampliQ QuEChERS EN extraction salt packet (p/n 5982-5650), containing 4 g anhydrous $MgSO_4$, 1 g NaCl, 1 g Na_3Citrate, and 0.5 g Na_2H Citrate sesquihydrate, was added directly to each tube. The salt bag was massaged carefully to loosen any clumped salts before pouring. No powders were left in the threads or rims of the tubes. Tubes were sealed tightly and shaken vigorously for 1 min by hand to ensure that the solvent interacted well with the entire sample and crystalline agglomerates were broken up sufficiently. Sample pH was checked and 5M NaOH solution was used to adjust the pH to 5–5.5, if necessary. Sample tubes were centrifuged at 4000 rpm for 5 min.

Dispersive SPE Cleanup

A 1 mL aliquot of upper ACN layer was transferred into Agilent SampliQ QuEChERS EN dispersive SPE 2 mL tube (p/n 5982-5021); or a 6 mL of aliquot was transferred into Agilent SampliQ QuEChERS EN dispersive SPE 15 mL tube (p/n 5982-5056). The 2 mL tube contained 25 mg of PSA and 150 mg of anhydrous $MgSO_4$; while the 15 mL tube contained 150 mg of PSA and 900 mg of anhydrous $MgSO_4$. The tubes were capped tightly and vortexed for 1 min. The 2 mL tubes were centrifuged with a micro-centrifuge at 13,000 rpm for 2 min, and the 15 mL tubes in a standard centrifuge at 4000 rpm for 5 min. A 500 µL portion of the extract was transferred into an autosampler vial and 25 µL of 1% FA in ACN was added immediately.

Figure 1 shows the flow chart for the QuEChERS EN sample extraction procedure.

Results and Discussion

Using the SampliQ QuEChERS kits, the entire procedure is fast, easy, and offers time and labor savings, while ensuring consistency. An analyst can process 40-50 samples in just a few hours. Agilent's SampliQ extraction salts are uniquely prepared in an anhydrous package. The addition of a food sample with a high content of water directly to the salts creates an exothermic reaction, which can affect analyte recoveries, especially for volatile pesticides. The unique SampliQ anhydrous salts packet allows salt addition **AFTER** the addition of organic solvent to the sample, as specified in the original QuEChERS methodology.

Our previous study demonstrated good performance of Agilent's SampliQ QuEChERS EN kits on the extraction of a broad variety of semi-polar to polar pesticides analyzed by LC/MS/MS. [5] It is also advantageous to evaluate the performance of the EN kit for the analysis of volatile and semi-volatile pesticides using GC/MS, since these classes of pesticides have been widely used for many years. The selectivity of GC/MS (SIM mode) is not as powerful as that of LC/MS/MS (MRM mode). Furthermore, the final QuEChERS prepared samples still contain some food matrix impurities, which can be observed in the GC/MS chromatogram of blank apple extract. Therefore, it is critical to carefully choose the selected ions of each compound for monitoring when setting up the SIM method. In general, the most abundant ions were selected in order to achieve the best sensitivity; however in a few instances the sensitivity was compromised to obtain better selectivity by using more unique but less abundant ions.

Another potential issue with the use of GC/MS for the analysis of QuEChERS samples is the contamination of the ionization source and deterioration of the GC column. QuEChERS food samples usually still contain high-boiling indigenous impurities, which can accumulate on the head of the column, causing peak tailing and retention time shift. Over time, these impurities can migrate to the mass spectrometer (MS) source, causing contamination of the source. Decreased sensitivity and peak shape distortion, especially for the semi-polar compounds, were observed when additional QuEChERS samples were injected into the GC/MS system. Therefore, column backflushing was employed to increase column life as well as preserve the MS source. Agilent's capillary flow technology makes column backflushing routine [10–12]. Several different capillary flow devices can be used for this purpose.

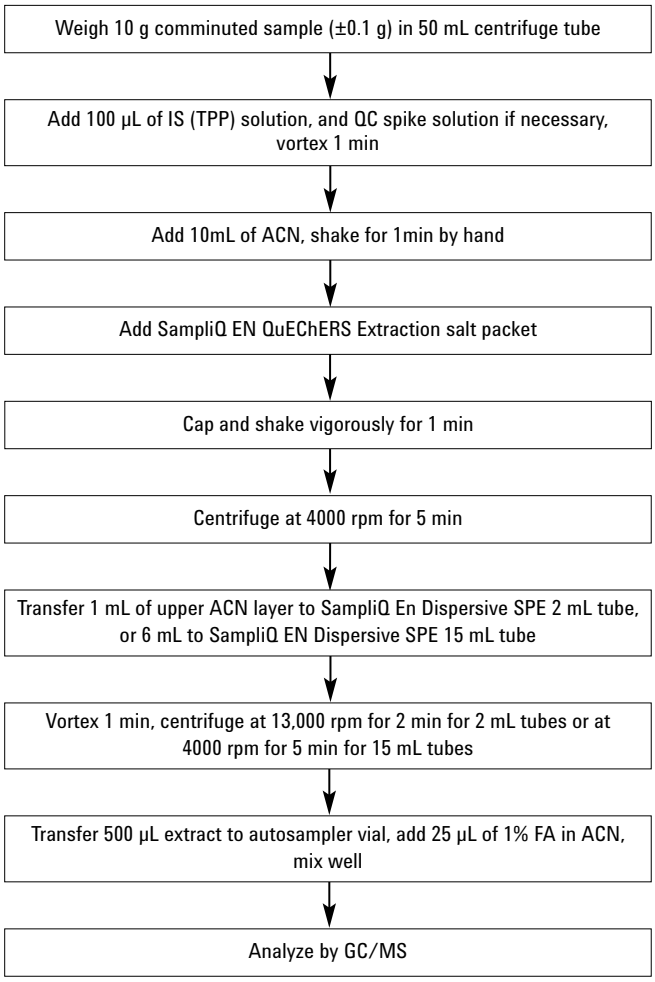


Figure 1. Flow chart of the Agilent SampliQ QuEChERS EN extraction procedure.

In this study, the GC/MS system used a Purged Ultimate Union. The analytical column was connected to the capillary flow device. A short restrictor (65 cm × 0.15 mm × 0.15 µm of DB-5ms Ultra Inert column) was used to couple the capillary flow device to the mass spectrometer. In a previous application note [10], there are figures showing the backflush system, that was used in this study.

Figure 2(a, b) shows the chromatograms of a blank apple extract and a 50 ng/g fortified apple extract. As shown in Figure 2a, interference peaks are found in the blank chromatogram; fortunately most pesticides are free of co-eluting interferences. There was an interference eluting at a retention time very close to that of σ-phenylphenol (peak 2), and cannot be differentiated for integration. The average response of this interference in the blank extract was 215 (n=4), while the average response of α-phenylphenol in the LOQ (10 ng/g)

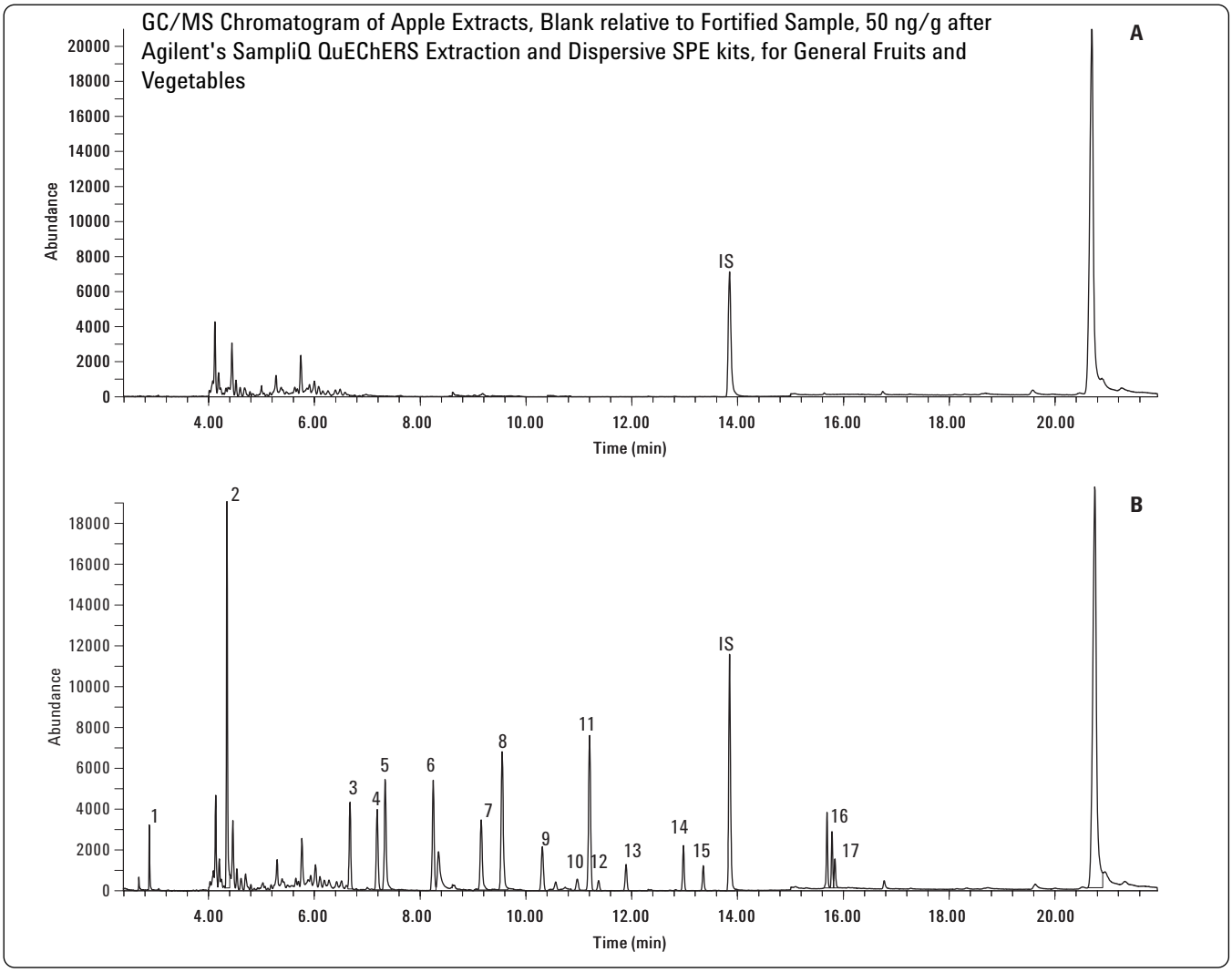


Figure 2. GC/MS chromatogram of apple extract. (A) apple extract blank; (B) 50ng/g fortified apple extract. Peak Identification: 1. Dichlorvos, 2. σ-Phenylphenol, 3. Lindane, 4. Diazinon, 5. Chlorothalonil, 6. Chlorpyrifos-methyl, 7. Dichlofluanid, 8. Dichlorobenzophenone, 9. Heptachlor epoxide, 10. γ-Chlordane, 11. DDE, 12. α-Chlordane, 13. Dieldrin, 14. Ethion, 15. Endosulfan sulfate, 16, Permethrin, 17. Coumaphos. IS. Triphenyl phosphate (TPP)

was 3196 (n=12). The interference response was less than 20% of the response of the σ-phenylphenol peak at the LOQ (10 ng/g) sample. Therefore the selectivity was considered acceptable for this compound.

Linearity and Limit of Quantification (LOQ)

The linear calibration range for all of the pesticides was 0–400 ng/g. Two different dispersive SPE volumes (1 mL and 6 mL) were used for evaluation and comparison; therefore, two calibration curves were generated from matrix blanks prepared from each size. Calibration curves were made at levels of 10, 20, 50, 100, 250, and 400 ng/g. The TPP was the internal standard (IS) at 100 ng/g in all cases. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). Table 1 shows that the 10 ng/g quantification limits LOQ (10 ng/g or 10 ppb) established for the pesticides are substantially lower than many MRLs for the pesticides in fruit and vegetables. The regression fit used for the calibration curves was the average response factor. Table 3 shows the linear term and RF relative standard deviation (%) for both 1 mL and 6 mL dispersive SPE samples. The RF relative SD is an important parameter for the evaluation of the linearity of calibration. In general, the smaller the value the better linearity of the curve, and it is usually acceptable for less than

Table 3. Linearity of 17 Pesticides in Apple Extract

	1 mL dispersive SPE		6 mL dispersive SPE	
Analytes	**Linear Term**	**RF Rel Std Dev (%)**	**Linear Term**	**RF Rel Std Dev (%)**
Dichlorvos	4.53e–001	7.9	5.52e–001	8.6
σ-Phenylphenol	2.41e+000	7.5	2.82e+000	9.3
Lindane	6.79e–001	11.5	8.09e–001	9.2
Diazinon	8.35e–001	15.0	9.32e–001	13.6
Chlorothalonil	1.39e+000	14.1	1.69e+000	14.1
Chlorpyrifos-mehyl	1.32e+000	14.7	1.31e+000	16.5
Dichlofluanid	1.03e+000	11.7	1.29e+000	12.9
Dichlorobenzophenone	6.08e–001	10.0	7.13e–001	10.4
Heptachlor epoxide	5.41e–001	12.4	5.58e–001	12.3
γ-Chlordane	1.77e–001	9.3	1.83e–001	9.1
DDE	2.44e+000	10.7	2.67e+000	9.5
α-Chlordane	1.34e–001	10.0	1.38e–001	9.4
Dieldrin	2.85e–001	9.8	3.09e–001	6.9
Ethion	7.06e–001	27.8	7.30e–001	27.9
Endosulfan sulfate	2.95e–001	11.2	3.29e–001	11.5
Permethrin	8.73e–001	11.8	8.20e–001	17.6
Coumaphos	2.36e–001	19.0	2.16e–001	28.7

20% RF relative SD. The data in Table 3 shows that the linearity of the calibration curve for most analytes is perfect with less than a 15% of RF relative SD value. However, the two standard curves of Ethion (1 mL and 6 mL) and one standard curve of Coumaphos generated RF relative SD values higher than 20%, possibly due to the matrix effect.

Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticides standards in comminuted apple sample at levels of 10, 50 and 200 ng/g. These QC samples were quantitated against the matrix spiked calibration curve. The analysis was performed in replicates of six (n=6) at each level. The recovery and reproducibility (shown as % RSD) data for 1 mL and 6 mL dispersive SPE sample are shown in Table 4 and 5, respectively. It can be seen from the results that all of the pesticides give good recoveries (average of 84.7% for 1 mL and 87.2% for 6 mL) and precision (average of 4.3% RSD for 1 mL and 5.1% RSD for 6 mL). Compared to the results of these pesticides extracted with AOAC QuEChERS method [13], the EN QuEChERS method gives slightly lower recovery (recovery 5–6% lower on average) but similar precision (RSD 4–5% on average for both methods). Variance may be possible due to a different buffering system and solvent volume used in the first extraction step.

Table 4. Recovery and Repeatability of Pesticides in Fortified Apple With Agilent's SampliQ Dispersive SPE Tube, 2 mL (p/n 5982-5021); Recovery 84.7%, RSD 4.3% (avg)

	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
Analytes	**Recovery**	**RSD (n=6)**	**Recovery**	**RSD (n=6)**	**Recovery**	**RSD (n=6)**
Dichlorvos	97.6	5.1	90.8	6.2	81.0	6.9
σ-Phenylphenol	94.4	5.5	83.1	6.6	76.3	5.2
Lindane	87.4	4.9	80.0	6.1	73.3	3.8
Diazinon	83.6	5.6	79.6	5.5	69.6	5.0
Chlorothalonil	68.3	4.9	71.8	5.8	69.6	5.0
Chlorpyrifos-mehyl	79.3	4.5	80.7	4.8	83.1	3.0
Dichlofluanid	91.8	5.6	85.8	6.9	65.2	4.7
Dichlorobenzo phenone	83.9	6.4	83.0	4.8	80.0	3.1
Heptachlor epoxide	80.0	4.7	82.9	5.0	81.4	1.7
γ-Chlordane	79.6	4.3	80.5	5.4	78.3	1.6
DDE	80.5	3.2	80.3	5.1	76.8	1.2
α-Chlordane	84.8	3.3	83.1	4.7	78.6	1.3
Dieldrin	83.4	3.1	80.5	4.3	76.2	1.1
Ethion	97.7	4.4	104.9	4.8	91.7	1.4
Endosulfan sulfate	93.2	5.4	88.5	4.5	87.9	1.1
Permethrin	88.8	6.2	93.9	4.6	104.3	0.7
Coumaphos	101.4	4.7	111.9	3.9	111.2	1.6

Table 5. Recovery and Repeatability of Pesticides in Fortified Apple With Agilent's SampliQ Dispersive SPE Tube, 15 ML (p/n 5982-5056), Recovery 87.2%, RSD 5.1% (avg)

	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
Analytes	**Recovery**	**RSD (n=6)**	**Recovery**	**RSD (n=6)**	**Recovery**	**RSD (n=6)**
Dichlorvos	99.4	8.2	90.9	2.6	85.7	4.4
σ-Phenylphenol	76.9	8.9	81.6	1.6	82.0	3.6
Lindane	87.8	7.0	88.9	2.7	86.3	2.7
Diazinon	87.0	8.3	86.6	1.8	89.3	2.7
Chlorothalonil	71.7	11.1	77.9	1.8	75.9	3.8
Chlorpyrifos-mehyl	77.7	9.8	82.7	2.3	86.7	2.2
Dichlofluanid	80.0	7.8	86.5	6.1	76.6	5.1
Dichlorobenzo phenone	86.2	6.1	87.6	2.4	85.7	1.5
Heptachlor epoxide	82.6	5.7	86.7	2.8	85.9	1.9
γ-Chlordane	89.6	7.1	85.1	2.9	83.6	2.1
DDE	91.9	5.5	88.7	3.5	83.8	1.8
α-Chlordane	90.3	4.4	88.0	3.1	84.0	1.3
Dieldrin	93.6	7.3	88.1	4.3	83.2	1.6
Ethion	81.0	6.7	94.2	4.0	91.1	1.7
Endosulfan sulfate	96.4	5.3	91.2	3.9	89.8	1.2
Permethrin	89.3	5.6	95.5	3.9	108.9	1.3
Coumaphos	89.0	10.8	90.7	6.6	97.1	2.0

Figure 3 shows the recovery and precision results for 1 mL dispersive SPE and 6 mL dispersive SPE. The two different dispersive SPE clean-ups were performed by transferring 1 mL or 6 mL of ACN extract from the same sample following the extraction step. In order to simplify the comparison, the average recovery and precision of three fortification concentrations were used for all of the pesticides. The results of each dispersive SPE clean-up appeared to be independent of volume used. Both approaches provided similar efficient sample clean-up and generated relatively equivalent results.

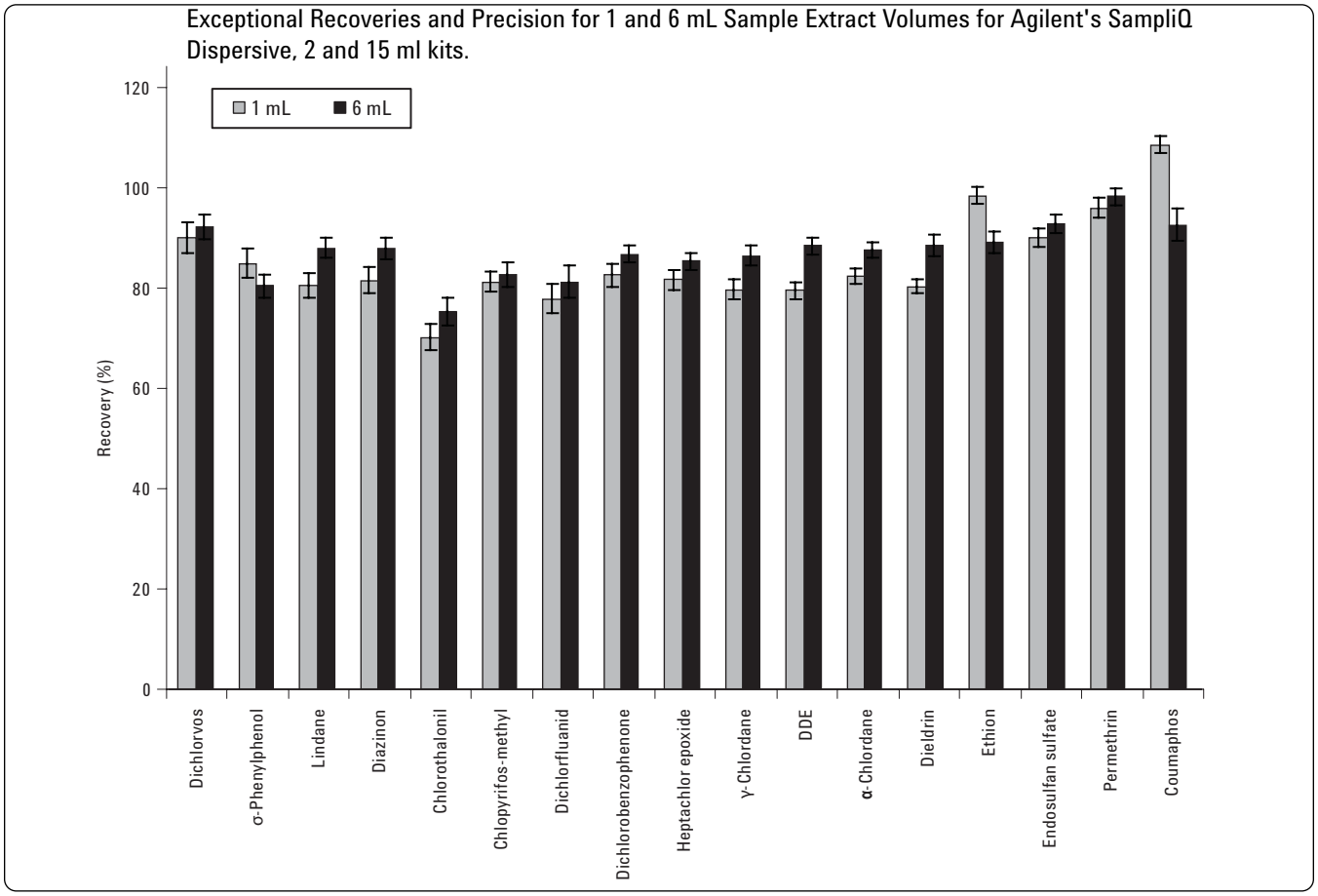


Figure 3. The recovery and precision results of 1 and 6 mL sample volumes employing Agilent's SampliQ Dispersive SPE, 2 and 15 mL kits, respectively.

Conclusions

Agilent's SampliQ QuEChERS EN Extraction and Dispersive SPE kits for General Fruits and Vegetables provide a simple, fast and effective method for the purification and enrichment of representative volatile to semi-volatile pesticides in apple. The recovery and reproducibility, based on matrix spiked standards, were acceptable for multi-class, multi-residue pesticide determination in apple. The impurities and matrix effects from apple did not interfere with the quantitation of target compounds. The LOQs of the pesticides were lower than regulated MRLs in apple. Since the selected pesticides represented a broad variety of different classes and properties, the Agilent SampliQ QuEChERS EN Extraction and Dispersive SPE kits for General Fruits and Vegetables is an excellent choice for other pesticides in similar food matricies

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Printed in the USA
June 30, 2009
5990-4073EN

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Analysis of Pesticide Residues in Apple Using Agilent SampliQ QuEChERS AOAC Kits by GC/MS

Application Note

Food Safety

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Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) AOAC sample preparation approach for extraction and cleanup of 17 GC-amenable pesticide residues from multiple classes, in apple. The method employed involves initial extraction in a buffered aqueous/acetonitrile system, an extraction/ partitioning step after the addition of salt, and a cleanup step utilizing dispersive solid phase extraction (dispersive SPE). The two different dispersive SPE clean-up approaches used either a 1 mL or 8 mL sample volume and were evaluated in parallel after sample extraction. The target pesticides in the apple extracts were then analyzed by gas chromatography/mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode. The method was validated in terms of recovery and reproducibility. The limit of quantitation (LOQ) for most pesticides is 10 ng/g; however, the pesticide Folpet has an LOQ of 50 ng/g in apple. This application employing SampliQ QuEChERS kits produced results well below the maximum residue limits (MRLs) for all the pesticides screened. The spiked levels for the recovery experiments were 10, 50, and 200 ng/g. Recoveries ranged between 70 and 136% (92.5% on average), with RSD below 15% (5.0% on average).

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Introduction

The QuEChERS method for pesticide analysis was first introduced by USDA scientists in 2003. [1] The method was modified to address problematic pesticides by including a buffered extraction system [2]. After a full validation for more than 200 pesticides, this improved method was formalized and adopted as AOAC Official Method 2007.01. [3] In summary, the method uses a single-step buffered acetonitrile (1% HAc) extraction while simultaneously salting out water from the sample using anhydrous magnesium sulfate ($MgSO_4$) to induce liquid-liquid partitioning. For cleanup, a dispersive solid phase extraction (dispersive SPE) step is employed using a combination of primary secondary amine (PSA) to remove fatty acids as well as other components, and anhydrous $MgSO_4$ to reduce the remaining water in the extract. After mixing and centrifugation, the upper layer is ready for analysis.

Gas Chromatography/Mass Spectrometry (GC/MS) has been widely used in pesticide analysis for many years, because many pesticides are volatile or semi-volatile they are GC-amenable. Previously, we evaluated the performance of a SampliQ AOAC buffered extraction kit and SampliQ AOAC dispersive SPE kits for the analysis of polar pesticides in apple using LC/MS/MS for detection and quantification. [4] In this study, the performance of the SampliQ AOAC Buffered Extraction kit (PN 5982-5755) and SampliQ AOAC dispersive-SPE kits for General Fruits and Vegetables (p/n 5982-5022 and 5982-5058) were evaluated for the extraction of volatile and semi-volatile pesticides. Analysis was performed by GC/MS. Seventeen GC-amenable pesticides were selected which represent multiple classes, including non-polar organochlorine pesticides (OCs), certain organophosphorus pesticides (OPs) and organonitrogen pesticides (ONs). The MRLs of these pesticides are a function of both the pesticide class and food matrix and have been set at 10 ng/g or higher. Table 1 shows the chemical and regulatory information for these pesticides in apple.

Experimental

Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN), and methanol (MeOH) were from Honeywell (Muskegon, MI, USA), and acetic acid (HAc) was from Sigma-Aldrich (St Louis, MO, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards and internal standard (triphenyl phosphate, TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), Chem Service (West Chester, PA, USA), or Ultra Scientific (North Kingstown, RI, USA).

Solutions and Standards

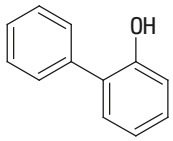
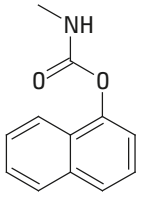
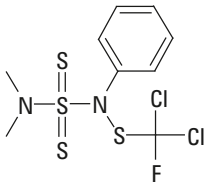
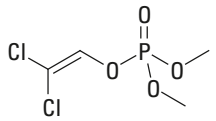
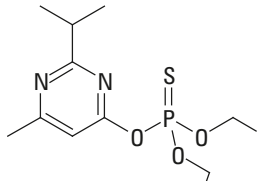
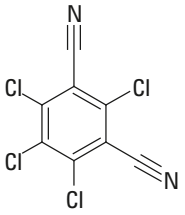
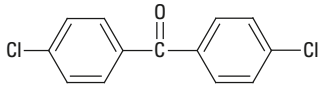
A 1% acetic acid solution in ACN was prepared by adding 10 mL of acetic acid to 1 L of ACN.

Standard and internal standard (IS) stock solutions (2 mg/mL of 11 pesticides) were made in MeOH, respectively, and stored at –20 °C. A commercially available mix of 6 pesticides, at 20 µg/mL in hexane was used directly. Three QC spiking solutions of 11 pesticides at 1.5, 7.5 and 30 µg/mL were made fresh daily in 1:1 ACN/H_2O containing 0.1% FA, while the 20 µg/mL of 6 pesticides mix was directly used for QC spike. A 2.5 µg/mL standard solution of 17 pesticides in ACN containing 0.1% FA was used to prepare the calibration curves in the matrix blank extract by appropriate dilution. A 15 µg/mL of TPP spiking solution in 1:1 ACN/H_2O containing 0.1% FA was used as the internal spiking standard (IS).

Equipment and Material

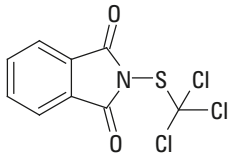
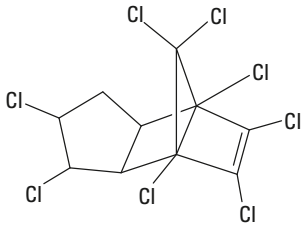
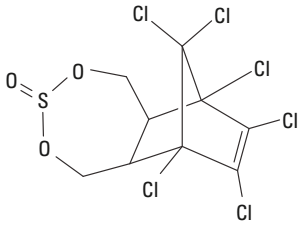
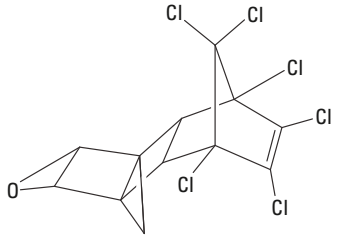
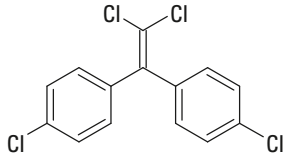
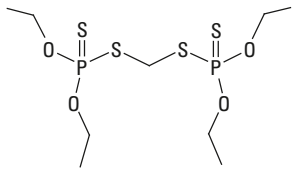
- Agilent Gas Chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA).
- Agilent 5975C Series GC/MSD (Agilent Technologies Inc., Santa Clara, CA, USA).
- Agilent SampliQ QuEChERS AOAC Extraction kits, p/n 5982-5755 (Agilent Technologies Inc., Wilmington, DE, USA).
- Agilent SampliQ QuEChERS AOAC dispersive SPE kits for General Fruits and Vegetables, p/n 5982-5022 and 5982-5058 (Agilent Technologies Inc., Wilmington, DE, USA).
- CentraCL3R Centrifuge (Thermo IEC, MA, USA)
- Bottle top dispenser (VWR, So Painfield, NJ, USA)
- Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)

Table 1. Pesticides Chemical and Regulatory Information [5–8]

Name	Category	Log P	pKa	Structure	MRLs in apple (ng/g)*
σ-Phenylphenol	Phenol	3.18	9.4		20
Carbaryl	Carbamate	2.36	10.4		50
Dichlofluanid	Sulphamide	3.7	NA		5000
Dichlorvos	Organophosphate	1.9	NA		10
Diazinon	Organophosphate	3.69	2.6		100
Chlorothalonil	Chloronitrile	2.94	NA		10
Dichlorobenzo phenone	Organochlorine	4.44	NA		500

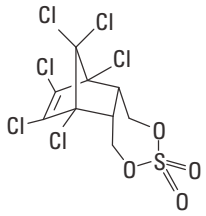
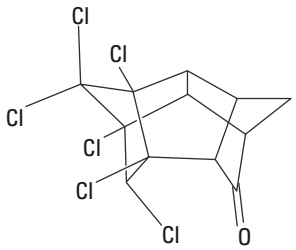
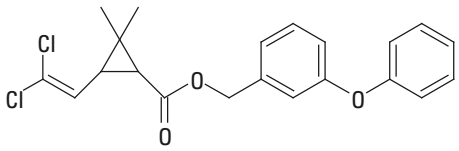
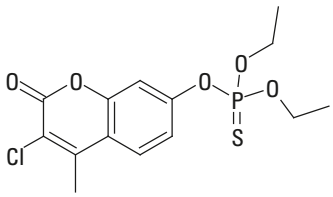
(Continued)

Table 1. Pesticides Chemical and Regulatory Information [5–8]

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
Folpet	Phthalimide	3.02	NA		3000
Chlordane	Cyclodiene organochlorine	2.78	NA		20
Endosulfan	Organochlorine	3.13	NA		50
Dieldrin	Chlorinated hydrocarbon	3.7	NA		10
DDE	Organochlorine	6.55	NA		50
Ethion	Organophosphate	5.07	NA		300

(Continued)

Table 1. Pesticides Chemical and Regulatory Information [5–8]

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
Endosulfan sulfate	Organochlorine	3.13	NA		50
Endrin ketone	Organchlorine	4.99	NA		10
Permethrins	Pyrethroid	6.1	NA		50
Coumaphos	Organothio phosphate	3.86	NA		100

*The MRLs numbers list in the table are for apple or lowest level in other fruit and vegetables. They could be higher in different commodities.

Instrument Condition

An Agilent GC/MS method for pesticides analysis was used for this study. [9]

GC conditions

Auto-sampler:	Agilent 7683 automatic liquid sampler
Inlet:	Splitless
Column:	Agilent 30 m × 0.25 mm × 0.25 µm HP-5MS Ultra Inert (p/n 19091S-433UI)
Carrier gas:	Helium in the constant pressure mode
Retention time locking:	Chlorpyrifos-methyl locked to 16.596 min (nominal column head pressure = 22.0 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 (11.5 min)
Injection volume:	1.0 µL

MS conditions

Tune file:	Atune.u
Mode:	SIM (refer to Table 2 for settings in detail)
Source, quad, transfer line temperature:	230 °C, 150 °C and 280 °C respectively,
Solvent delay:	3.00 min
Multiplier voltage:	Autotune voltage

Sample preparation

Sample comminution

Organically grown, pesticide-free apples were purchased from a local grocery store. Approximately three pounds of apples were chopped into small, bean sized cubes. Skin was included, but the seeds were discarded. The chopped apple cubes were then placed into a clean plastic bag and frozen at –20 °C overnight. The bag was massaged occasionally to make sure the cubes remained separate. The following day, only the required amount of frozen apple cubes was removed and thoroughly blended. Dry ice was added while comminuting, when possible. Samples were comminuted thoroughly to get the best sample homogeneity, ensuring there were no pieces of apple visible in the final sample.

Extraction/Partitioning

A 15 g (± 0.1g) amount of previously homogenized sample was placed into a 50 mL centrifuge tube (from the SampliQ QuEChERS extraction kit). QC samples were fortified with 100 µL of appropriate QC spiking solution (11 pesticides) and 7.5, 37.5, and 150 µL of 20 µg/mL stock solution (6 pesticides mixture), respectively, yielding QC samples with concentra-

Table 2. Instrument Acquisition Data Used for the Analysis of 17 Pesticides by GC/MS.

Analyte	SIM	Collection window (min)	RT (min)
(1) Dichlorvos	184.9	3.0 – 6.5	5.8
(2) σ-Phenylphenol	170.1, 169.1	6.5 – 9.5	8.8
(3) Diazinon	137.1, 179.1	13.5 – 14.65	14.5
(4) Chlorothalonil	265.9, 263.9	14.65 – 16.0	14.8
(5) Carbaryl	144	16.0 – 17.5	16.8
(6) Dichlofluanid	123, 167.1	17.5 – 18.8	18.4
(7) Dichlorobenzophenone	139, 249.9	18.8 – 20.5	19.2
(8) Folpet	259.9, 261.9	21.35 – 21.8	21.6
(9) γ-Chlordane	372.9, 374.9	21.8 – 22.3	22.0
(10) Endosulfan	240.8, 238.8	22.3 – 23.2	22.6
(11) Dieldrin	262.8	23.2 – 25.0	23.9
(12) DDE	245.9, 317.9	23.2 – 25.0	24.0
(13) Ethion	230.9	25.0 – 26.4	26.0
(14) Endosulfan sulfate	273.8	26.4 – 27.2	26.8
TPP (IS)	325.1, 326.1	27.2 – 28.0	27.7
(15) Endrin ketone	316.9	28.0 – 28.5	28.2
(16) Permethrin	183.1	30.0 – 32.5	31.4, 31.6
(17) Coumaphos	362.0	30.0 – 32.5	31.7

tions of 10, 50 and 200 ng/g. A 100 µL amount of internal standard spiking solution (15 µg/mL of TPP) was added to all samples except the control blank to yield a 100 ng/g concentration in each sample. Tubes were capped and vortexed for 1 min. A 15 mL amount of 1% HAc in ACN was added to each tube using the dispenser. An Agilent SampliQ QuEChERS extraction salt packet from the kit (PN 5982-5755) containing 6 g of anhydrous $MgSO_4$, and 1.5 g of anhydrous NaOAc was added directly to the tubes. The salt bag was massaged carefully to break up any salt clumps before pouring. The tubes were examined to ensure that no powder was left in the threads or rims of the tubes. Sample tubes were sealed tightly and shaken vigorously for 1 min by hand to ensure that the solvent interacted with the entire sample and crystalline agglomerates were dispersed. Sample tubes were centrifuged at 4000 rpm for 5 min.

Dispersive SPE Cleanup

A 1 mL aliquot of the upper ACN layer was transferred to an Agilent SampliQ QuEChERS dispersive SPE 2 mL tube (p/n 5982-5022). An 8 mL aliquot was transferred to an Agilent SampliQ QuEChERS dispersive SPE 15 mL tube (p/n 5982-5058). The 2 mL tube contained 50 mg of PSA and 150 mg of anhydrous $MgSO_4$; while the 15 mL tube contained 400 mg of PSA and 1200 mg of anhydrous $MgSO_4$. The tubes were tightly capped and vortexed for 1 min. The 2 mL tubes were centrifuged with a micro-centrifuge at 13,000 rpm for 2 min, and 15 mL tubes in a standard centrifuge at 4000 rpm for 5 min. An aliquot from the extract, 500 µL was transferred into an autosampler vial, and analyzed by GC/MS.

Figure 1 shows the flow chart for the QuEChERS AOAC sample extraction procedure.

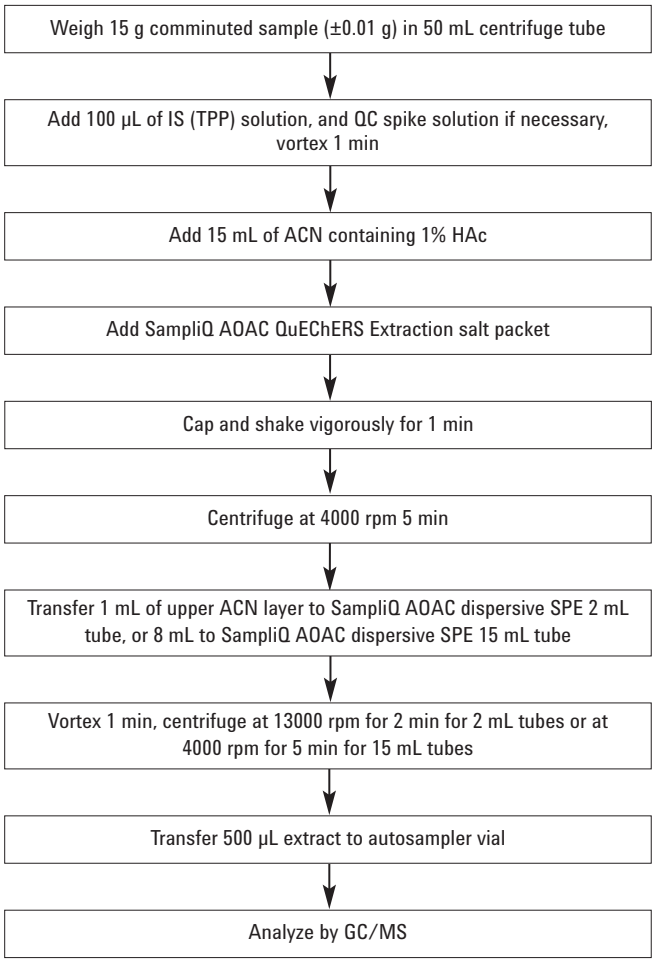


Figure 1. Flow chart of the Agilent SampliQ QuEChERS AOAC extraction procedure.

Results and Discussion

Using the SampliQ QuEChERS kits, the entire procedure is faster, easier, offers time and labor savings, while ensuring consistency. An analyst can process 40–50 samples in just a few hours. The addition of a food sample with a high content of water directly to the salts creates an exothermic reaction, which can affect analyte recoveries, especially for volatile pesticides. Agilent's SampliQ extraction salts are uniquely prepared in an anhydrous package. The unique SampliQ anhydrous salts packet allows addition after adding organic solvent to the sample, as specified in the original QuEChERs method.

In our previous study, the new design of SampliQ QuEChERS AOAC kits demonstrated excellent recovery and precision for a broad variety of semi-polar to polar pesticides using LC/MS/MS. [4] There are many semi-volatile and volatile pesticides, so the use of GC/MS is applicable for the performance evaluation of the AOAC kits for the analysis of these groups of pesticides. The selectivity of GC/MS (SIM mode) is not as effective as that of LC/MS/MS (MRM mode). Furthermore, the final QuEChERS samples still contained food matrix impurities, which can be observed in the GC/MS chromatogram of blank apple extract. Therefore, it is important to carefully choose the monitored ions of each compound when setting up the SIM method. In general, the most abundant ions were selected in order to achieve the best sensitivity; however in a few instances the sensitivity was compromised to obtain better selectivity by using more unique but less abundant ions. As shown in Figure 2a, there are interference peaks apparent in the blank chromatogram; fortunately most pesticides are free of co-eluting interferences. There was an interference eluting at a retention time very close to that of σ-phenylphenol, and can not be differentiated for quantitation.

The response of this interferent within the blank was integrated to be less than 20% response of σ-phenylphenol peak at the LOQ (10 ng/g) sample. Therefore, the selectivity was considered acceptable for this compound. Figure 2 (a, b) shows the chromatograms of a blank apple extract and 50 ng/g fortified apple extract.

Linearity and Limit of Quantification (LOQ)

The linear calibration range for all of the pesticides was 0–400 ng/g; excluding Folpet at 50–400 ng/g due to poor sensitivity. Two different dispersive SPE volumes (1 mL and 8 mL) were used for evaluation and comparison; therefore, two calibration curves were generated from matrix blanks prepared from each size. Each calibration curve was made at levels of 10, 20, 50, 100, 250, and 400 ng/g. The TPP was the internal standard (IS) at 100 ng/g in all cases. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). Table 1 shows that the 10 ng/g quantification limits LOQ (10 ppb) and 50 ng/g LOQ for Folpet (50 ppb) established for pesticides are substantially lower than many MRLs for the pesticides in fruit and vegetables. The regression fit used for the calibration curves was the average response factor. Table 3 shows the linear term and RF relative standard deviation (%) for both 1 mL and 8 mL dispersive SPE.

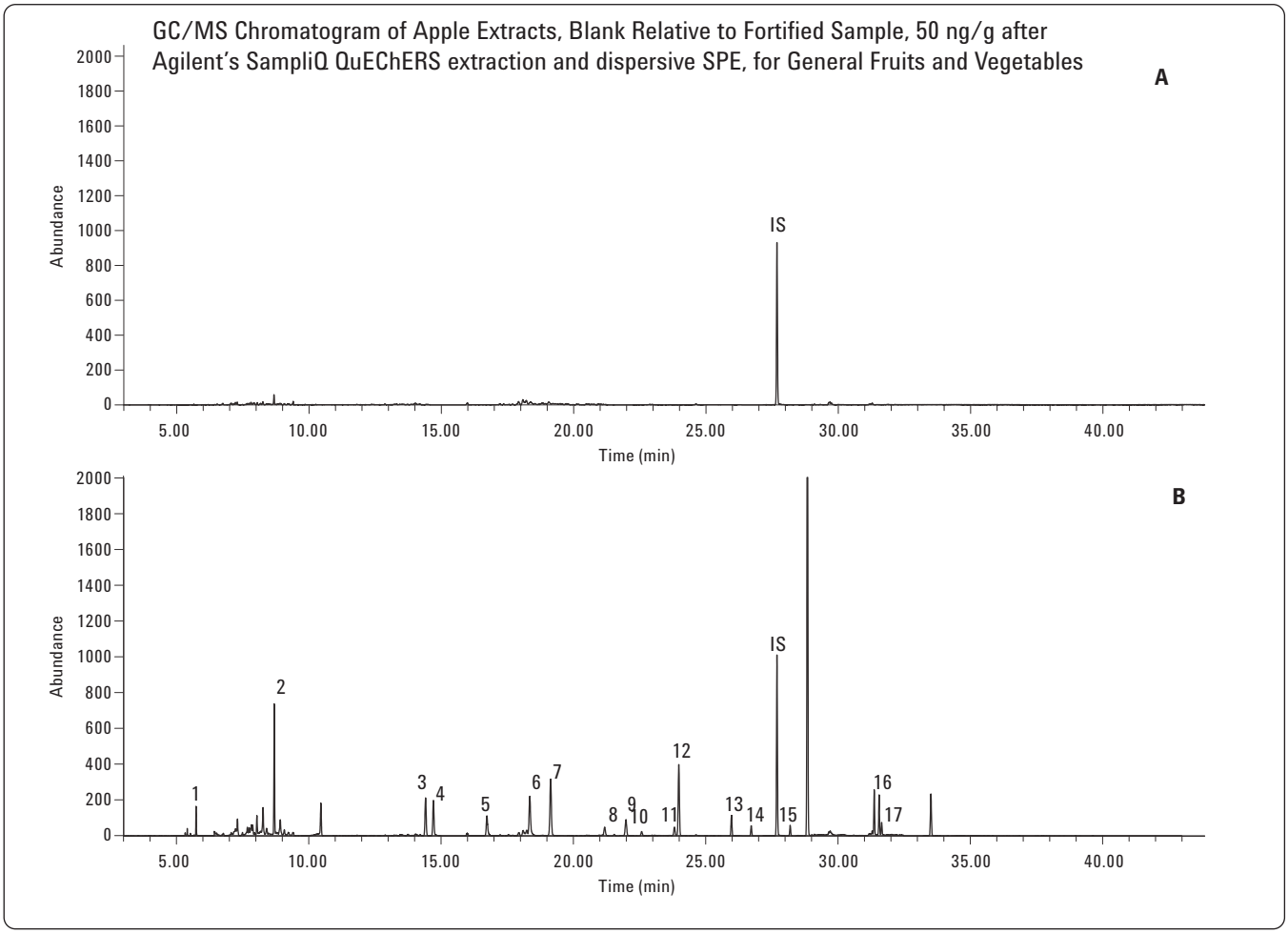


Figure 2. GC/MS chromatogram of apple extract. (A) apple extract blank; (B) 50-ng/g fortified apple extract. Peak Identification: 1. Dichlorvos, 2. σ-Phenylphenol, 3. Diazinon, 4. Chlorothalonil, 5. Carbaryl, 6. Dichlofluanid, 7. Dichlorobenzophenone, 8. Folpet, 9. γ-Chlordane, 10. Endosulfan, 11. Dieldrin, 12. DDE, 13. Ethion, 14. Endosulfan sulfate, 15. Endrin ketone, 16, Permethrin, 17. Coumaphos. IS. Triphenyl phosphate (TPP).

Table 3. Linearity of 17 Pesticides in Apple Extract

	1 mL dispersive SPE		8 mL dispersive SPE	
Analytes	**Linear Term**	**RF Rel Std Dev (%)**	**Linear Term**	**RF Rel Std Dev (%)**
Dichlorvos	3.47e-001	11.4	3.87e-001	4.6
σ-Phenylphenol	1.37e-000	10.7	1.50e-000	11.4
Diazinon	7.04e-001	10.9	7.39e-001	6.5
Chlorothalonil	6.84e-001	13.7	8.02e-001	8.9
Carbaryl	8.07e-001	14.1	1.01e-000	10.8
Dichlofluanid	1.04e-000	12.8	1.08e-000	8.6
Dichlorobenzophenone	4.55e-001	11.4	4.60e-001	8.2
Folpet	3.88e-002	19.5	4.52e-002	20.1
γ-Chlordane	3.23e-001	10.4	3.31e-001	9.2
Endosulfan	8.56e-002	15.2	8.26e-002	8.8
Dieldrin	2.71e-001	6.2	2.59e-001	5.9
DDE	1.43e-000	8.4	1.39e-000	7.5
Ethion	5.87e-001	19.7	5.63e-001	17.0
Endosulfan sulfate	2.72e-001	9.6	2.74e-001	9.5
Endrin ketone	2.75e-001	10.1	2.75e-001	7.8
Permethrin	9.71e-001	9.4	9.29e-001	8.0
Coumaphos	2.70e-001	15.6	2.72e-001	15.7

Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticides standards in comminuted apple sample at levels of 10, 50 and 200 ng/g. These QC samples were quantitated against the matrix-spiked calibration curve. The analysis was performed in replicates of six (n=6) at each level. The recovery and reproducibility (shown as % RSD) data for 1 mL and 8 mL volume dispersive SPE are shown in Table 4 and Table 5, respectively. It can be seen from the results that all of the pesticides give excellent recoveries (average of 90.8% for 1 mL and 94.2% for 8 mL) and precision (average of 5.7% RSD for 1 mL and 4.3% RSD for 8 mL). As mentioned above, an interferent was eluted very closely with σ-phenylphenol. The selectivity was still acceptable because the interferent contributed less than 20% of LOQ; however, the contribution of the interference peak resulted in the higher recovery of this compound at low levels. Folpet is a notoriously unstable pesticide, and the main problems dealing with degradation and instability come from the N-trihalomethylthio functional group. [3, 10] Folpet was quantified, but the LOQ was found to be 50 ng/g due to poor sensitivity, however, recovery and reproducibility at 50 ng/g and above were acceptable (average recovery 85.5%, average reproducibility 10%).

Table 4. Recovery and Repeatability of Pesticides in Fortified Apple With Agilent SampliQ 2 mL Dispersive SPE Tube (p/n 5982-5022); Recovery 90.8%, RSD 5.7% (avg)

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Dichlorvos	86.8	7.0	83.9	11.6	81.5	5.5
σ-Phenylphenol	113.4	6.3	96.3	6.5	100.5	3.6
Diazinon	98.6	2.3	87.3	2.8	90.4	4.9
Chlorothalonil	86.1	10.0	84.4	5.3	93.2	7.6
Carbaryl	96.1	9.0	93.8	8.3	99.1	8.2
Dichlofluanid	90.0	7.0	84.6	2.9	94.6	5.0
Dichlorobenzo phenone	97.8	7.6	95.0	6.2	102.2	4.3
Folpet	–	–	74.4	9.1	95.7	11.0
γ-Chlordane	79.6	4.4	88.9	4.3	95.3	4.4
Endosulfan	69.8	9.2	91.2	5.3	96.2	5.2
Dieldrin	90.6	10.9	86.6	3.2	92.8	4.8
DDE	84.0	4.8	89.4	3.8	95.4	4.5
Ethion	90.9	1.8	103.5	1.4	116.5	5.0
Endosulfan sulfate	79.8	1.9	80.4	4.6	86.8	5.6
Endrin ketone	85.2	12.0	80.7	3.6	91.8	4.5
Permethrin	87.9	2.8	93.8	2.0	94.0	4.4
Coumaphos	87.8	5.1	89.7	3.0	90.0	6.4

Table 5. Recovery and Repeatability of Pesticides in Fortified Apple With Agilent SampliQ 15 mL Dispersive SPE Tube (p/n 5982-5058); Recovery 94.2%, RSD 4.3% (avg)

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Dichlorvos	103.4	4.2	85.6	8.1	97.2	7.2
σ-Phenylphenol	125.8	8.7	99.2	4.4	105.4	5.0
Diazinon	96.0	4.5	82.3	2.1	88.4	6.3
Chlorothalonil	96.5	3.0	82.8	5.2	97.7	4.5
Carbaryl	97.7	3.9	91.4	4.4	101.9	5.0
Dichlofluanid	91.7	5.1	83.7	1.0	93.7	5.1
Dichlorobenzo phenone	98.8	9.3	96.2	4.7	105.3	4.3
Folpet	–	–	88.4	4.0	72.5	6.0
γ-Chlordane	80.9	3.5	87.5	3.3	94.8	5.0
Endosulfan	80.3	7.3	84.1	3.6	98.6	3.0
Dieldrin	81.2	3.4	93.1	2.0	98.7	3.9
DDE	86.1	1.8	92.4	3.4	98.9	3.9
Ethion	106.5	3.6	122.2	2.0	136.3	4.2
Endosulfan sulfate	91.6	4.6	87.7	4.0	93.0	4.1
Endrin ketone	76.2	3.3	82.4	3.9	91.8	4.1
Permethrin	97.9	1.6	104.7	1.1	106.6	4.2
Coumaphos	82.3	6.7	86.5	2.5	89.3	5.1

Figure 3 shows the recovery and precision results for 1 mL dispersive SPE and 8 mL dispersive SPE. The two different dispersive SPE clean-ups were performed by transferring 1 mL or 8 mL of ACN extract from the same sample following the extraction step. In order to simplify the comparison, the average recovery and precision of three fortification concentrations were used for all pesticides. The results of each dispersive SPE clean-up appeared to be independent of volume used. Both approaches provided efficient and similar sample clean-up, and thus generated relatively equivalent results.

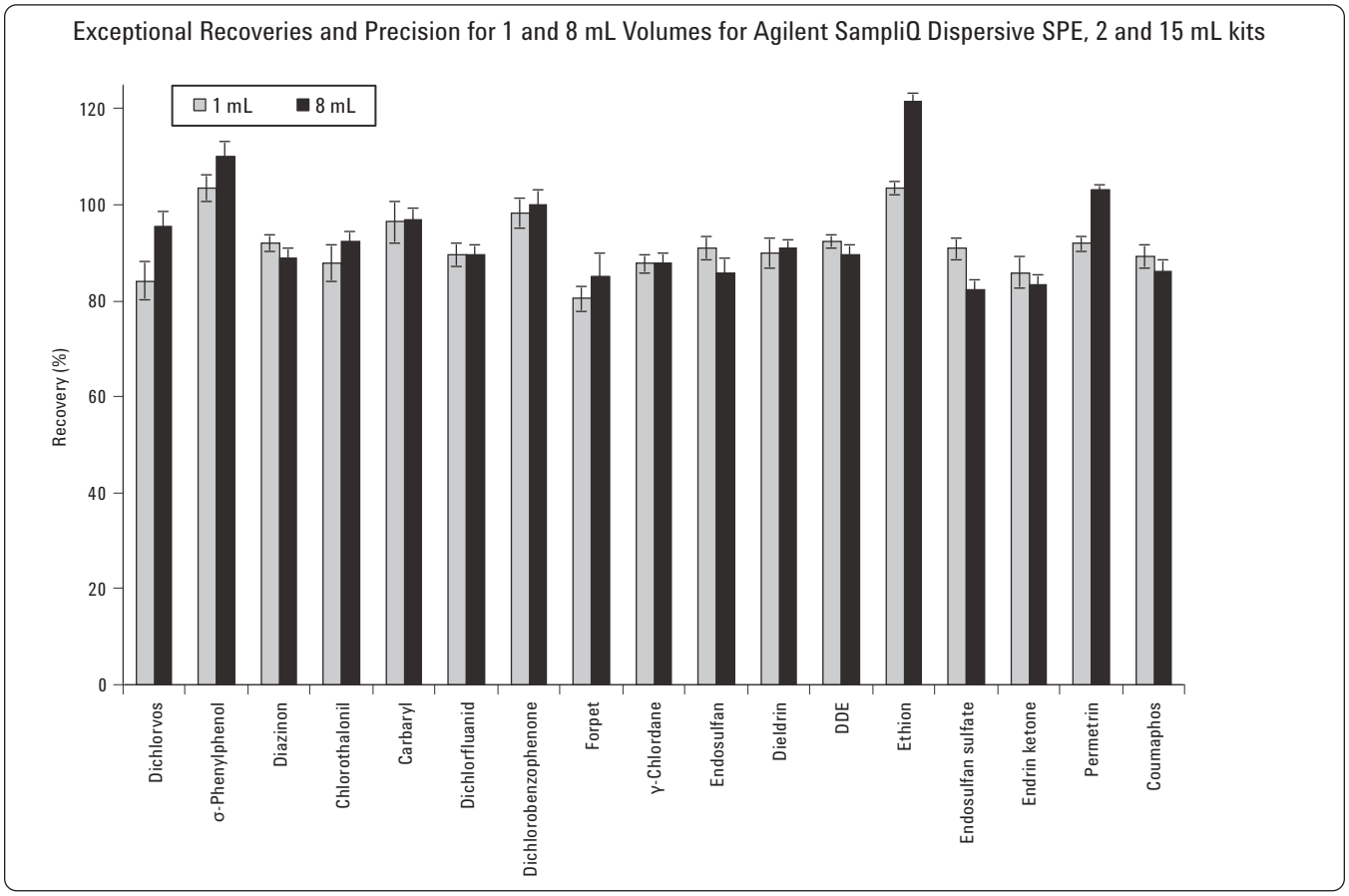


Figure 3. Recoveries and precision for 1 and 8 mL sample volumes employing Agilent SampliQ Dispersive SPE, 2 and 15 mL kits, respectively.

Conclusions

Agilent SampliQ QuEChERS AOAC method for General Fruits and Vegetables: Extraction and Dispersive SPE kits provided a simple, fast and effective method for the purification and enrichment of representative volatile to semi-volatile pesticides in apple. The recovery and reproducibility, based on matrix spiked standards, were acceptable for multiclass, multi-residue pesticide determination in apple. The impurities and matrix effects from apple did not interfere with the quantitation of target compounds. The LOQs of the pesticides were lower than regulated MRLs in apple. As the selected pesticides represented a broad variety of different classes and properties, the Agilent SampliQ QuEChERS AOAC Extraction and Dispersive SPE kits for General Fruits and Vegetables is an excellent choice for other pesticides in similar food matricies.

Acknowledgements

The authors wish to thank Dr. Meng, Chin K. for his help with the GC/MS instrument, and Dr. Ball, Carol H. for many valuable suggestions.

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Printed in the USA
April 20, 2010
5990-4068EN

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Analysis of Pesticide Residues in Apples using Agilent SampliQ QuEChERS AOAC Kit by LC/MS/MS Detection

Application Note

Food Safety

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Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS), Association of Analytical Communities (AOAC) Official Method 2007.01; sample preparation approach for extraction and cleanup of 16 pesticide residues in apple. The 16 pesticides chosen represent various classes of interest. The method employed involves initial extraction in a buffered aqueous/acetonitrile system, an extraction/partitioning step after the addition of salt, and then a cleanup step utilizing dispersive solid phase extraction (dispersive SPE). The two different dispersive SPE clean-up approaches (1 mL and 8 mL) were evaluated simultaneously after sample extraction. The target pesticides in the apple extracts were then determined by liquid chromatography coupled to an electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode. The method was validated in terms of recovery and reproducibility. The 5 ng/g limit of quantitation (LOQ) for pesticides in apple shown in this application was well below the maximum residue limits (MRLs). The spiking levels for the recovery experiments were 10, 50, and 200 ng/g. Mean recoveries ranged between 76 and 117% (95.4% on average), with RSD below 15% (4.3% on average).

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Introduction

Multi-residue analysis of pesticides in fruits, vegetables, and other foods is the primary function of many regulatory, industrial, and contract laboratories throughout the world. Because of the wide variety of pesticides and complexity of food matrices, the sample must be initially cleaned up using a sample preparation technique prior to analysis. Without question, the most efficient approach to pesticide analysis involves the use of multiclass, multi-residue methods. Once the preliminary analytical quality requirements, including accuracy, precision, sensitivity, selectivity and dynamic range, have been met to suit the needs of a particular analysis, other considerations should be evaluated. These additional considerations include sample throughput, ruggedness, ease of use, cost of materials and labor, toxic solvent usage, and waste generation.

The QuEChERS method was introduced first by USDA scientists in 2003. [1] The method was then modified to address some problematic pesticides by using a buffered extraction system. [2] After a full validation for more than 200 pesticides, this improved method was formalized and adopted as AOAC Official Method 2007.01. [3] In summary, the method uses a single-step buffered acetonitrile (1% HAc) extraction while salting out water from the sample using anhydrous magnesium sulfate ($MgSO_4$) to induce liquid-liquid partitioning. After removing an aliquot from the organic layer, for further cleanup a dispersive solid phase extraction (dispersive SPE) is conducted using a combination of primary secondary amine (PSA) to remove fatty acids from other components and anhydrous $MgSO_4$ to reduce the remaining water in the extract. After mixing and centrifugation, the upper layer is ready for analysis.

In this study, 16 pesticides were used for evaluating the performance of the Agilent AOAC Buffered Extraction kit (p/n 5982-5755) and SampliQ QuEChERS AOAC dispersive SPE kit for General Fruits and Vegetables (p/n 5982-5022 and 5982-5058), suitable for common fruit and vegetable applications. Apple was selected as the fruit matrix for the evaluation. Most of the pesticides are from the original "representative pesticides" list [2]. According to their experience, a method working well for these representative pesticides should work equally well for nearly all of the other pesticides that are routinely monitored in multiclass, multi-residue methods. These pesticides are from 9 different pesticide classes, including acidic, basic, neutral, base-sensitive and acid-labile pesticides. Furthermore, the selected pesticides are suitable for LC/MS/MS analysis. The MRLs of these pesticides are a function of both the pesticide class and food matrix and have been set at 10 ng/g or higher. Table 1 shows the chemical and regulatory information for these multiple class pesticides in apple.

Experimental

Reagents and Chemicals

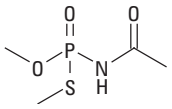
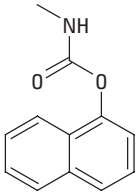
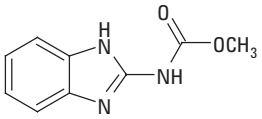
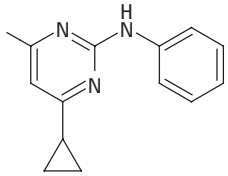
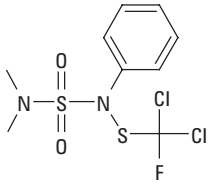
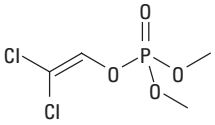
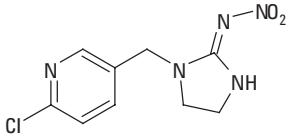
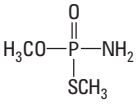
All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN), methanol (MeOH) were from Honeywell (Muskegon, MI, USA). Dimethyl sulfoxide (DMSO) and acetic acid (HAc) were from Sigma-Aldrich (St Louis, MO, USA). Ammonium acetate (NH_4OAc) was from Fisher Chemicals (Fair Lawn, NJ, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards and internal standard, triphenyl phosphate (TPP), were purchased from Sigma-Aldrich (St Louis, MO, USA), ChemService (West Chester, PA, USA), Ultra Scientific (North Kingstown, RI, USA), or AlfaAesar (Ward Hill, MA, USA).

Solutions and Standards

A stock solution of 1M ammonium acetate pH 5 was made by dissolving 19.27 g NH_4OAc powder in 250 mL Milli-Q water. The pH was adjusted to 5 with HAc monitored with a pH meter. The solution was stored at 4 °C. MeOH/H_2O (20:80) containing 5 mM NH_4OAc pH 5 was made by combining 200 mL MeOH and 800 mL Milli-Q water, adding 5 mL of 1M NH_4OAc pH 5 stock solution. 5 mM NH_4OAc in ACN was prepared by adding 5 mL of 1M NH_4OAc pH 5 stock solution to 1 L ACN, mixing well and sonicating 5 min. 1% HAc in ACN was prepared by adding 10 mL of acetic acid to 1 L of ACN.

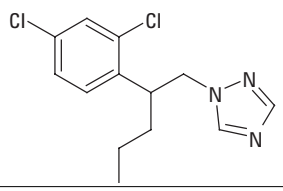
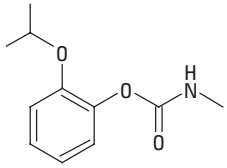
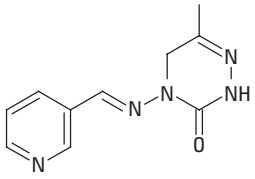
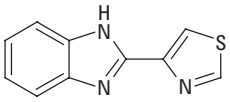
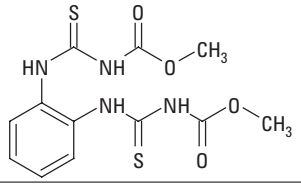
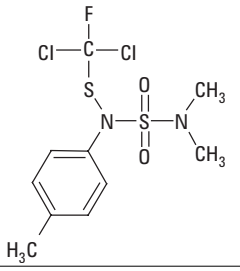
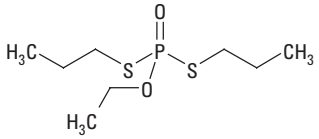
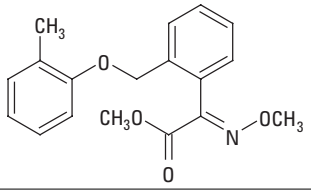
Standard and internal standard (IS) stock solutions (2.0 mg/mL for all, except 0.5 mg/mL for carbendazim) were made in MeOH, 0.1% FA in ACN, or DMSO, respectively, and stored at –20 °C. Three QC spiking solutions of 1.5, 7.5, and 30 µg/mL were made fresh daily in 1:1 ACN/H_2O (0.1% FA).

Table 1. Pesticides Chemical and Regulatory Information [4–6]

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
Acephate	Organophosphate	–0.89	8.35		20
Carbaryl	Carbamate	2.36	10.4		50
Carbendazim	Benzimidazole	1.48	4.2		100
Cyprodinil	Anilinopyrimidine	4	4.44		50
Dichlofluanid	Sulphamide	3.7	NA		5000
Dichlorvos	Organophosphate	1.9	NA		10
Imidacloprid	Neonicotinoid	0.57	NA		500
Methamidophos	Organophosphate	-0.79	NA		10

(Continued)

Table 1. Hormones Used in this Study

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
Penconazole	Triazole	3.72	1.51		50
Propoxur	Carbamate	0.14	NA		1000
Pymetrozine	Pyridine	-0.19	4.06		20
Thiabendazole	Benzimidazole	2.39	4.73 12.00		50
Thiophanate-methyl	Benzimidazole	1.45	7.28		100
Tolylfluanid	Sulphamide	3.9	NA		3000
Ethoprophos	Organophosphate	2.99	NA		5
Kresoxim-methyl	Strobilurin	3.4	NA		50

*The MRLs numbers list in the table are for apple or lowest level in other fruit and vegetables. They could be higher in different commodities.

A 10 µg/mL standard solution in 1:1 ACN/H_2O (0.1% FA) was made for preparation of calibration curves in the matrix blank extract by appropriate dilution. A 15 µg/mL of TPP in 1:1 ACN/H_2O (0.1% FA) was used as an internal standard (IS).

Equipment and Material

- Agilent 1200 HPLC with Diode Array Detector (Agilent Technologies Inc., Santa Clara, CA, USA).
- Agilent 6410 Triple Quadrupole LC/MS/MS system with Electrospray Ionization (Agilent Technologies Inc., Santa Clara, CA, USA).
- Agilent SampliQ Buffered QuEChERS AOAC Extraction kit, p/n 5982-5755, and SampliQ QuEChERS AOAC Dispersive SPE kit for General Fruits and Vegetables, p/n 5982-5022 and 5982-5058 (Agilent Technologies Inc., Wilmington, DE, USA).
- CentraCL3R Centrifuge (Thermo IEC, MA, USA)
- Bottle top dispenser (VWR, So. Plainfield, NJ, USA)
- Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)
- Grinder (St. Joseph, MI, USA)

Instrument Condition

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl, 3.0 x 150 mm, 3.5 µm (p/n 959963-312)
Flow rate:	0.3 mL/min
Column Temperature:	30 °C
Injection volume:	10 µL
Mobile Phase:	A: 5mM NH_4OAc, pH 5.0 in 20:80 MeOH/H_2O B: 5 mM NH_4OAc, pH 5.0 in ACN
Needle wash:	1:1:1:1 ACN/MeOH/IPA/H_2O (0.2% FA)

Gradient:

Time	% B	Flow rate (mL/min)
0	20	0.3
0.5	20	0.3
8.0	100	0.3
10.0	100	0.3
10.01	20	0.5
12.0	100	0.5
13.0	STOP	

Post run:	4 min
Total cycle time:	17 min

MS conditions

Positive mode	
Gas Temperature:	350 °C
Gas Flow:	10 L/min
Nebulizer:	40 psi
Capillary:	4000 V

Other MS conditions relating to the analytes are listed in Table 2.

Table 2. Instrument Acquisition Data Used for the Analysis of 16 Pesticides by LC/MS/MS

Analyte	MRM channels (*m/z*)	Fragmentor (V)	CE (V)	RT (min)
Acephate	1) 184.0 > 94.9 2) 184.0 > 111.0	60	3 15	2.55
Methamidophos	1) 142.0 > 94.0 2) 142.0 > 124.9	60	8 8	2.54
Pymetrozine	1) 218.1 > 105.0 2) 218.1 > 78.0	115	20 50	2.97
Carbendazim	1) 192.1 > 160.0 2) 192.1 > 105.0	95	18 40	5.07
Dichlorvos	1) 221.0 > 109.0 2) 221.0 > 95.0	110	13 40	6.57
Thiophanate methyl	1) 343.1 > 151.0 2) 343.1 > 117.9	105	17 65	7.08
Propoxur	1) 210.1 > 111.0 2) 210.1 > 92.9	50	12 15	6.89
Carbaryl	1) 202.0 > 145.0 2) 202.0 > 115.0	50	3 40	7.30
Cyprodinil	1) 226.1 > 93.0 2) 226.1 > 108.0	120	35 35	9.23
Dichlorfluanid	1) 333.0 > 123.0 2) 333.0 > 223.9	85	28 5	9.40
Ethoprophos	1) 243.1 > 130.9 2) 243.1 > 172.9	80	15 15	8.50
Penconazole	1) 284.1 > 158.9 2) 284.1 > 172.9	80	32 32	8.95
Tolyfluanid	1) 347.0 > 136.9 2) 347.0 > 238.0	60	25 3	9.73
Thiabendazole	1) 202.1 > 175.0 2) 202.1 > 131.0	110	27 38	5.65
Imidacloprid	1) 256.1 > 209.1 2) 256.1 > 175.0	60	12 18	5.53
TPP	1) 327.1 > 77.0 2) 327.1 > 151.9	70	45 45	9.49
Kresoxim methyl	1) 314.0 > 222.1 2) 314.0 > 235.0	70	10 10	9.44

1) Quantifier transition channel
2) Qualifier transition channel

Sample preparation

Sample comminution

In order to get the most reliable statistical results, it is important to spend the necessary effort and time on conducting proper sampling and homogenization procedures. Organically grown, pesticide-free apples were purchased from a local grocery store. Approximately three pounds of apples were chopped into small, bean-sized cubes. Skin was included, but pit was discarded. The chopped apple cubes were put into a clean plastic bag and frozen at –20 °C overnight. The bag was massaged occasionally to make sure the cubes were frozen loosely, to avoid clumping. The following day, a portion of frozen apple cubes were removed and thoroughly blended. Certain precautions were exercised while blending the sample. First, the chopped apple cubes remained in the freezer until the point of blending. Only the portion of apple cubes necessary for homogenizing were removed; the rest were kept in the freezer until the next comminution. Dry ice was added, when possible, while comminuting to keep the temperature low. Second, the blender container was kept dry to prevent clumping. In between blending, the container was rinsed and dried. Third, samples were comminuted thoroughly to obtain the best sample homogeneity. No pieces of apple were visible in the final sample.

Extraction/Partitioning

A 15 g (±0.05 g) previously homogenized sample was placed into a 50 mL centrifuge tube from the SampliQ QuEChERS Extraction kit. QC samples were fortified with 100 µL of appropriate QC spiking solution yielding QC samples with concentrations of 10, 50, and 200 ng/g. One hundred microliters of IS spiking solution (15 µg/mL of TPP) were added to all samples except the control blank to yield a 100 ng/g concentration in each sample. Tubes were capped and vortexed for 1 min. Fifteen milliliters of 1% HAc in ACN were added to each tube using the dispenser. To each tube, an Agilent AOAC Buffered Extraction packet from the kit (p/n 5982-5755) containing 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous NaOAc, was added directly to the tubes. No powders were left in the threads or rims of the tubes. Tubes were sealed tightly and shaken vigorously for 1 min by hand to ensure that the solvent interacted well with the entire sample and crystalline agglomerates were broken up. Sample tubes were centrifuged at 4000 rpm for 5 min.

Dispersive SPE Cleanup

A 1 mL aliquot of the upper ACN layer was transferred into a SampliQ QuEChERS AOAC 2 mL dispersive SPE tube (p/n 5982-5022) or 8 mL aliquot were transferred into an SampliQ QuEChERS AOAC 15 mL dispersive SPE tube (p/n 5982-5058). The 2 mL tube contained 50 mg of PSA and 150 mg of anhydrous $MgSO_4$; while the 15 mL tube contained 400 mg of PSA and 1200 mg of anhydrous $MgSO_4$. The tubes were tightly capped and vortexed for 1 min. The 2 mL tubes were centrifuged with a micro-centrifuge at 13000 rpm for 2 min, and the 15 mL tubes were centrifuged in a standard centrifuge at 4000 rpm for 5 min. Two hundred microliters of extract were transferred into an autosampler vial. Then 800 µL of water or another appropriate standard solution (prepared in water) were added. The samples were capped and vortexed thoroughly. The samples were then ready for LC/MS/MS analysis.

The flow chart in Figure 1 illustrates the sample preparation procedure.

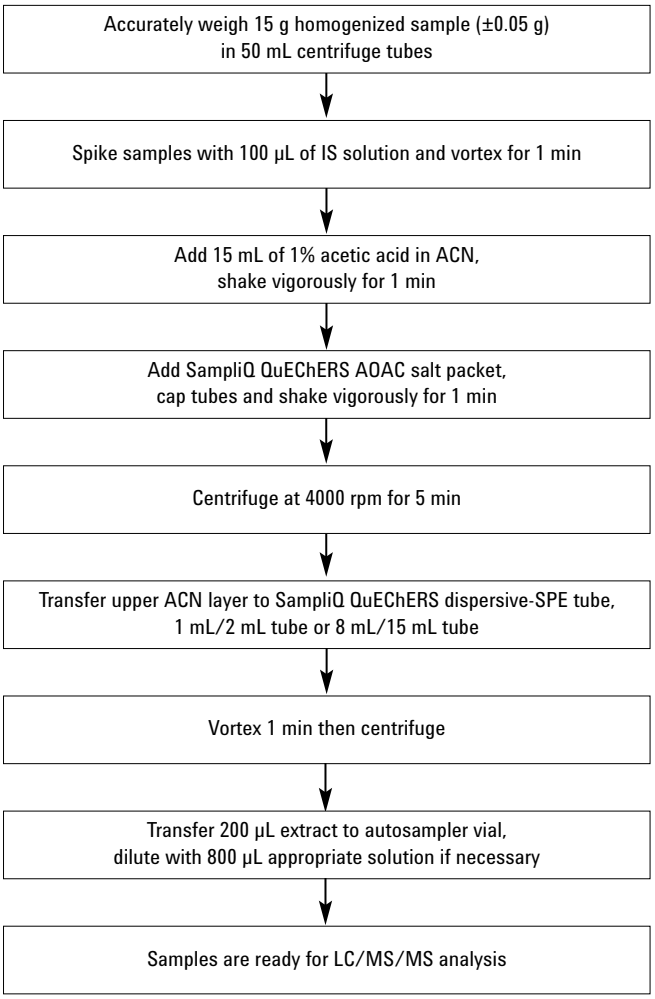


Figure 1. QuEChERS AOAC sample preparation procedures flow chart.

Results and Discussion

In addition to being fast, easy, cheap, effective, rugged and safe, an additional key feature of the QuEChERS method is the potential for the simultaneous analysis of multi-pesticide residues. With the new design of SampliQ QuEChERS kits, the whole procedure is even faster, easier, and offers more time and labor savings, while ensuring consistency. An analyst can process 40–50 samples in just a few hours. Adding a food sample with a high percentage of water directly to the salts may create an exothermic reaction that can affect analyte recovery. Agilent's SampliQ salts and buffers are uniquely prepared in anhydrous packages. This allows addition **AFTER** adding solvent to the sample, as specified in the QuEChERS methodology. The final QuEChERS sample may contain food matrix impurities because it is a very simple sample extraction and cleanup procedure. The final apple extract appeared light green. But with the powerful selectivity of LC/MS/MS multiple reaction monitoring (MRM) mode, the extracted apple blank appeared to be clean and free of impurities, indicating the blank apple extract did not contribute any interferences with the target compounds. Figure 2 shows the chromatograms of a blank apple extract and a 10 ng/g fortified apple extract.

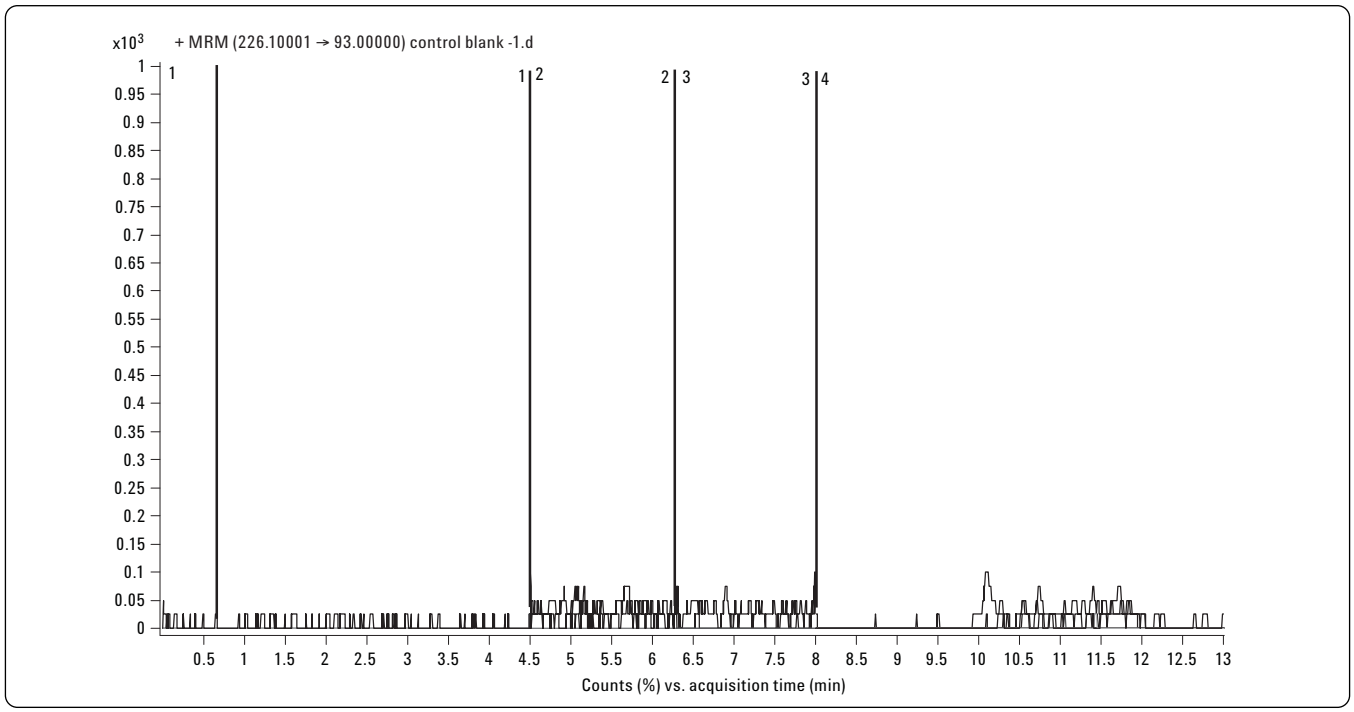


Figure 2a Chromatograms of apple extract blank. No interference was found in the blank.

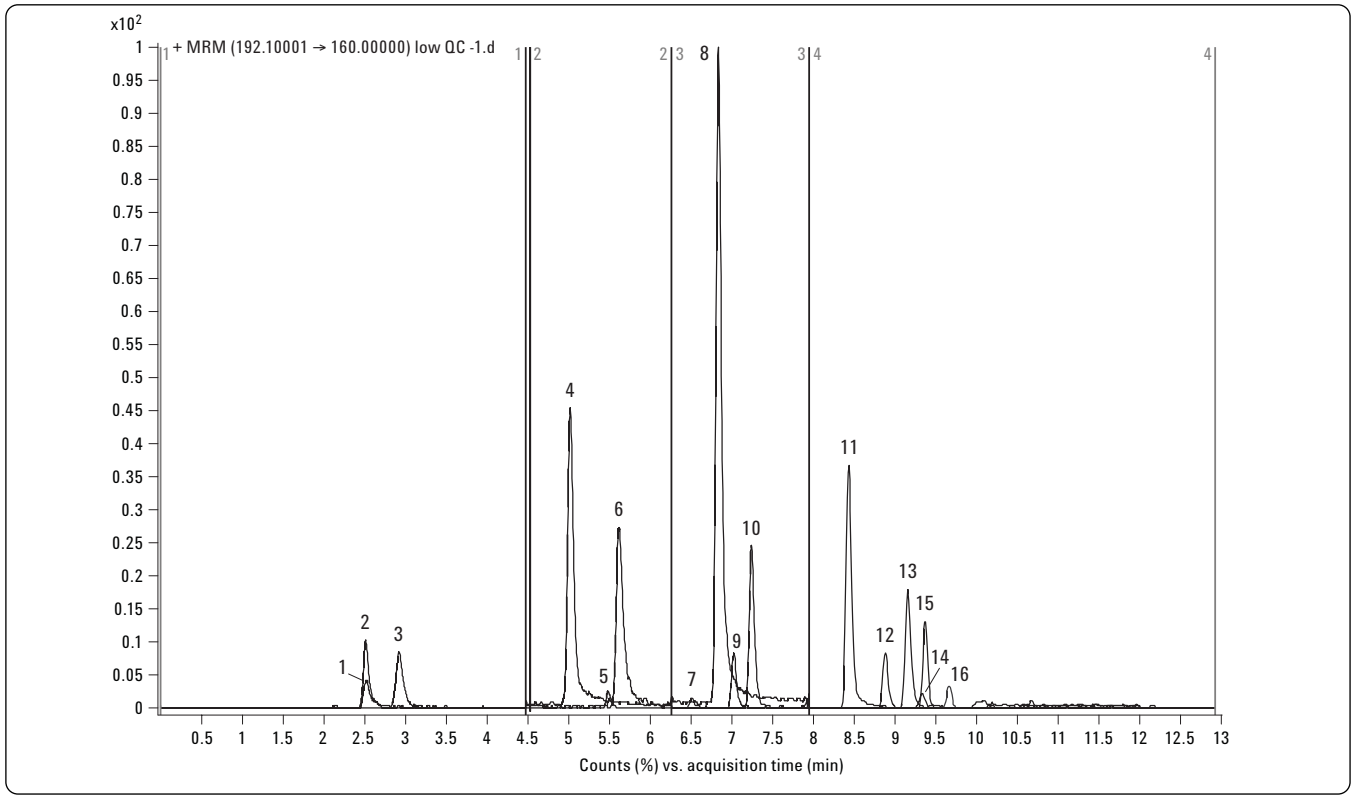


Figure 2b. Chromatogram of 10 ng/g fortified apple extract. Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid, 6. Thiabendazole, 7. Dichlorvos, 8. Propoxur, 9. Thiophanate methyl, 10. Carbaryl, 11. Ethoprophos, 12. Penconazole, 13. Cyprodinil, 14. Dichlofluanid, 15. Kresoxim methyl, 16, Tolyfluanid.

Linearity and Limit of Quantification (LOQ)

The linear calibration range for all the pesticides was 5 – 250 ng/g. Since two different dispersive SPE volumes (1 mL and 8 mL) were used for evaluation and comparison, two sets of calibration curves were generated respectively. Matrix blanks were prepared for each size. Calibration curves, spiked in matrix blanks, were made at levels of 5, 10, 50, 100, 200, and 250 ng/g. The TPP (IS) was used at 100 ng/g level.

The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). Table 1 shows that the 5 ng/g quantification limits LOQ (5 ppb) established for all of the pesticides is lower than the MRLs of these pesticides in fruit and vegetables. Table 3 shows the regression equation and correlation coefficient (R^2) for both 1 mL and 8 mL dispersive SPE volumes.

Table 3. Linearity of Pesticides in Apple Extract

Analytes	1 mL dispersive SPE Regression equation	R^2	8 mL dispersive SPE Regression equation	R^2
Methamidophos	Y = 0.2349X - 0.0013	0.9949	Y = 0.2300X - 0.0007	0.9981
Acephate	Y = 0.1118X - 0.0012	0.9881	Y = 0.1094X - 0.0014	0.9980
Pymetrozine	Y = 0.2671X - 0.0016	0.9950	Y = 0.2290X - 0.0014	0.9975
Carbendazim	Y = 0.9441X + 0.0063	0.9895	Y = 0.8583X + 0.0006	0.9968
Imidacloprid	Y = 0.0513X - 0.0009	0.9905	Y = 0.0500X - 0.0007	0.9933
Thiabendazole	Y = 0.7049X + 0.0044	0.9868	Y = 0.6198X + 0.0043	0.9961
Dichlorvos	Y = 0.0265X + 0.0001	0.9884	Y = 0.0247X + 0.0006	0.9439
Propoxur	Y = 2.0348X - 0.0091	0.9951	Y = 2.0264X - 0.0090	0.9965
Thiophanate methyl	Y = 0.2024X - 0.0054	0.9307	Y = 0.5090X - 0.0041	0.9682
Carbaryl	Y = 0.4984X - 0.0002	0.9965	Y = 0.4889X - 0.0029	0.9976
Ethoprophos	Y = 0.8203X - 0.0064	0.9952	Y = 0.8536X - 0.0076	0.9971
Penconazole	Y = 0.1775X - 0.0006	0.9903	Y = 0.1783X - 0.0019	0.9848
Cyprodinil	Y = 0.3529X - 0.0023	0.9960	Y = 0.3528X - 0.0022	0.9958
Dichlorfluanid	Y = 0.0453X - 0.0004	0.9869	Y = 0.0460X - 0.0006	0.9954
Kresoxim methyl	Y = 0.2498X - 0.0024	0.9932	Y = 0.2490X - 0.0013	0.9927
Tolyfluanid	Y = 0.0718X - 0.0016	0.9823	Y = 0.0755X - 0.0006	0.9788

Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticide standards in homogeneous apple samples at levels of 10, 50, and 200 ng/g. These QC samples were quantitated against the matrix spiked calibration curve. The analysis was performed in replicates of six (n = 6) at each level. The recovery and reproducibility (RSD) data of 1 mL and 8 mL dispersive SPE sample volumes are shown in Tables 4 and 5, respectively. It can be seen from the results that all of the pesticides give acceptable recoveries (average of 97.5% for 1 mL and 93.3% for 8 mL) and precision (average of 4.5% RSD for 1 mL and 4.1% RSD for 8 mL). The notoriously base-sensitive pesticides such as dichlorfluanid and tolyfluanid showed excellent recovery and precision. Acid labile pesticide, pymetrozine, also showed acceptable recovery and precision.

Table 4. Recovery and Repeatability of Pesticides in Fortified Apple With 2 mL Dispersive SPE Tube (p/n 5982-5022)

	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
Analytes	**Recovery**	**RSD (n=6)**	**Recovery**	**RSD (n=6)**	**Recovery**	**RSD (n=6)**
Methamidophos	83.6	5.6	81.3	2.6	83.4	1.4
Acephate	106.8	5.8	95.6	2.3	97.3	2.0
Pymetrozine	78.3	11.4	76.6	11.6	108.1	5.3
Carbendazim	101.0	6.5	98.5	4.3	91.0	2.6
Imidacloprid	107.0	6.5	97.6	3.4	107.4	3.0
Thiabendazole	106.2	6.6	103.7	2.6	95.5	2.0
Dichlorvos	78.2	11.4	94.2	7.2	95.8	1.8
Propoxur	106.3	0.8	105.7	1.2	101.2	1.6
Thiophanate methyl	79.0	15.4	76.7	15.4	102.2	8.1
Carbaryl	93.4	1.9	98.4	2.2	97.5	1.1
Ethoprophos	95.8	4.5	96.1	1.8	94.7	1.3
Penconazole	117.0	4.8	111.9	2.3	111.0	1.6
Cyprodinil	106.9	4.0	102.0	2.8	102.4	1.8
Dichlorfluanid	92.5	6.5	96.3	2.2	99.4	2.6
Kresoxim methyl	98.2	9.3	101.9	2.7	104.1	1.8
Tolyfluanid	96.6	9.5	105.1	1.8	102.2	1.7

Table 5. Recovery and Repeatability of Pesticides in Fortified Apple With 15 mL Dispersive SPE Tube (p/n 5982-5058)

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Methamidophos	80.6	9.3	79.4	2.9	83.1	2.5
Acephate	94.6	7.0	93.7	3.4	95.1	2.5
Pymetrozine	88.8	12.1	87.7	10.1	118.4	5.5
Carbendazim	85.9	3.9	90.4	2.7	85.5	2.2
Imidacloprid	101.8	3.5	99.3	3.7	106.0	0.9
Thiabendazole	92.5	6.4	92.2	2.6	89.5	1.5
Dichlorvos	73.7	14.8	91.8	7.3	95.5	2.0
Propoxur	96.2	1.6	98.2	0.6	97.2	1.2
Thiophanate methyl	81.4	4.9	78.2	13.4	102.3	5.8
Carbaryl	86.5	2.6	90.3	1.4	91.1	1.2
Ethoprophos	89.6	2.9	92.1	1.0	94.1	1.1
Penconazole	102.1	2.5	106.0	3.0	111.0	1.6
Cyprodinil	93.9	3.7	97.4	0.9	99.7	2.0
Dichlorfluanid	81.7	8.7	96.9	5.6	98.1	2.6
Kresoxim methyl	91.8	5.8	93.9	2.0	98.3	1.2
Tolyfluanid	94.1	7.9	95.2	4.0	97.5	2.6

Figure 3 shows the recovery and precision results for 1 mL and 8 mL dispersive SPE. The two different dispersive SPE clean-ups were performed by using 1 mL or 8 mL of ACN extract from the same sample tube after the extraction step. In order to simplify the comparison, the average recovery and precision of three fortification concentrations were used for all pesticides. The results of two dispersive SPE clean-up approaches appeared to be independent of volume used. Both approaches provided efficient sample clean-up, and generated relatively equivalent results.

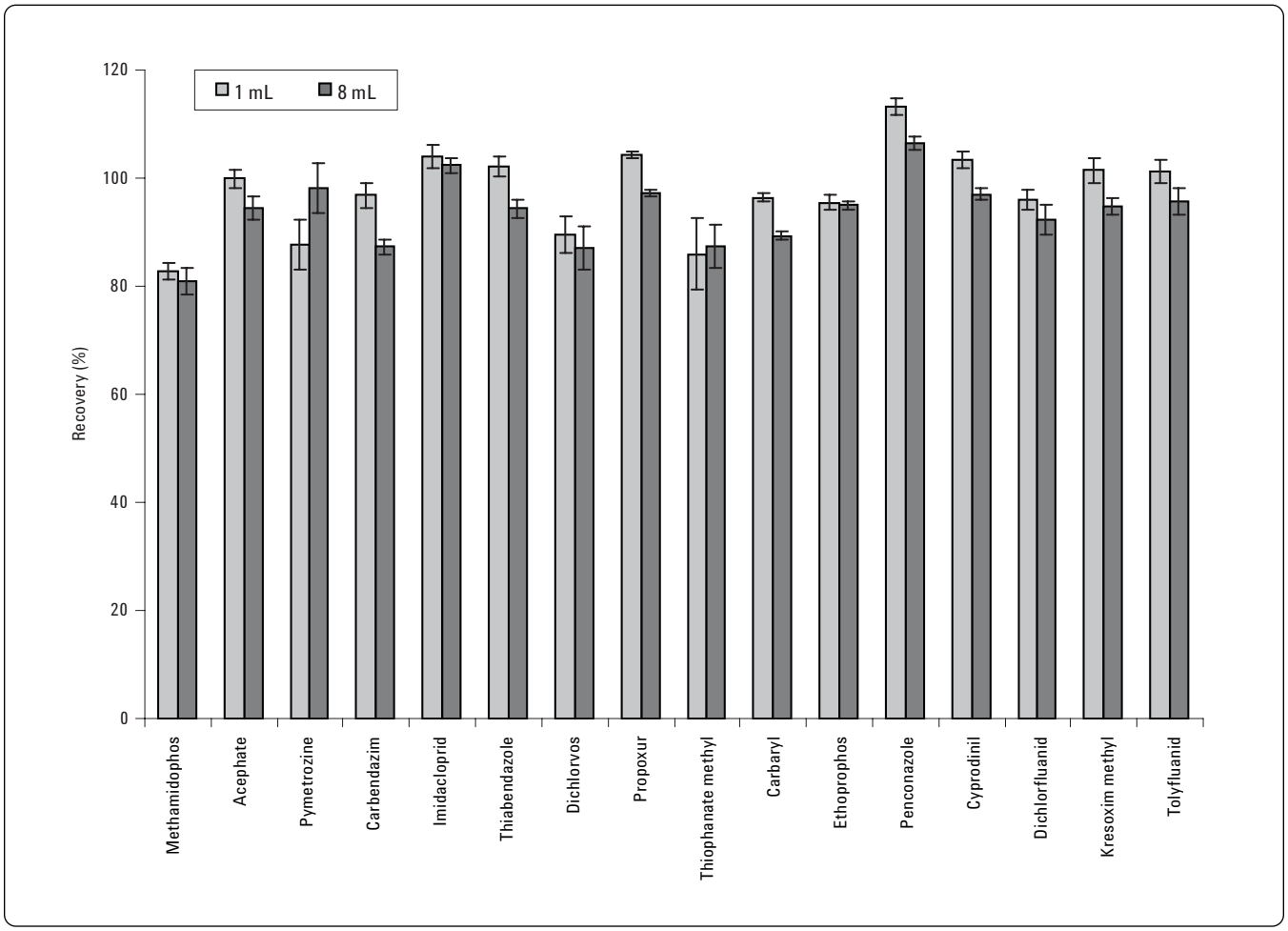


Figure 3. Results comparison of 1 mL and 8 mL dispersive SPE sample volume.

Conclusions

Agilent SampliQ AOAC Buffered Extraction kit and SampliQ AOAC dispersive SPE kit for General Fruits and Vegetables provided a simple, fast, and effective method for the purification of representative pesticides in apple. The recovery and reproducibility, based on matrix spiked standards, were acceptable for multiclass, multi-residue pesticide determination in apple. The impurities and matrix effects from apple were minimal and did not interfere with the quantitation of any target compound. The LOQs of the pesticides were significantly lower than their regulated MRLs in apple. As the selected pesticides represented a broad variety of different classes and properties, the Agilent SampliQ QuEChERS AOAC Extraction and Dispersive kit for General Fruits and Vegetables can be used for other pesticides in similar fruit matrices.

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Introduction

Multi-residue methods are efficient and cost-effective for analysis of pesticide residues. For methods with a very wide scope, generic sample preparation procedures are usually employed. Inherent to this approach is that clean up of extracts is only possible to a limited extent[1]. When applying such methods to complex matrices like baby food, herbs, spices and tobacco, enhanced selectivity in detection is required to make up for the low selectivity in sample preparation.

Your Challenges

The analytical challenge is to maximize the number of pesticides, minimize the variety of methods, keep run times short and achieve limits of detection (LOD's) at or below the maximum residue limits (MRLs) which are specified for pesticides under EU legislation.

As regulations in the European Union require very low MRLs for pesticide residues, the latest challenge has been to reach part-per-billion level concentrations for hundreds of pesticides in complex matrices, which in turn has required greater sensitivity and efficiency in pesticide screening. Quantitation and confirmation of identity of trace level compounds can be complicated by the matrix, resulting in qualifier ion ratios out of range, or target ions buried in the high chemical background noise. With single quadrupole mass spectrometry, selected ion monitoring (SIM) is often used to improve the detection limit and quantitative reproducibility. In SIM mode, only a few ions are monitored for each target within the retention time (RT) range that the target elutes from the column. SIM may not work well for trace levels in matrix as the interferences in SIM are the same as in full scan mode.

Our Integrated Approach

Triple quadrupole mass spectrometry allows for drastic reduction or elimination of matrix interferences that limit the accuracy and detection limits of SIM methods. This process, referred to as Multiple Reaction Monitoring (MRM), has two fundamental advantages over SIM. First, detection is based secondary "product ion" produced by the collisional dissociation of an analyte "precursor ion". The analyte precursor ion (isolated in Q1 by a SIM mechanism) has the same selectivity as SIM, but there is a high probability that at least one of the resultant product ions will be unique to the precursor and not the interference. The increase selectivity of MRM is often apparent by the reduced offset of the baseline as compared to SIM. Secondly, during the mass filtering process in Q1, all lower m/z ions from the sample are eliminated. The unique product ions from the collisional dissociation are measured in this "zero" noise region of the spectrum. The combination of a unique product ions (more selectivity) and the elimination of background noise results in consistently low limits of detection even for complex matrices.

This application brief describes the analysis of pesticides in fruit and vegetable extracts using the Agilent 7000 Series Triple Quadrupole GC/MS system in MRM mode and in combination with Retention Time Locking[2] and Agilent Capillary Flow Technology to provide backflushing of high-boiling materials.[3]

Column backflushing is essential for the analysis of complex samples such as food extracts[4] because they usually contain high-boiling indigenous compounds. In just a few runs, these materials can collect on the head of the column, causing peak tailing, retention time shifts and increased chemical noise. Over time, they can migrate from the column to the ionization source, which would eventually have to be cleaned. Agilent's proprietary capillary flow technology makes column backflushing routine and easy to setup for non experts.

The method robustness is drastically increased and the analysis cycles are shortened[5]. In conclusion the system up-time is maximized allowing significant productivity gains. The need for maintenance is reduced by keeping the chromatographic system and MS ion source cleaner between each injection.

Figure 1
Agilent 7890A/7000A Triple Quadrupole GC/MS system with the new high capacity 7693 ALS.

Experimental

Samples were prepared using the QuEChERS[6] method. QuEChERS stands for Quick Easy, Cheap, Effective, Rugged and Safe and is a food sample preparation for multi-class, multi-residue pesticide analysis. See more at www.agilent.com/chem/Quechers

Instrumentation

The Triple quadrupole GC/MS system used for these experiments are described in Table 1 and shown in Figure 1.

Instrument conditions

Instrumentation	
GC/MS Triple Quadrupole:	Agilent 7000A
GC:	Agilent 7890A
Inlet:	PTV, in splitless mode, 1µL Injection, Multi-baffle liner 80 °C for 0.5 min, then 500 °C/min to 280 °C for 2 min
Capillary flow technology device:	3-way splitter with analytical column in and restrictor out to the triple quadrupole helium pressure provided by Aux EPC at 1 psi
Column:	Agilent J&W HP-5ms Ultra Inert 30 m x 0.25 mm ID, 0.25 µm HP-5MSUI
Restrictor:	80 cm x 0.180 mm deactivated fused silica
Carrier gas:	Helium 30.883 psi (constant pressure mode)
Oven temperature:	70 °C (1 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)
Backflush:	Time 5 min, inlet press. 1 psi, Aux EPC 80 psi, oven temp. 280 °C
Retention time locking:	Chlorpyrifos-methyl locked to 16.53 min
Collision cell gases:	N_2 2.60 psi and He 6.25 psi
Inert source temperature:	260 °C
Quadrupole temperature:	150°C

Table 1
Instrument conditions.

Method Development

In order to maximize the response of the instrument for each residue the choice of precursor ion, product ion and collision energy were optimized. The spectra of a typical pesticide in full scan mode (50-500 m/z) e.g. Dicloran (MW = 206) is displayed in Figure 2. The product ion scan spectra of 206 m/z at different collision energy are displayed in Figure 3.

Results

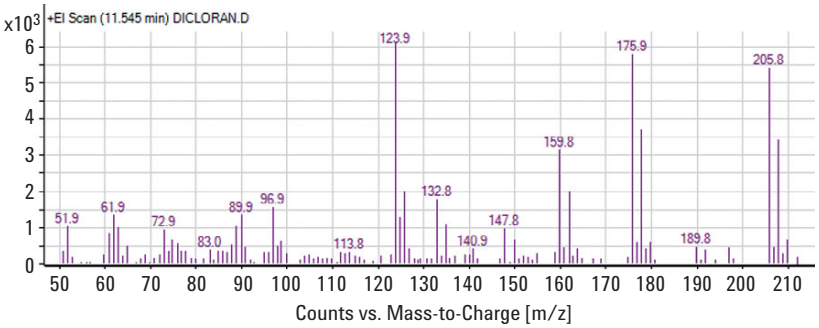

Figure 2
Full scan spectrum of Dicloran under EI ionization mode.

Results

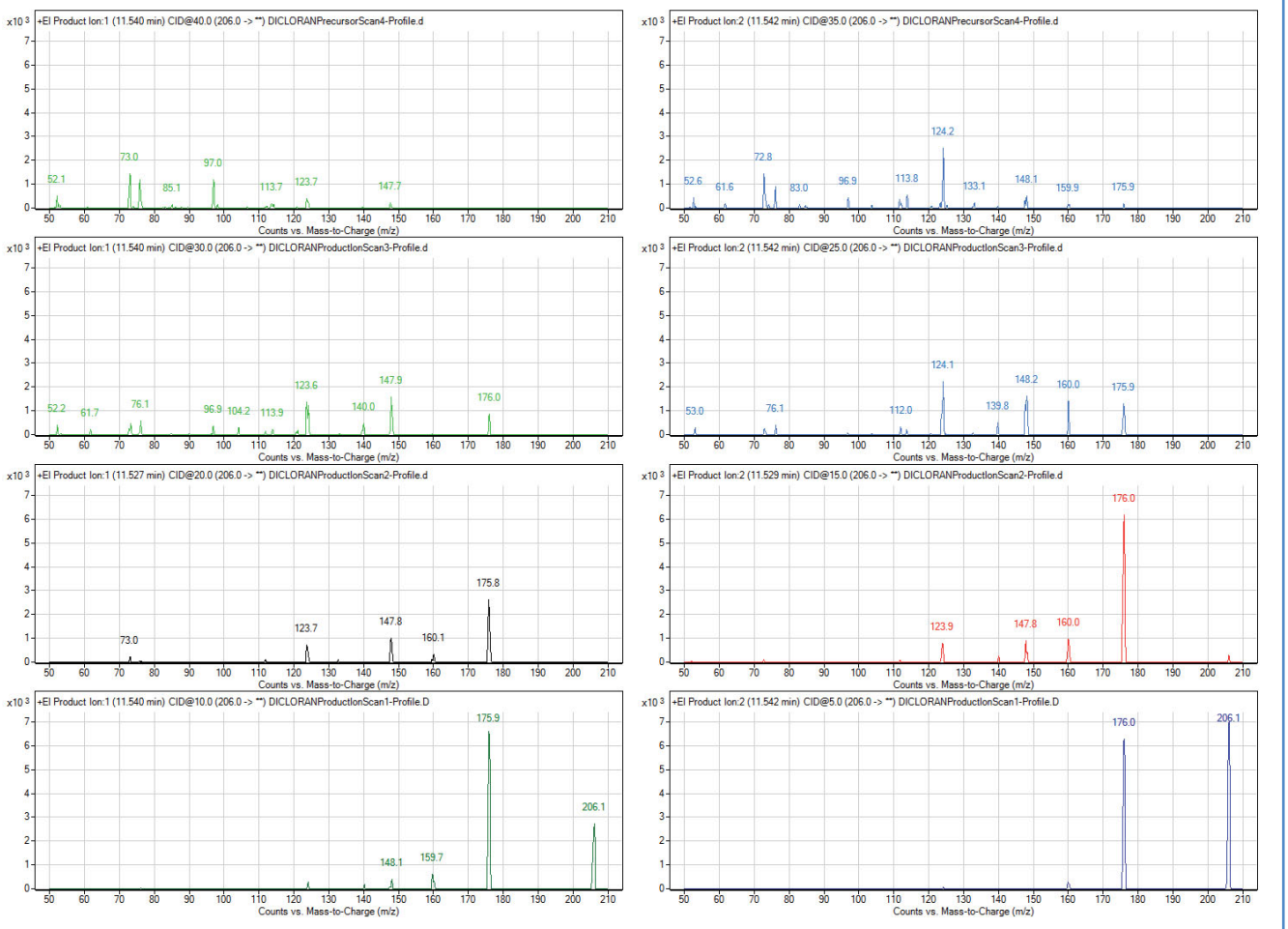

Figure 3
Product ion scan of Dicloran for the Precursor (206 m/z) at different collision energies (5-40 V).

The optimum collision energy for the 206>176 transition product ion was found to be at 10V and the resulting MRM chromatogram is shown in Figure 4.

Results and discussion

Figure 5 shows the TIC chromatogram acquired in MRM mode for 360 pesticides. Each MRM segment is indicated by a grey marker line. An enhanced view on a selected part of the analysis with an overlay of all MRM for the compounds in this part is shown Figure 6. The MRM mode allows for accurate quantification of many coeluting analytes as shown between 13.6 and 14.2 minutes. Figure 7 demonstrates the identity confirmation of Diclobenil in a peppermint extract at 10 pg on column using two MRM transitions. The dashed lines indicate the allowed range of the ion ratio as specified in the method.

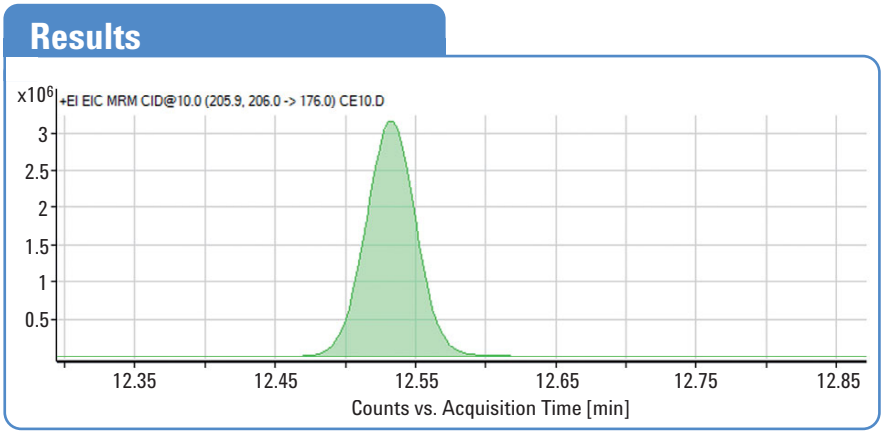

Figure 4
MRM chromatogram of Dicloran at 10ppb.

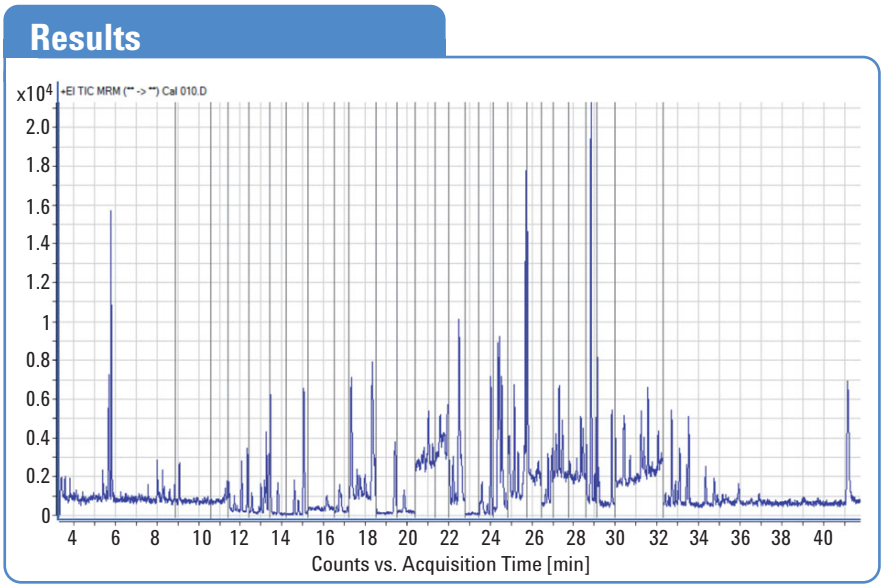

Figure 5
Total ion chromatogram of the vegetable extract by GC/MS/MS.

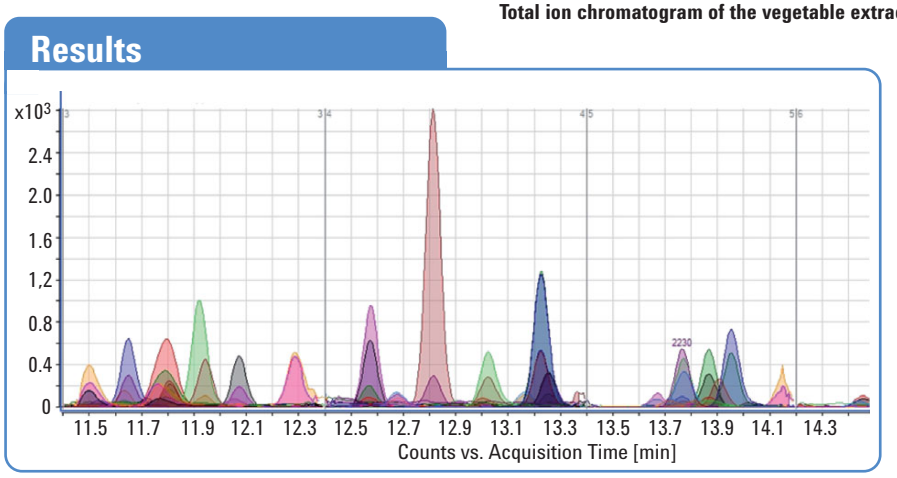

Figure 6
Overlay of extracted MRM transitions.

Results

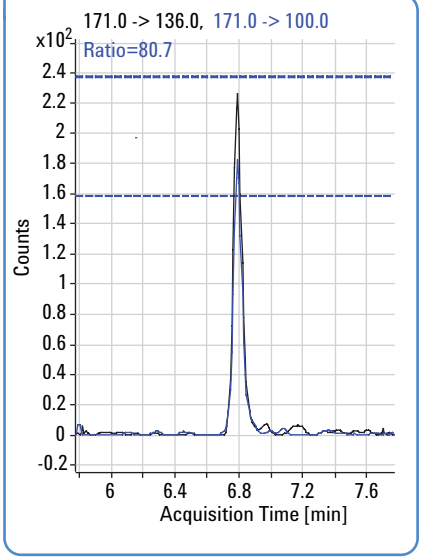

Figure 7
Two transitions identifying Diclobenil in a peppermint extract at 10 pg on column. The dashed lines indicate the allowed range of the ion ratio.

Linearity was also tested with five levels between 1 and 200 ppb for 360 pesticides and Figure 8 shows the calibration curves for Diclobenil and Chlormefos. The correlation coefficients of the external standard calibration curve were 0.99 on average. The LOD was estimated based on the calculated S/N of the 10 pg standard. For the majority of pesticides, LODs were below 2 pg on column (based on S/N >3:1 Peak to Peak).

Retention time reproducibility was also tested to demonstrate the robustness of the analytical method. Figure 9 shows the outstanding retention time stability of one representative compound: Trifluralin. Calculated %RSD is 0.0306 at 6.073 min for one hundred consecutive injections of lettuce extract into the GC/MS/MS system. Only 3 minute Backflush was necessary to remove all high boiling matrix compounds, the total cycle time for this stability test was 21 hours.

Results

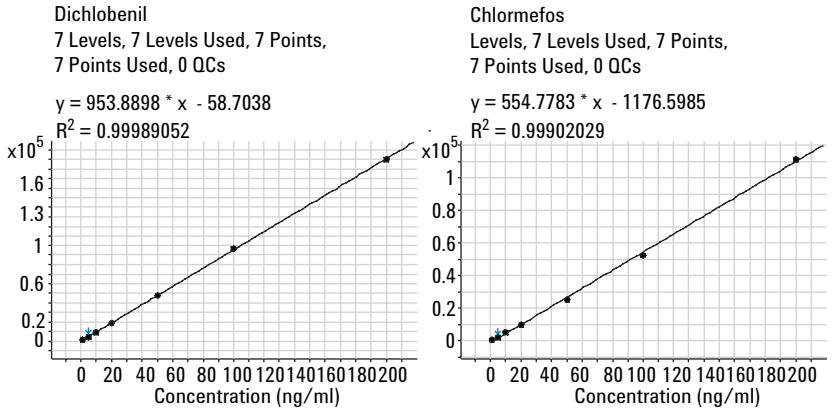

Figure 8
Calibration curves showing excellent linearity over the concentration from 1 ppb to 200 ppb range R^2= 0.999 respectively for Dichlobenil and Chlormefos.

Results

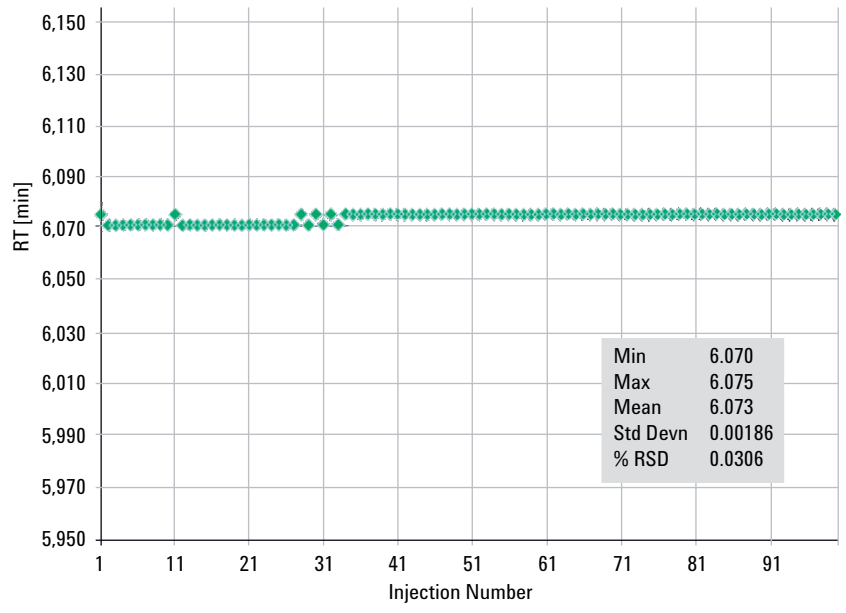

Figure 9
Exceptional retention time stability of Trifluralin with 100 injections of lettuce extract thanks to column backflushing.

Conclusion

- Agilent's 7000 Series Triple Quadrupole GC/MS in combination with the 7890 GC is a sensitive and rugged tool for target pesticide analysis in complex matrices. The single multi-residue method we developed also meets the performance and identity confirmation criteria defined by the stringent EU regulations.

- Excellent selectivity has been achieved to allow unambiguous confirmation of identity for these 360 pesticides even in very complex food matrices and generic sample clean up. Agilent SampliQ QuEChERS kits enable you to prepare food samples for multiresidue, multi-class pesticide analysis with just a few simple steps.

- For this new GC/MS/MS method, the Agilent Retention Time Locking (RTL) database was used to calibrate the retention times of all pesticides. Therefore, the presented GC/MS/MS method can be easily transferred to other Agilent 7000 Series Triple Quadrupole systems with minimum effort and time.

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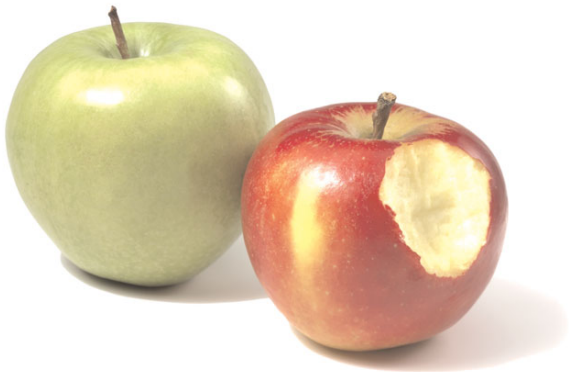

www.agilent.com/chem/7000B

Published November 1, 2009
Publication Number 5990-5044EN

Agilent Technologies

A Method for the Trace Analysis of 175 Pesticides Using the Agilent Triple Quadrupole GC/MS/MS

Application Note

Food Safety

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Abstract

A GC/MS/MS multiple reaction monitoring (MRM) method has been developed on the Agilent 7890A/7000A GC triple quadrupole mass spectrometer system (GC/QQQ) for 175 commonly analyzed pesticides. Numerous fruit and vegetable extracts were analyzed by this method and by a GC single quadrupole mass spectrometer (GC/Q) for comparison. The GC/Q was operated in the selected ion monitoring (SIM) mode and in the scan mode. Scan results were evaluated using Agilent's Deconvolution Reporting Software (DRS) with the RTL Pesticide and Endocrine Disruptor Database. The GC/Q instrument was equipped with a Multimode inlet and injections of 5 µL were made in the cold splitless mode. These were compared to 1 µL injections of the same extracts on the GC/QQQ. The GC/QQQ was found to be far more sensitive and selective than either GC/Q approach, primarily because there is much less interference from co-extracted matrix. There is, however, still a need for GC/Q methods that use DRS to screen for more than 900 pesticides and other contaminants since the GC/QQQ in the MRM mode is only for target compound analysis.

Agilent Technologies

Introduction

Pesticide residue analysis is a complex task requiring the analyst to search for dozens, or even hundreds, of compounds in a wide variety of crop matrices. Extraction techniques, such as the QuEChERS method [1–3] leave large amounts of indigenous materials in the extract. The use of more extensive cleanup steps risks removing pesticide residues in addition to the matrix. As required detection limits for many pesticides fall to 10 µg/Kg (10 ppb) or lower, more sophisticated analytical tools are needed.

For GC-amenable pesticides, many laboratories are using two complementary techniques for screening and confirmation purposes. For broad screening at the 5 to 100 ppb level, GC/single quadrupole (GC/Q) is employed with Deconvolution Reporting Software (DRS) and the RTL Pesticide and Endocrine Disruptor library from Agilent Technologies [4–6]. This is a scan method to screen for 927 GC-amenable pesticides and endocrine disruptors in a single GC/MS run. Detection limits for most pesticides vary from approximately 5 to 100 ppb, depending upon the matrix and the injection volume [4]. For target pesticide analysis in the most complex matrices, the Agilent 7890A/7000A GC/triple quadrupole (GC/QQQ) is unmatched.

This paper compares three mass spectral techniques for the analysis of pesticide residues in a variety of crop matrices. Spiked and unspiked samples were analyzed by GC/Q in the selected ion monitoring (SIM) mode and in the scan mode with DRS analysis. The same samples were also analyzed by GC/QQQ using a multiple reaction monitoring (MRM) method for 175 pesticides. The objective was to compare the ability of these GC/Q and GC/QQQ methods to detect low levels of pesticides in several different crop matrices.

Experimental

Samples

Spiked and unspiked extracts of fresh produce were provided by the U.S. Food and Drug Administration (U.S. FDA, CFSAN, College Park, MD) and the U.S. Department of Agriculture (USDA ARS, ERRC, Wyndmoor, PA). Samples from the FDA were prepared using the QuEChERS [1–3] method modified to include the use of activated carbon as an additional sorbent. The resultant toluene solution contained 4.5 grams of produce per milliliter of extract. Samples from the USDA were extracted using the published QuEChERS method and contained 1 gram of produce per milliliter of acetonitrile solvent.

Instrumentation

The GC/Q and GC/QQQ systems used for these experiments are described in Tables 1 and 2.

Table 1. Instrumentation and Analytical Conditions for the GC/Q System

GC	Agilent 7890A Series
Autosampler	Agilent 7693A Injector and sample tray
Inlet	Multimode inlet
Carrier gas	Helium
Inlet pressure	18.420 psi (constant pressure mode) during run 2.0 psi (during backflush)
Splitless Mode Inlet Parameters	
Temperature	250 °C
Inlet liner	Helix double taper, deactivated (P/N 5188-5398)
Injection volume	1 µL
Purge flow to split vent	30 mL/min at 0.75 min
Cold Splitless Mode Inlet Parameters	
Temperature program	60 °C (0.01 min), 700 °C/min to 280 °C (hold)
Inlet liner	Helix double taper, deactivated (P/N 5188-5398)
Injection volume	5 µL
Purge flow to split vent	30 mL/min at 1.25 min
Oven temperature program	70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C/min to 200 °C (0 min), 16 °C/min to 280 °C (5 min)
Capillary flow technology	2-way splitter with one port capped – used for backflushing the analytical column and retention gap
Pneumatic Control Module (PCM)	Helium plumbed to 2-way splitter
PCM pressure	4.0 psi during run, 60.0 psi during backflush
Analytical column	Agilent J&W HP-5ms UI 15 m × 0.25 mm × 0.25 µm (P/N 19091S-431UI)
Connections	Between retention gap and 2-way splitter
Retention gap	2.0 m × 0.25 mm Siltek deactivated fused silica tubing (Restek, Bellefonte, PA)
Connections	Between inlet and analytical column using an Ultimate Union (P/N G3182-61580) to couple the retention gap to the column
Restrictor	80 cm × 0.15 mm deactivated fused silica tubing (Agilent)
Connections	Between the 2-way splitter and the MSD
Initial column flow rate	2.705 mL/min (nominal)
Retention time locking	Chlorpyrifos-methyl locked to 8.298 min
Mass selective detector	Agilent 5795C Series with performance turbo pump
Mode	Electron impact
Transfer line temperature	280 °C
Source temperature	230 °C
Quadrupole temperature	150 °C
Threshold	100
Sampling rate	A/D = 4
Gain factor	1
SIM dwell times	Variable from 4 to 25 ms
Trace ion detection	On
Solvent delay	2.5 min
Backflushing Conditions	
Timing	5 min duration during post-run
Oven temperature	280 °C

(Continued)

Table 1. Instrumentation and Analytical Conditions for the GC/Q System (continued)

Aux EPC pressure	60 psi
Inlet pressure	2 psi
Software	
GC/MSD	Agilent GC/MS ChemStation control and data analysis software (P/N G1701EA E.02.00 SP1)
Deconvolution Reporting Software	Agilent P/N G1716AA (Ver. A.04.00)
Library Searching Software	NIST MS Search (Ver 2.0d) (comes with NIST mass spectral library – Agilent P/N G1033A)
Deconvolution software	Automated Mass Spectral Deconvolution and Identification Software (AMDIS_32 version 2.62 or greater; comes with NIST mass spectral library – Agilent P/N G1033A)
MS Libraries	NIST 08 mass spectral library (Agilent P/N G1033A) Pesticide and Endocrine Disruptor Database (Agilent P/N G1672AA)

Table 2. Instrumentation and Analytical Conditions for the GC/QQQ system

GC	Agilent 7890A Series
Autosampler	Agilent 7683A Injector and sample tray
Inlet	Split/splitless
Inlet liner	Helix double taper, deactivated (P/N 5188-5398)
Carrier gas	Helium
Inlet pressure	18.350 psi (constant pressure mode) during run 1.0 psi (during backflush)
Inlet temperature	250 °C
Injection volume	1 µL
Purge flow to split vent	30 mL/min at 0.75 min
Gas saver	On (20 mL/min at 2.0 min)
Oven temperature program	70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C/min to 200 °C (0 min), 16 °C/min to 280 °C (5.5 min)
Capillary flow technology	Purged Ultimate Union (P/N G3186B) – used for backflushing the analytical column and retention gap
Aux EPC gas	Helium plumbed to Purged Ultimate Union
Aux EPC pressure	4.0 psi during run, 80.0 psi during backflush
Analytical column	Agilent J&W HP-5ms UI 15 m × 0.25 mm × 0.25 µm (P/N 19091S-431UI)
Connections	Between retention gap and Purged Ultimate Union (P/N G3186B)
Retention gap	2.0 m × 0.25 mm Siltek deactivated fused silica tubing (Restek, Bellefonte, PA)
Connections	Between inlet and analytical column using ultimate union (P/N G3182-61580) to couple the retention gap to the column
Restrictor	65 cm × 0.15 mm deactivated fused silica tubing (Agilent)
Connections	Between the Purged Ultimate Union (P/N G3186B) and the MSD
Initial column flow rate	2.688 mL/min (nominal)
Retention time locking	Chlorpyrifos-methyl locked to 8.298 min
Triple Quadrupole Mass Spectrometer	Agilent 7000A Series
Mode	Electron impact
Transfer line temperature	280 °C
Solvent delay	2.3 min
Source temperature	300 °C
Quadrupole temperature	Q1 and Q2 = 150 °C
MRM Mode Conditions	
MS1 resolution	1.2 u
MS2 resolution	1.2 u
Collision gas flows	Nitrogen at 1.5 mL/min, Helium at 2.35 mL/min
Backflushing Conditions	
Timing	3 min duration during post-run
Oven temperature	280 °C
Aux EPC pressure	80 psi
Inlet pressure	1 psi
Software	
Data acquisition	Agilent MassHunter Data Acquisition Software (Ver. B.04.00)
Qualitative analysis	MassHunter Workstation Software for Qualitative Analysis (Ver. B.03.01)
Quantitative analysis	MassHunter Workstation Software for Quantitative Analysis (Ver. B.03.01)

Results and Discussion

GC Configuration

Both GC systems used a 15-m × 0.25 mm × 0.25 µm Agilent J&W HP-5ms UI column and were running the standard Agilent pesticide method [7] at 2X speed. This method uses an initial oven temperature of 70 °C, which works for most GC solvents without using a retention gap. However, 1-µL injections of samples in toluene lead to poor peak shape, so a 2-m deactivated retention gap was coupled to the front of the column. This improved the peak shapes.

Column backflushing is essential for the analysis of food extracts [4, 8–9] because they usually contain high-boiling indigenous compounds. In just a few runs, these materials can collect on the head of the column (or retention gap), causing peak tailing and retention time shifts. Over time, they can migrate from the column to the mass spec source, which would eventually have to be cleaned.

Agilent's capillary flow technology makes column backflushing routine (4, 8–9) and several different capillary flow devices can be used for the purpose. The GC/QQQ system used a Purged Ultimate Union, while the GC/Q system used a two-way splitter (with one port capped). In both cases, the analytical column was connected to the capillary flow device. A short restrictor was used to couple the capillary flow device to the mass spectrometer. Figure 1 shows the configuration of each instrument.

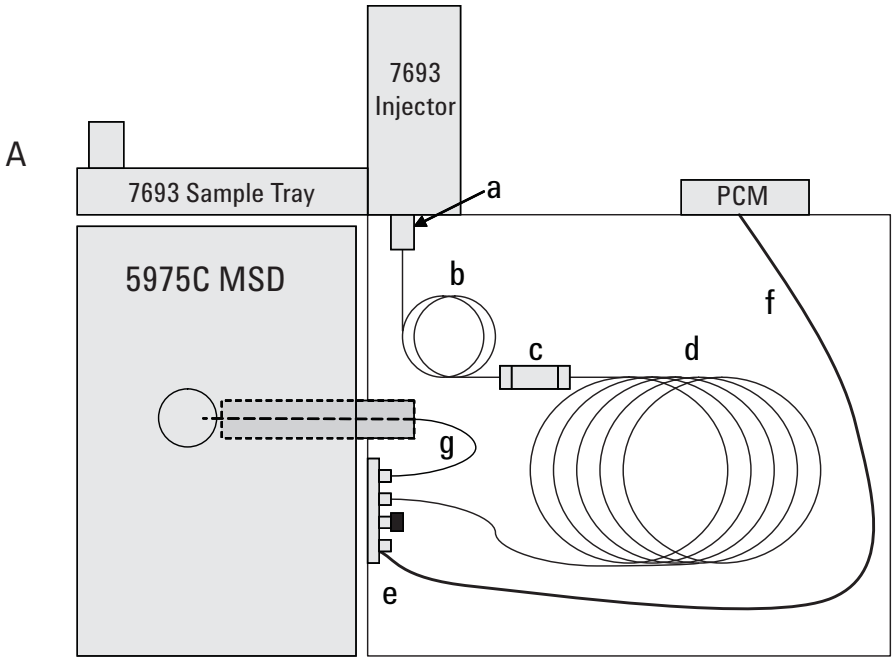

Figure 1A. The GC/MSD used for scan and SIM analyses was configured with a) Multimode inlet, b) 2 m × 0.25 mm deactivated retention gap, c) Ultimate Union, d) 15 m × 0.25 X 0.25 µm Agilent J&W HP-5ms UI column, e) two-way purged splitter with one port capped, f) helium purge flow controlled by a pneumatic control module (PCM), and g) 80 cm × 0.15 mm deactivated restrictor.

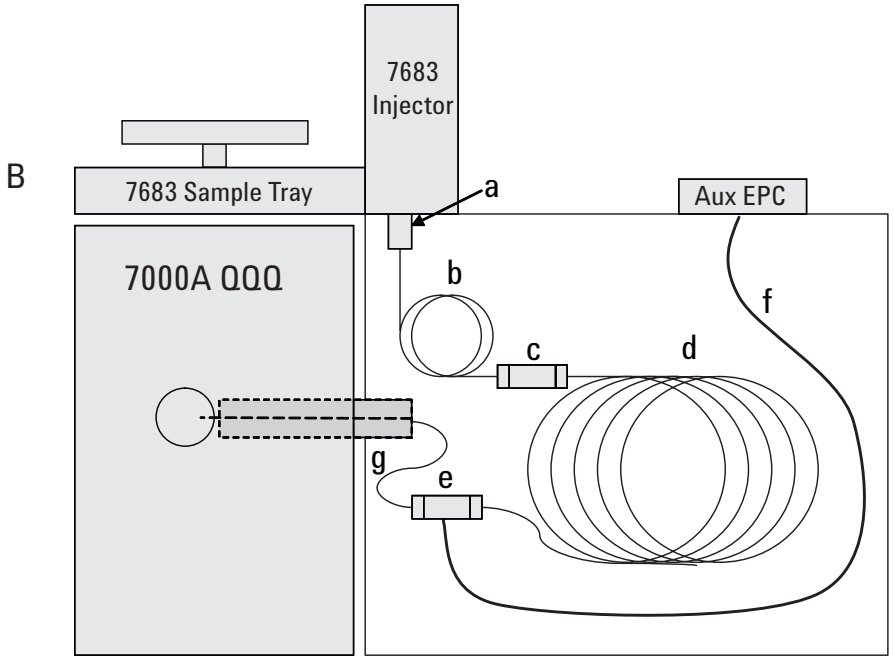

Figure 1B. The GC/QQQ used for MRM analyses was configured with a) split/splitless inlet, b) 2 m × 0.25 mm deactivated retention gap, c) Ultimate Union d) 15 m × 0.25 × 0.25 µm Agilent J&W HP-5ms UI column, e) Purged Ultimate Union, f) helium purge flow, and g) 65 cm × 0.15 mm deactivated restrictor.

MRM Method

A method was developed for the analysis of 175 commonly analyzed pesticides. Two transitions were determined for each compound and the collision energy was optimized for each. Since the method was locked to the Agilent Pesticide method (running at twice the original speed), the retention times correspond to those recorded in Agilent's RTL Pesticide and Endocrine Disruptor Database (P/N G1672AA) divided by two. There are small differences in RT between the database and values shown here because this method used a retention gap, capillary flow device, and a restrictor. Table 3 lists the pesticides in alphabetical order with their retention times, quant and qual transitions, and the collision energies for each.

Table 3. Target and Qualifier Transitions for 175 Pesticides

		Quant transition			Qual transition		
Compound name	**RT (min)**	**Precursor ion**	**Product ion**	**CE**	**Precursor ion**	**Product ion**	**CE**
Acrinathrin	15.371	181.1	152.1	25	181.1	127.1	30
Akton	11.403	282.9	219.0	10	282.9	184.0	25
Alachlor	8.507	188.1	130.1	40	188.1	160.1	10
Aldrin	9.247	262.9	192.9	40	262.9	190.9	40
Allethrin	10.908	123.1	81.1	10	123.1	79.1	20
Atrazine	6.581	200.1	122.1	10	200.1	104.0	20
Azamethidaphos (Azamethiphos)	13.248	215.0	171.0	15	215.0	128.0	30
Azinphos-methyl	14.835	160.1	77.1	20	160.1	132.1	0
Benfluralin	5.842	292.1	264.0	10	292.1	160.1	20
BHC, α-	6.025	181.0	145.0	15	181.0	109.0	30
BHC, ß-	6.595	181.0	145.0	15	181.0	109.0	30
BHC, δ-	7.266	181.0	145.0	15	181.0	109.0	30
Bifenthrin	14.428	181.1	165.1	30	181.1	166.1	15
Bromacil	9.186	205.0	132.0	30	205.0	187.9	20
Bromophos	10.020	330.9	315.9	20	330.9	285.9	35
Bromophos-ethyl	11.261	358.9	302.9	15	358.9	284.8	35
Bromopropylate	14.320	183.0	155.0	15	183.0	76.0	35
Captan	10.617	79.1	77.1	10	79.1	51.1	25
Carbophenothion	13.316	157.0	121.0	25	157.0	75.1	40
Chlordane, *cis*-	11.410	372.9	265.9	40	372.9	263.9	30
Chlordane, *trans*-	11.010	372.9	265.9	20	372.9	263.9	25
Chlordene, α-	8.562	230.0	160.0	40	230.0	195.0	25
Chlordene, ß-	9.376	230.0	160.0	35	230.0	195.0	25
Chlordene, γ-	9.314	230.0	160.0	40	230.0	195.0	25
Chlorfenvinphos, ß-	10.779	267.0	159.0	20	267.0	81.0	40
Chlorobenzilate	12.706	139.0	111.0	15	139.0	75.0	30
Chloroneb	4.323	191.0	113.0	15	191.0	141.0	10
Chlorothalonil	7.395	265.9	133.0	40	265.9	230.9	20
Chlorpyrifos	9.606	196.9	168.9	15	196.9	107.0	40
Chlorpyrifos-methyl	8.284	286.0	93.0	25	286.0	270.9	20
Chlorthiophos	13.051	268.9	205.0	15	268.9	177.0	25
Coumaphos	15.859	362.0	109.0	15	362.0	81.0	40
Cyanazine	9.694	212.1	123.1	20	212.1	151.1	10
Cyanophos	6.887	243.0	109.0	10	243.0	79.0	30
Cyfluthrin 1	16.144	163.0	127.1	5	163.0	91.1	15
Cyfluthrin 2	16.212	163.0	91.1	15	163.0	127.1	5

(Continued)

Compound name	RT (min)	Quant transition			Qual transition		
		Precursor ion	Product ion	CE	Precursor ion	Product ion	CE
Cyfluthrin 3	16.273	163.0	127.1	5	163.0	91.1	15
Cyfluthrin 4	16.307	163.0	127.1	5	163.0	91.1	15
Cyhalothrin, λ-	15.208	181.1	152.1	30	181.1	127.1	35
Cypermethrin 1	16.381	181.1	152.1	25	181.1	127.1	35
Cypermethrin 2	16.463	181.1	152.1	30	181.1	127.1	35
Cypermethrin 3	16.531	181.1	152.1	25	181.1	127.1	30
Cypermethrin 4	16.558	181.1	152.1	25	181.1	127.1	30
Dacthal (DCPA) (Chlorthal-Dimethyl)	9.721	300.9	222.9	25	300.9	166.9	40
DDD, o,p'-	12.170	235.0	165.1	30	235.0	199.1	15
DDD, p,p'-	12.841	235.0	165.1	25	235.0	199.1	20
DDE, o,p'-	11.241	246.0	176.1	40	246.0	211.0	20
DDE, p,p'-	12.007	246.0	176.1	40	246.0	175.1	40
DDT, o,p'-	12.882	235.0	165.1	30	235.0	199.1	20
DDT, p,p'-	13.492	235.0	165.1	30	235.0	199.1	20
DEF (Tribufos)	12.054	169.0	57.1	5	169.0	112.9	5
Deltamethrin	18.016	181.1	152.1	25	181.1	127.1	25
Demeton-S	6.303	88.1	60.0	5	88.1	59.0	20
Demeton-S-methyl	5.230	88.1	60.0	5	88.1	59.0	15
Dialifos	15.432	208.0	102.1	40	208.0	89.0	40
Diallate 1	5.957	234.1	150.0	20	234.1	192.0	10
Diallate 2	6.127	234.1	150.0	20	234.1	192.0	10
Diazinon	7.226	179.1	121.1	40	179.1	137.2	20
Dicapthon	9.694	262.0	216.0	15	262.0	123.0	40
Dichlofenthion	8.067	279.0	223.0	15	279.0	205.0	30
Dichlofluanid	9.199	123.0	77.1	20	123.0	51.1	40
Dichlorobenzophenone, 4,4'-	9.593	139.0	111.0	15	139.0	75.1	30
Dichlorvos	2.905	109.0	79.0	5	109.0	47.0	15
Diclobenil	3.367	171.0	100.0	25	171.0	136.0	15
Dicloran	6.269	206.0	176.0	10	206.0	124.0	30
Dieldrin	11.926	262.9	192.9	40	262.9	190.9	35
Dimethachlor	8.080	134.1	105.1	15	134.1	77.1	30
Dioxathion	15.934	125.0	97.0	5	125.0	65.0	25
Disulfoton	7.260	88.1	60.0	5	88.1	59.0	25
Ditalimfos	11.586	130.0	102.1	15	130.0	75.0	30
Edifenphos	13.377	173.0	109.0	15	173.0	65.1	40
Endosulfan ether	7.660	240.9	205.9	20	240.9	203.9	20
Endosulfan I	11.308	240.9	205.9	15	240.9	136.0	40
Endosulfan II	12.570	195.0	125.0	25	195.0	159.0	10
Endosulfan sulfate	13.377	271.9	236.9	20	271.9	116.9	40
Endrin	12.366	262.9	193.0	35	262.9	190.9	35
Endrin aldehyde	12.956	249.9	214.9	35	249.9	141.9	40
Endrin ketone	14.116	316.9	101.0	20	316.9	245.0	20
EPN	14.333	157.0	77.1	25	157.0	110.0	15
Ethalfluralin	5.632	276.1	105.1	35	276.1	202.0	20
Ethion	12.997	231.0	128.9	25	231.0	174.9	10
Ethoprop (Ethoprophos)	5.357	158.0	97.0	15	158.0	114.0	5
Etridazole	3.963	183.0	139.9	20	183.0	108.0	40

(Continued)

Compound name	RT (min)	Quant transition			Qual transition		
		Precursor ion	Product ion	CE	Precursor ion	Product ion	CE
Famphur	13.329	218.0	109.0	15	218.0	79.0	30
Fenamiphos (Phenamiphos)	11.803	303.1	80.0	40	303.1	154.0	20
Fenarimol	15.222	139.0	111.0	15	139.0	75.0	35
Fenchlorphos (Ronnel)	8.650	284.9	269.9	15	284.9	239.9	35
Fenitrothion	9.030	277.0	109.0	20	277.0	260.0	5
Fenpropathrin	14.503	181.1	152.1	30	181.1	127.1	35
Fensulfothion	12.780	292.0	156.0	25	292.0	109.0	20
Fenthion	9.552	278.0	109.0	20	278.0	125.0	20
Fenvalerate 1	17.202	167.1	125.0	15	167.1	89.1	40
Fenvalerate 2	17.412	167.1	125.0	10	167.1	89.1	35
Fluchloralin	7.321	306.1	264.1	5	306.1	206.0	15
Flucythrinate 1	16.571	199.1	107.1	30	199.1	157.1	10
Flucythrinate 2	16.741	199.1	107.1	25	199.1	157.1	5
Fluridone	16.944	328.1	259.0	30	328.1	189.1	40
Fluvalinate τ- 1	17.412	250.1	55.1	15	250.1	200.1	20
Fluvalinate τ- 2	17.480	250.1	55.1	15	250.1	200.1	25
Folpet	10.807	147.1	103.1	5	147.1	76.0	30
Fonophos	6.934	246.1	109.0	15	246.1	137.0	5
Heptachlor	8.379	271.9	236.8	25	271.9	116.9	40
Heptachlor exo-epoxide isomer A	10.474	183.0	118.9	30	183.0	154.9	15
Heptachlor exo-epoxide isomer B	10.352	352.9	262.8	25	352.9	281.9	20
Hexachlorobenzene	6.168	283.9	213.9	35	283.9	248.8	25
Hexazinone	13.702	171.1	71.1	15	171.1	85.1	15
Iprobenfos (IBP)	7.660	204.0	91.1	10	204.0	121.0	40
Iprodione	14.211	187.0	124.0	25	187.0	159.0	15
Isazophos	7.517	161.1	119.0	10	161.1	146.0	5
Isofenfos	10.813	213.1	121.0	20	213.1	185.0	5
Jodfenphos (Iodofenphos)	11.776	376.9	361.9	20	376.9	93.0	35
Leptophos	14.876	171.0	77.1	25	171.0	124.1	10
Lindane (γ-BHC)	6.710	181.0	145.0	15	181.0	109.0	30
Malathion	9.396	173.1	99.0	15	173.1	117.0	10
Methidathion	11.146	145.0	85.1	5	145.0	58.1	15
Methoxychlor, o,p'-	13.730	227.1	121.1	15	227.1	91.1	35
Methoxychlor, p,p'-	14.442	227.1	141.1	40	227.1	169.1	30
Metolachlor	9.450	162.1	133.1	15	162.1	132.1	25
Mevinphos	3.782	127.0	109.0	10	127.0	95.0	15
Mirex	14.923	271.9	236.9	15	271.9	116.9	40
Nonachlor, *cis*-	12.848	408.8	109.0	20	408.8	299.9	25
Nonachlor, *trans*-	11.539	408.8	299.8	25	408.8	301.8	30
Oxadiazon	12.210	175.0	112.0	15	175.0	76.1	40
Parathion	9.633	291.1	109.0	10	291.1	81.0	40
Parathion methyl	8.284	263.0	109.0	10	263.0	79.0	35
Pentachloroaniline	7.761	264.9	193.9	30	264.9	155.9	30
Pentachlorobenzene	4.459	249.9	214.9	25	249.9	142.0	40
Pentachlorobenzonitrile	6.866	274.9	239.9	20	274.9	204.9	35
Pentachlorophenyl methyl ester	6.283	264.9	236.9	10	264.9	142.9	40
Pentachlorothioanisole	9.016	295.9	245.8	40	295.9	262.9	15

(Continued)

Compound name	RT (min)	Quant transition			Qual transition		
		Precursor ion	Product ion	CE	Precursor ion	Product ion	CE
Permethrin, *cis-*	15.703	183.1	153.1	15	183.1	168.1	15
Permethrin, *trans-*	15.798	183.1	155.1	10	183.1	165.1	10
Phenanthrene-d10	6.863	188	160	10	188	186	10
Phenothrin	14.713	183.1	153.1	15	183.1	168.1	15
Phenthoate	10.861	274.0	121.0	10	274.0	125.0	20
Phorate	5.961	231.0	128.9	25	231.0	174.9	10
Phosalone	14.855	182.0	111.0	15	182.0	75.1	40
Phosmet	14.259	160.0	77.1	30	160.0	133.0	15
Pirimiphos ethyl	10.332	318.1	166.1	15	318.1	182.1	15
Pirimiphos methyl	9.138	290.1	125.0	25	290.1	233.0	10
Procymidone	10.983	283.0	96.1	10	283.0	67.1	40
Profenofos	11.953	207.9	63.1	40	207.9	99.0	25
Propachlor	5.164	120.1	77.1	20	120.1	92.1	5
Propargite	13.858	135.1	107.1	15	135.1	77.1	30
Propazine	6.676	214.1	172.0	10	214.1	104.0	20
Propetamphos	6.948	138.0	110.0	5	138.0	64.0	15
Propyzamide	6.975	173.0	145.0	15	173.0	109.0	35
Prothiophos	11.878	162.0	63.1	40	162.0	98.0	20
Pyraclofos	15.439	360.0	96.9	35	360.0	194.0	15
Pyrazophos	15.351	221.1	193.1	10	221.1	149.1	15
Pyridaphenthion	14.272	340.1	199.1	5	340.1	97.0	40
Quinalphos	10.827	146.1	118.1	10	146.1	91.1	30
Quintozene	6.832	236.9	118.9	25	236.9	142.9	30
Resmethrin	13.994	123.1	81.1	5	123.1	95.1	5
Simazine	6.473	201.1	173.1	5	201.1	138.1	10
Sulfotep-ethyl	5.902	322.0	146.0	25	322.0	65.0	40
Sulprofos	13.180	322.0	97.0	30	322.0	156.0	5
Tebupirimfos	7.687	261.1	137.1	15	261.1	153.1	20
Tecnazene (TCNB)	5.110	202.9	83.0	25	202.9	142.9	20
Tefluthrin	7.524	177.1	127.1	20	177.1	137.0	20
Temephos	20.525	125.0	47.0	20	125.0	79.0	10
Terbufos	6.890	231.0	128.9	25	231.0	174.9	10
Terbuthylazine	6.907	214.1	104.0	20	214.1	132.0	10
Tetrachloroaniline, 2,3,5,6-	5.293	230.9	158.0	25	230.9	122.0	40
Tetrachlorvinphos	11.478	329.0	109.0	25	329.0	79.0	35
Tetramethrin I	14.299	164.1	107.1	15	164.1	135.1	10
Tetramethrin II	14.421	164.1	107.1	10	164.1	135.1	5
Thiometon	6.161	125.0	47.0	20	125.0	79.0	10
Tolclofos methyl	8.392	265.0	250.0	15	265.0	93.0	25
Tolyfluanid	10.623	137.0	91.1	20	137.0	65.1	35
Triallate	7.470	268.0	183.9	25	268.0	226.0	15
Triazophos	13.241	161.0	134.1	10	161.0	91.1	20
Trifluralin	5.808	306.1	264.0	5	306.1	160.0	30
Triphenyl phosphate	13.865	326.1	169.1	35	326.1	233.0	10
Vinclozolin	8.311	212.0	145.0	25	212.0	109.0	40

Carrot Extract

A carrot extract with incurred pesticide residues was analyzed in the scan and SIM modes with the GC/Q. In each case, 5-µL injections were made using Agilent's new Multimode inlet operated in the cold splitless mode. Three SIM methods were used to monitor > 170 compuonds with about 60 pesticides in each method. Four ions were monitored for each compound. The scan data were analyzed automatically using Agilent's Deconvolution Reporting Software, together with the 927-compound RTL Pesticide and Endocrine Disruptor Database.

The same carrot sample was also analyzed on the 7890A/7000A GC/QQQ system using the MRM transitions listed in Table 3. An 11-point calibration curve was prepared in carrot matrix for 170 pesticides from 3.33 µg/kg (ppb) to 6670 µg/kg. Table 4 shows the results of these analyses.

Table 4. Results from the Analysis of a Carrot Extract with Incurred Pesticides by GC/MS in the Scan Mode with DRS Analysis, by GC/MS in the SIM Mode, and by GC/MS/MS in the MRM Mode (An X implies that the compound was found by that method.)

	GC/Q 5 µL (Multimode inlet)		GC/QQQ[a] 1 µL
Pesticide	Cold SL scan + DRS	Cold SL SIM	Hot SL (ppb)
Diclobenil			0.38[b]
Pentachlorobenzene			0.75[b]
Trifluralin			2.3[b]
Tefluthrin			0.53[b]
4,4'-Dichlorobenzophenone			1.2[b]
Chlorpyrifos			24.7
o,p'-DDE			3.7
p,p'-DDE	X	X	240
o,p'-DDD			9
p,p'-DDD o,p'-DDT	X X		Sum = 45
p,p'-DDT	X	X	130
Fenazaquin	X	Not in method	Not in method

a. The actual concentration of these compounds was lower in the original carrot sample by a factor of 4.5 since the extraction method results in 4.5 g of produce per mL of extract.

b. The reported values fall below the lowest point on the calibration curve.

The single quad methods were not quantitative, so Table 4 only indicates (with an X) if a pesticide was found, either by DRS or by manual examination of the SIM data. Since the triple quad method was calibrated, the amount of each pesticide could be determined. The amounts reported are those found in the extract. Because the extraction method concentrated this sample by a factor of 4.5:1 (4.5 g of carrot to 1.0 mL of final extract), the pesticide concentrations in the original carrot samples were actually lower by this factor.

The scan method with DRS analysis has the capability to find any of the 927 compounds in the database, while the SIM and MRM methods are limited to the 175 target compounds listed in Table 3. DRS found fenazaquin, a pesticide that was not in the SIM or MRM methods. This demonstrates the advantage of using GC/MS with DRS for screening purposes in combination with GC/MS/MS for target compound analysis.

In spite of the concentrated carrot matrix, the GC/QQQ was able to detect three pesticides below 1 ppb (1 µg/kg) and three more below 5 ppb. The lowest level calibration standard was prepared at 3.33 ppb, so numbers reported below that level are extrapolated values. The optimal MRM transitions for p,p'-DDD and o,p'-DDT are the same and, since these two compounds were only partially resolved chromatographically, they are reported together.

Figure 2A shows the extracted quant ion (*m/z* 246) for p,p'-DDE from the scan analysis of the carrot sample. Interferences in these chromatograms make it harder to do an accurate quantitative analysis without first deconvoluting the spectrum. After deconvolution (Figure 2B), ChemStation integration is trivial. Figure 2C shows the EIC (*m/z* 246) from the GC/MS SIM analysis of the same sample. Although the signal/noise ratio (S/N) is 10-fold better, there appear to be more interferences.

It is easy to see the advantage of the GC/QQQ for target compound analysis. A 1-µL injection of the carrot extract on this instrument gave a clean MRM chromatographic peak (Figure 2D) with better S/N (434) than was obtained for the 5-µL GC/Q SIM analysis (S/N = 375)(Figure 2C).

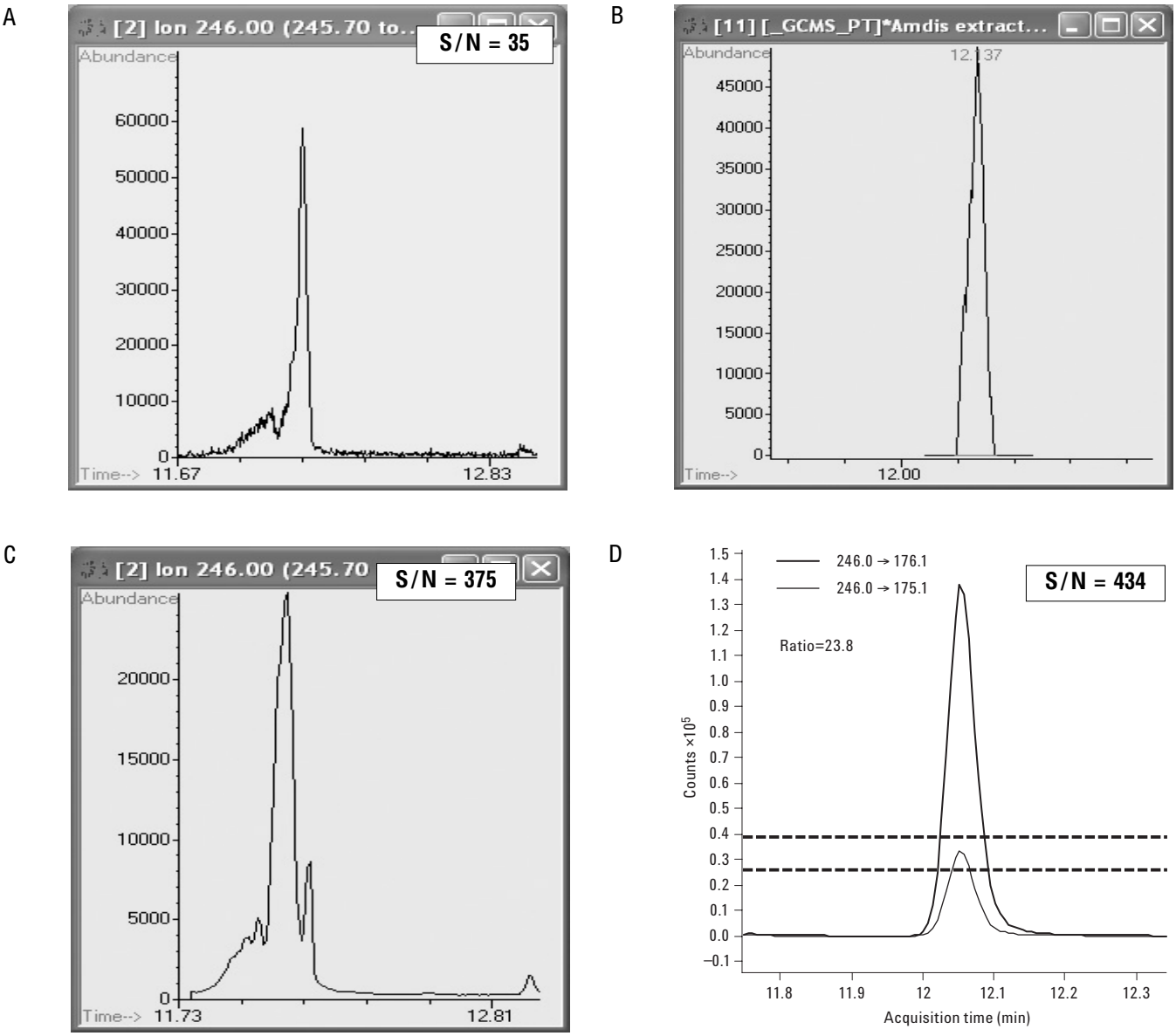

*Figure 2. A) p,p'-DDE quant ion (*m/z *246) extracted from the scan chromatogram obtained from a 5-µL cold splitless injection of a carrot extract with incurred pesticides. B) Same as in (A) but after deconvolution. C) p,p'-DDE quant ion (*m/z *246) extracted from a SIM chromatogram obtained from a 5-µL cold splitless injection of the same sample. D) Quant and qualifier transitions (246.0 → 176.1 and 246.0 → 175.1, respectively) for the GC/MS/MS analysis of a 1-µL hot splitless injection of the same carrot extract. Peak-to-peak signal/noise ratios for the extracted ions and the quant transition are shown. The ratio of the two transition ions (D) is 23.8, confirming the presence of p,p'-DDE.*

Comparing GC/MS SIM to GC/MS/MS MRM – Various Matrices

Figure 3 compares GC/MS SIM results to GC/MS/MS MRM results for p,p'-DDE spiked into various commodities at 10 ppb. On the left, the SIM EICs for the quant ion (*m/z* 246) show increasing amounts of matrix interference from the apple, cabbage, ginseng, orange, and spinach samples. In contrast, the p,p'-DDE GC/MS/MS transitions shown on the right have no interferences from any of the extracts. The large S/N values shown for the quant transition (246.0 → 176.1) suggest that one should be able to detect p,p'-DDE at the sub-ppb level.

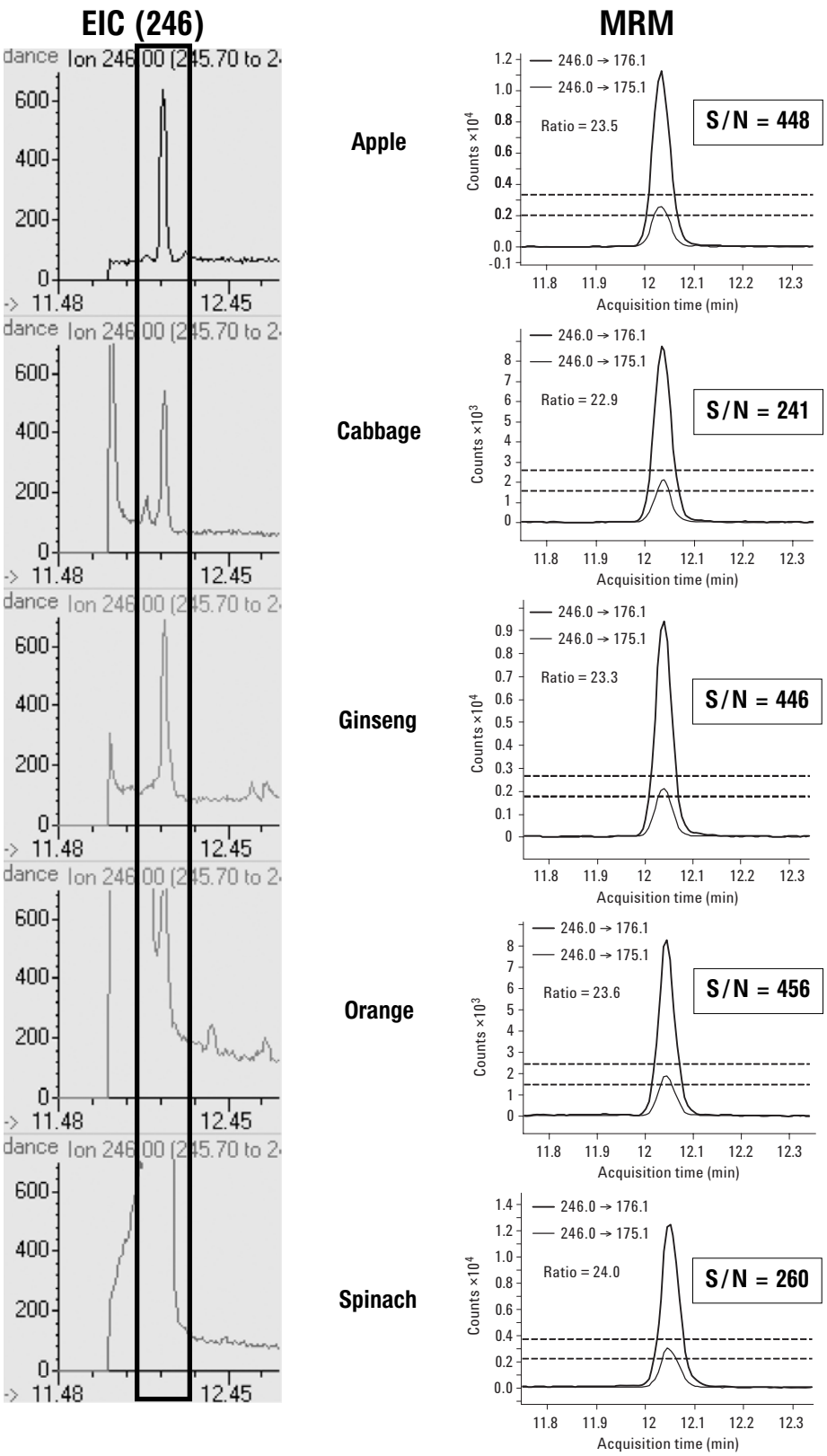

*Figure 3. A comparison of GC/MS SIM and GC/MS/MS MRM analysis for p,p'-DDE spiked at 10 ppb (10 µg/kg) into five different produce extracts. On the left, the EICs for the p,p'-DDE quant ion (*m/z *246) show increasing amounts of interference from the matrix. The transitions on the right (246.0 → 176.1 and 246.0 → 175.1) for p,p'-DDE are clean, with peak-to-peak S/N values ranging from 241 to 448. All injections were 1 µL.*

Tomato Extract

All three techniques being discussed were able to identify incurred chlorothalonil in a tomato extract, which was present at 1 ppm. However, only the GC/QQQ was able to identify pentachlorobenzonitrile, a chlorothalonil metabolite, which it measured at 9.3 ppb. Figure 4 shows the MRM transitions for pentachlorobenzonitrile and a calibration curve for the compound ranging from 3.33 ppb to 6670 ppb.

Backflushing the Column

The norm when analyzing dirty samples by GC/MS is to replace the inlet liner and clip the column frequently. Many labs do this daily. Otherwise, matrix accumulates in the liner and column, degrading the chromatography. Over time, these materials migrate through the GC column and contaminate the source, which then needs to be cleaned. This problem may be compounded with a GC/QQQ instrument because one does not see much evidence of the matrix and the temptation is to ignore maintenance until the source (and sometimes the first quadrupole) needs to be cleaned.

The Agilent 7000A Series triple quad MS uses the same inert source and gold-plated quartz quadrupole that are found in the 5975C MSD. These can be heated up to 350 °C and 200 °C, respectively, which greatly minimizes the need for cleaning, even when high-boiling matrix compounds do reach the detector.

The best way to prevent chromatographic degradation and reduce the need for source cleaning is to backflush the GC column during or after each run. With the configurations shown in Figure 1, backflushing is done for 3 to 5 minutes after the run by raising the pressure at the capillary flow device (two-way splitter or the Purged Ultimare Union) and lowering the inlet pressure. This reverses the flow through the column and purges high-boiling matrix components from the head of the column and out through the inlet's split vent.

During the course of this work, approximately 100 1-µL injections of concentrated food extracts were made into the GC/QQQ system with no evidence of column or MS performance problems. Nearly 300 µL of these same extracts were injected into the GC/Q system before column and inlet maintenance was required. With the capillary flow device installed, you can do this maintenance without venting the mass spectrometer.

Conclusions

Agilent's 7890A/7000A triple quadrupole MS system is a sensitive and rugged tool for target pesticide analysis. There is far less interference from matrix than one sees in single quadrupole methods, making it much easier to quantify pesticides at the low ppb levels required by today's legislation. In many cases a 1-µL injection into the GC/QQQ produced far better results than a 5-µL injection into the GC/Q. Nevertheless, there is still a need for screening methods that look for hundreds of pesticides. For this, we recommend using large-volume injection with Agilent's new Multimode inlet, GC/Q analysis in the scan mode, and data analysis using Deconvolution Reporting Software with Agilent's Pesticide and Endocrine Disruptor Database. The combination of these two approaches is the best way to screen for more than 900 contaminants (by GC/Q with DRS) while performing ultra-trace analysis for a smaller list of target compounds (using GC/QQQ). Both approaches benefit from column backflushing, which is highly recommended when analyzing dirty samples, such as food extracts.

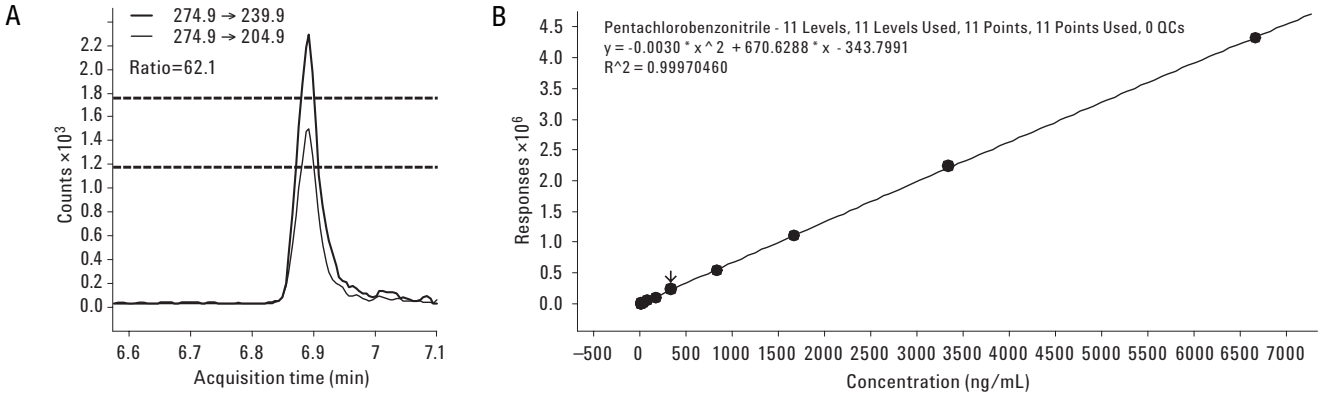

Figure 4. A) MRM transitions identifying pentachlorobenzonitrile at 9.3 ppb in a tomato extract. B) A calibration curve for pentachlorobenzonitrile from 3.33 to 6,670 ppb with a quadratic curve fit > 0.999.

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Acknowledgements

The authors wish to thank Dr. Jon Wong of the U.S. Food and Drug Administration (College Park, MD, USA) and Dr. Steven Lehotay of the U.S. Department of Agriculture (ARS, ERRC, Wyndmoor, PA, USA) for providing numerous standards and food extracts.

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Printed in the USA
February 25, 2009
5990-3578EN

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/sec, 1.5 mL/min 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.

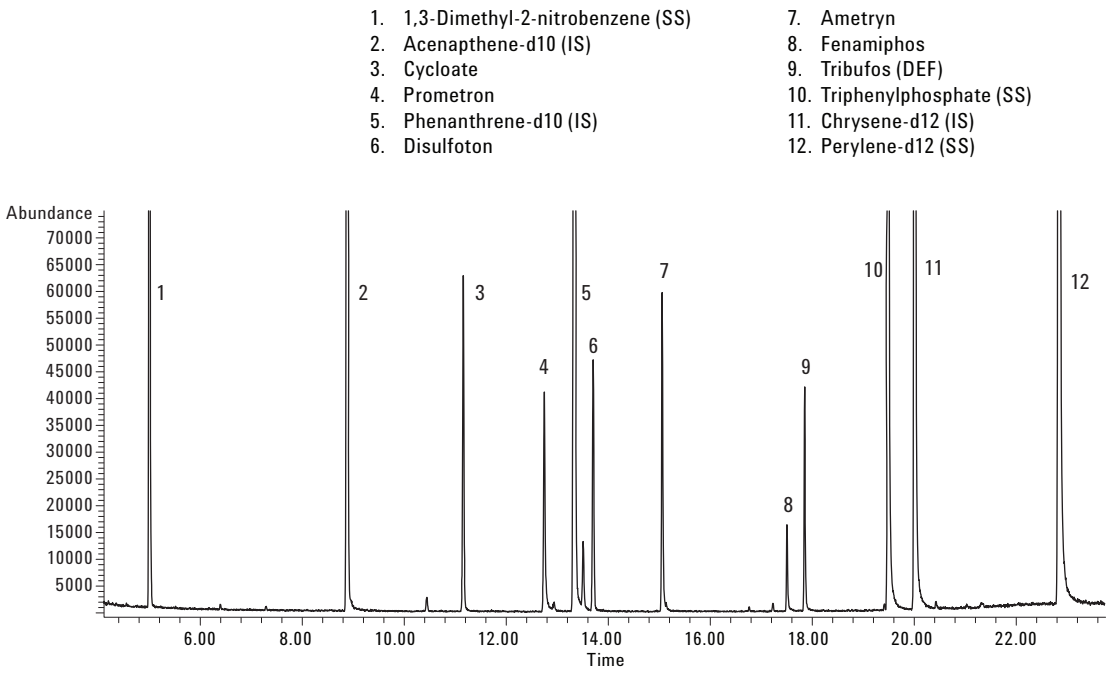

Figure 1. Total ion chromatogram (scan mode) of the 1-ng on-column EPA Method 525.2 standard solution loading on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). Chromatographic conditions are listed in Table 1A.

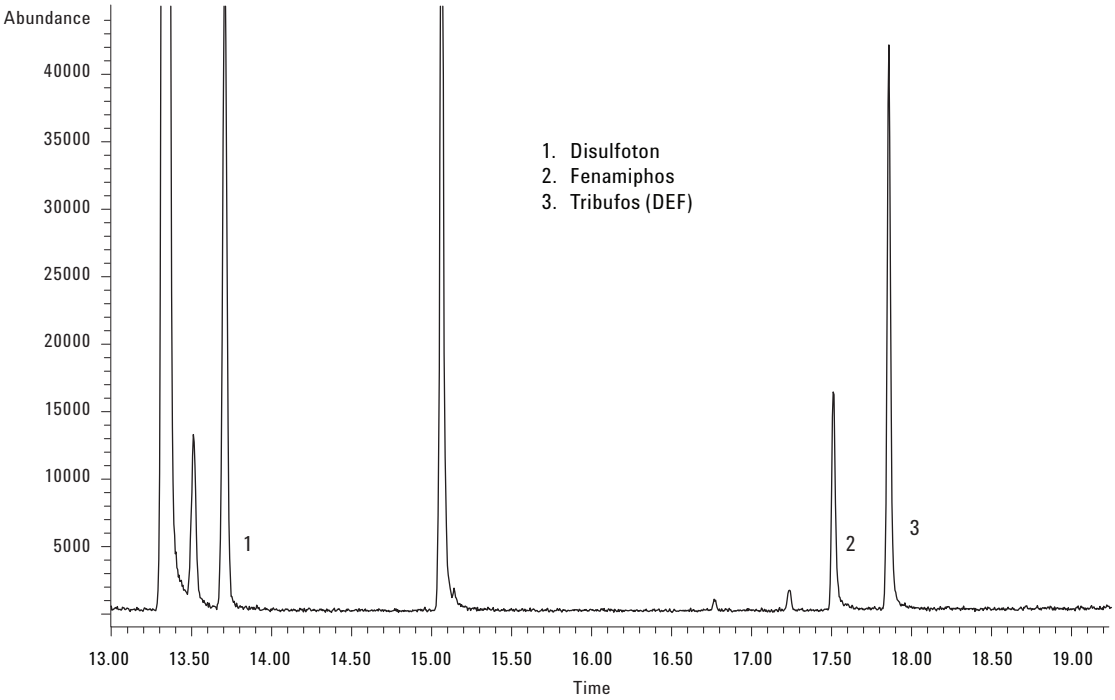

Figure 2. Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 1.0 µg/mL EPA Method 525.2 standard pesticide mix. The peaks noted in the figure are the three organophosphorus pesticides of interest. Chromatographic conditions are listed in Table 1A.

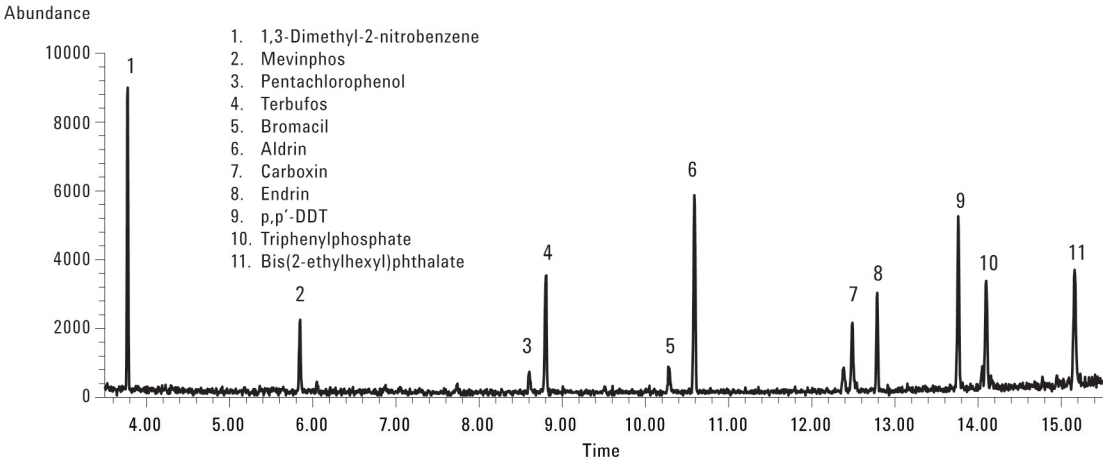

Figure 3. Total ion chromatogram (scan mode) of the 0.1-ng on-column Ultra Scientific standard solution loading on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). Chromatographic conditions are listed in Table 1B.

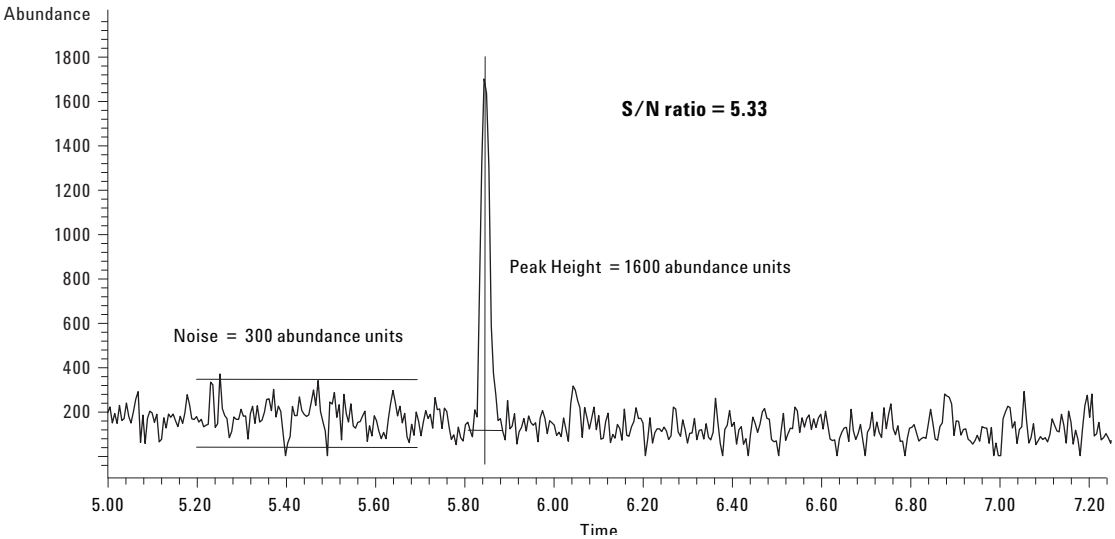

Figure 4. Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 0.1 µg/mL Ultra Scientific standard pesticide mix on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 122-5532UI). The peak in the figure is mevinphos, an organophosphorus pesticide 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

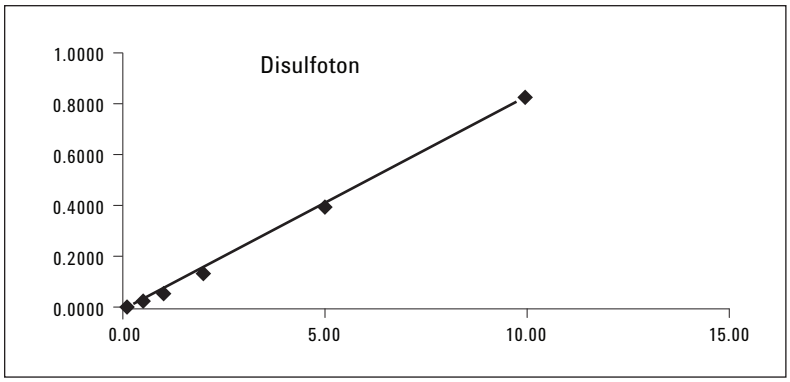

Figure 5. Correlation coefficients for the EPA Method 525.2 pesticide components over the 0.1 to 10 µg/mL range of this study and an example linear regression plot for disulfoton.

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

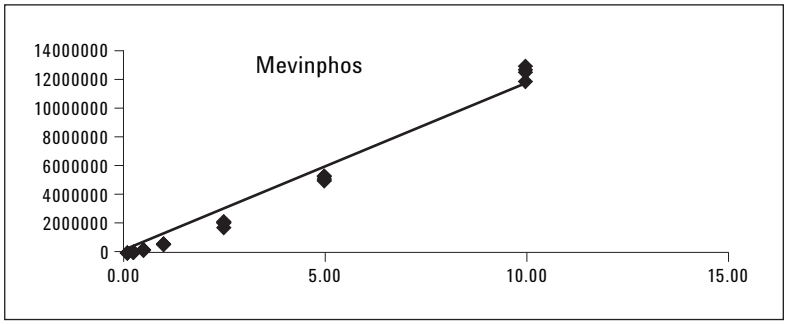

Figure 6. Correlation coefficients for the Ultra Scientific pesticide components over the 0.1 to 10 µg/mL range of this study and an example linear regression plot for mevinphos.

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
March 30, 2010
5989-9879EN

Agilent Technologies

The Analysis of Chlorinated Pesticides and PCBs Using the HP-608 Capillary Column

Application Note
228-236

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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.

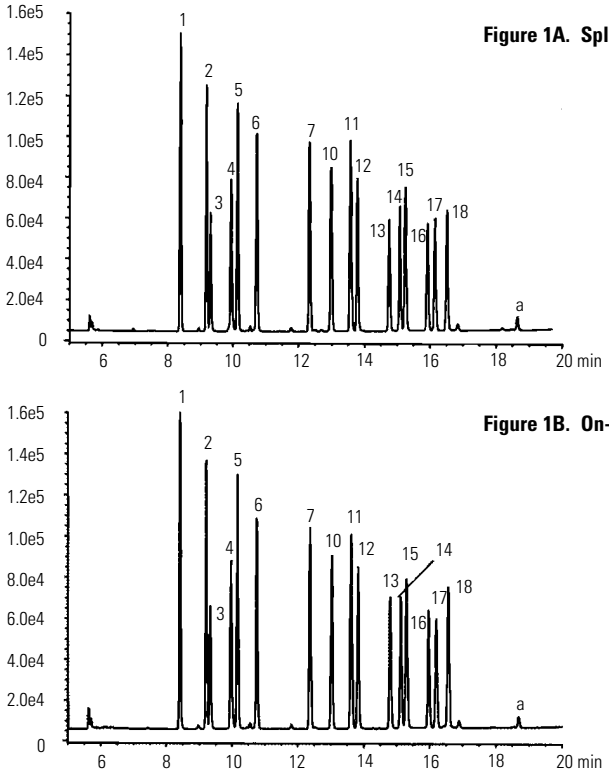


Table 2. Chlorinated Pesticides

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.

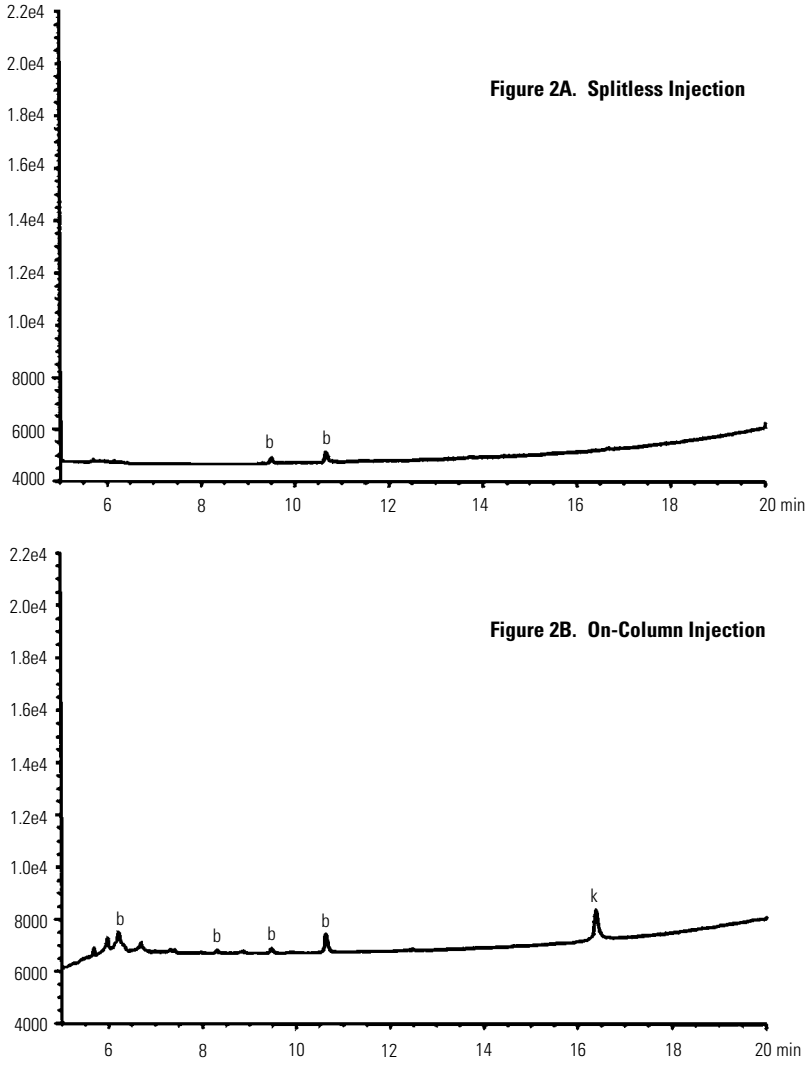
Use of the Merlin™ Microseal[5] and the deactivated glass liner also contributed directly to the low degradation rate in the splitless mode. The Microseal is designed to provide a good inlet seal without using a conventional septum. By eliminating the introduction of particulates into the inlet liner 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)

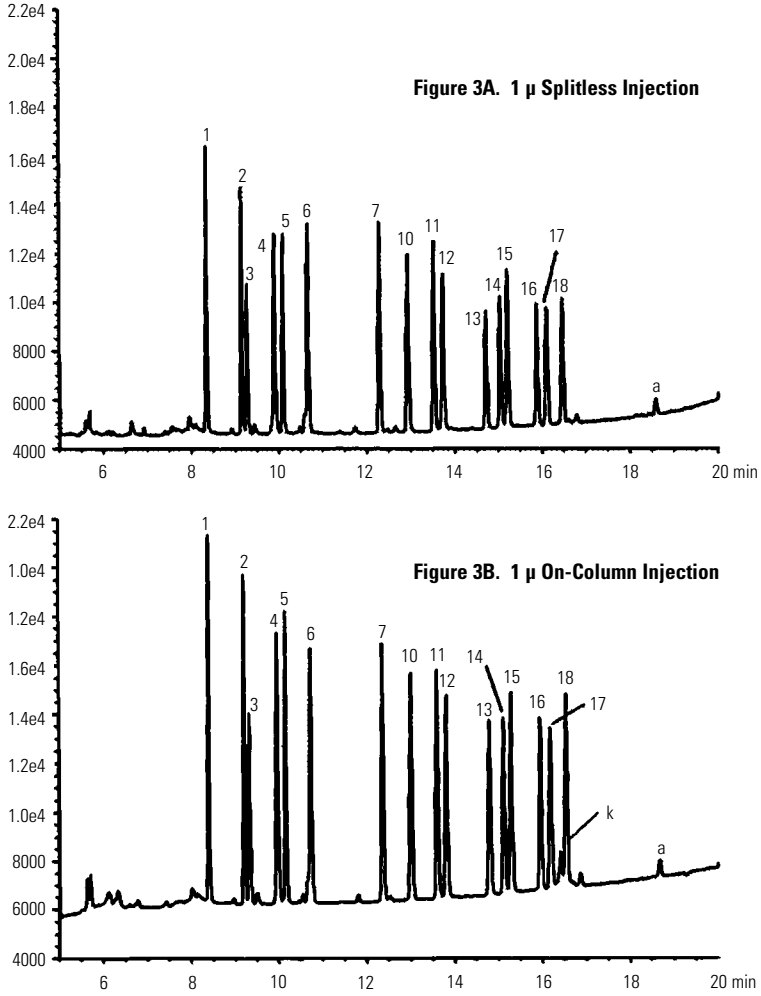
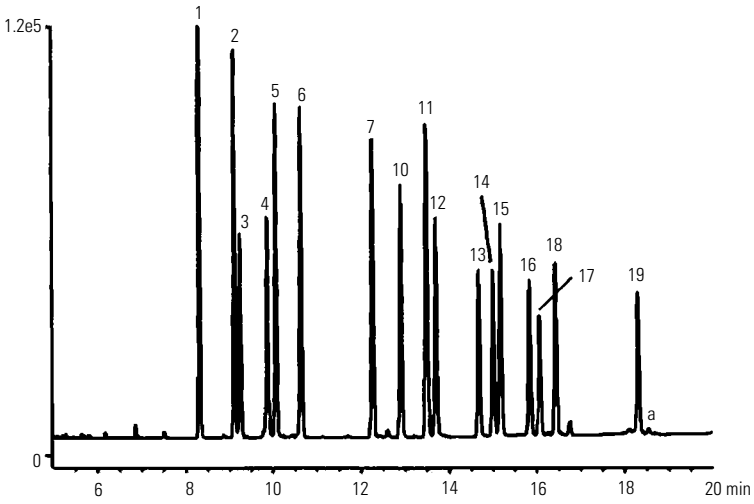


Figure 4. Chromatograms of the EPA-Method 8080 pesticides under optimum GC conditions. Splitless injection of 100–200 pg per component. (Peak ID, see Table 2)



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)

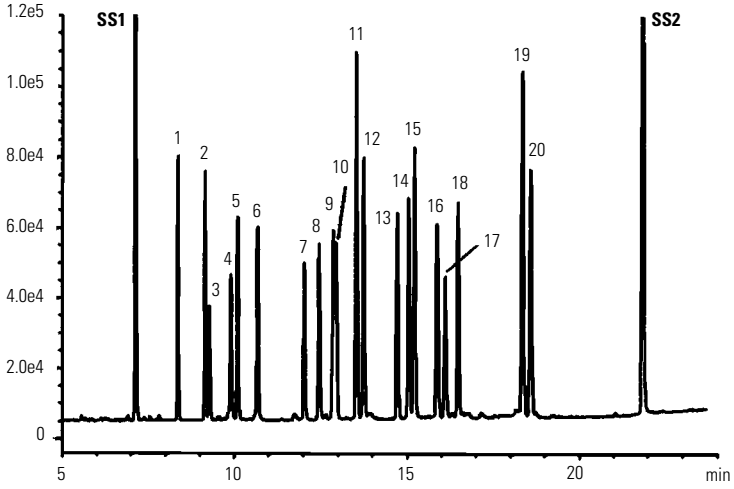


Figure 6. Chromatogram of technical grade toxaphene and chlordane under optimum GC conditions. Splitless injection of 1 µl 2.5 ppm mix

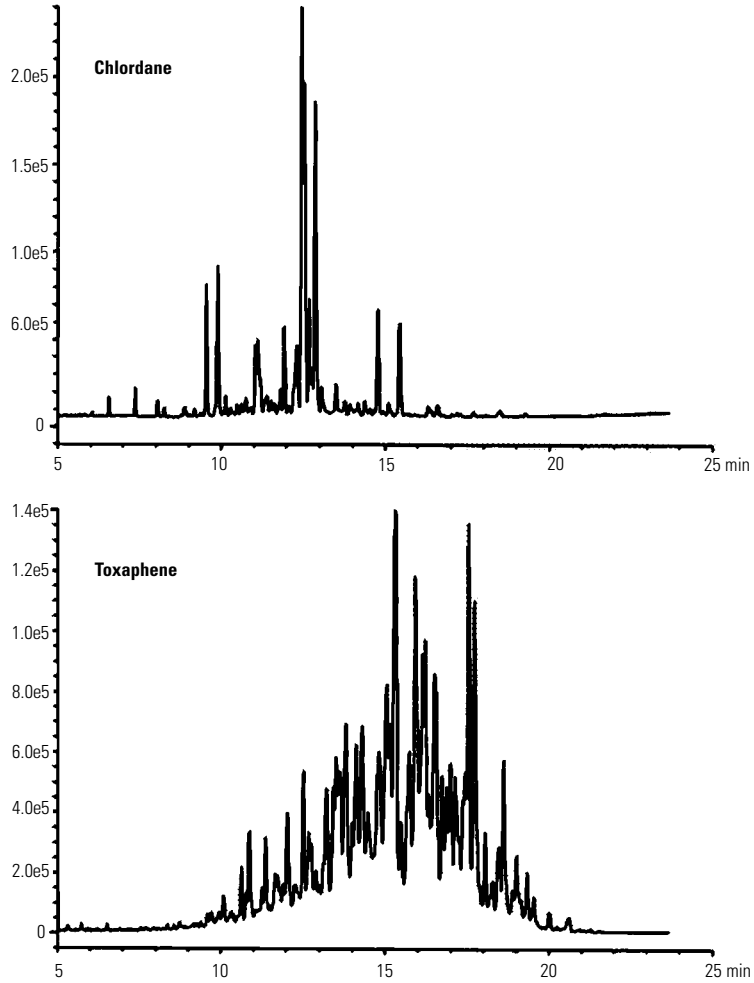
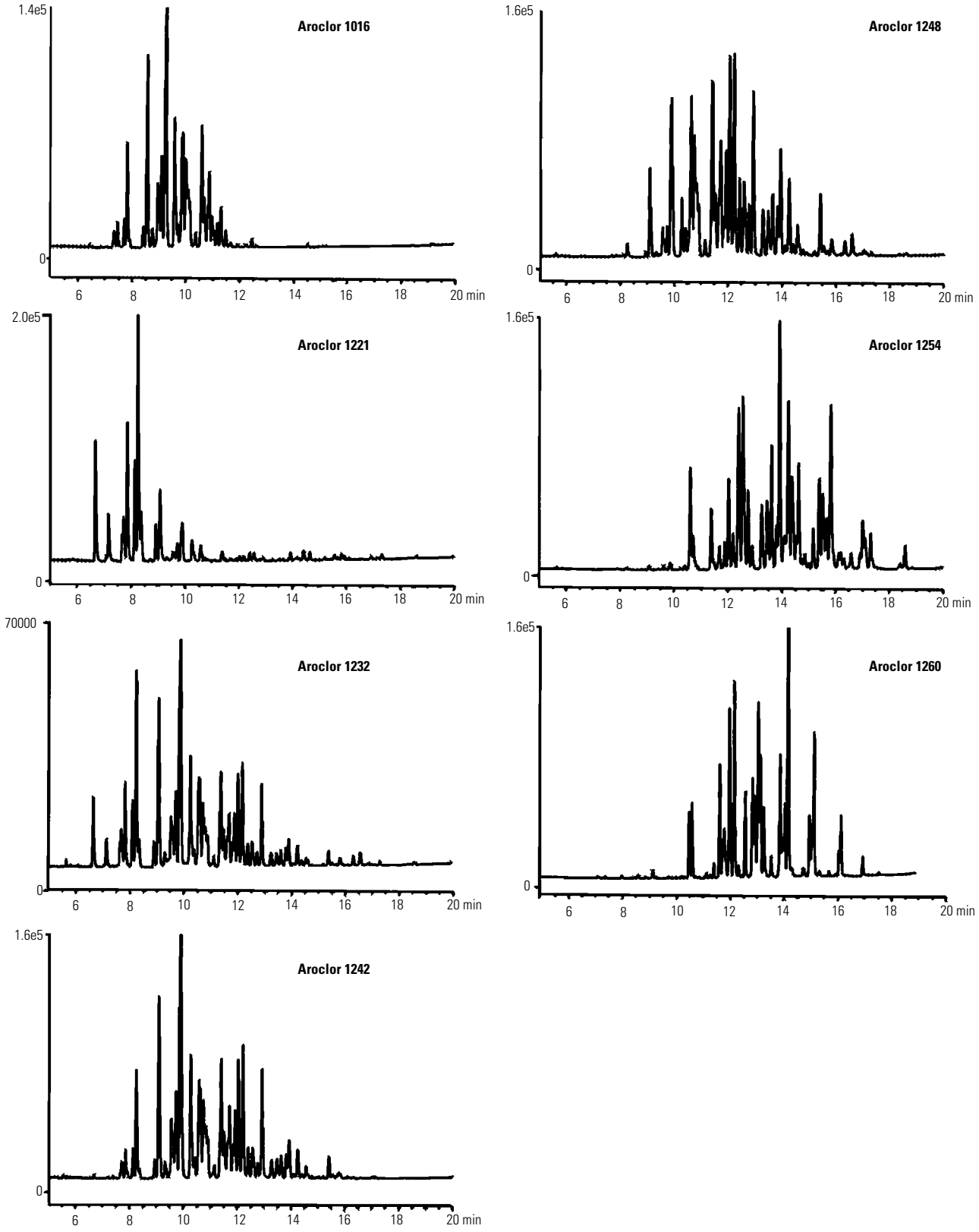


Figure 7. A comparison of seven PCBs under optimum GC conditions. Splitless injection of 1 µl 2.5 ppm each



Acknowledgment

The authors wish to thank Dr. D. Pautler for his many helpful discussions.

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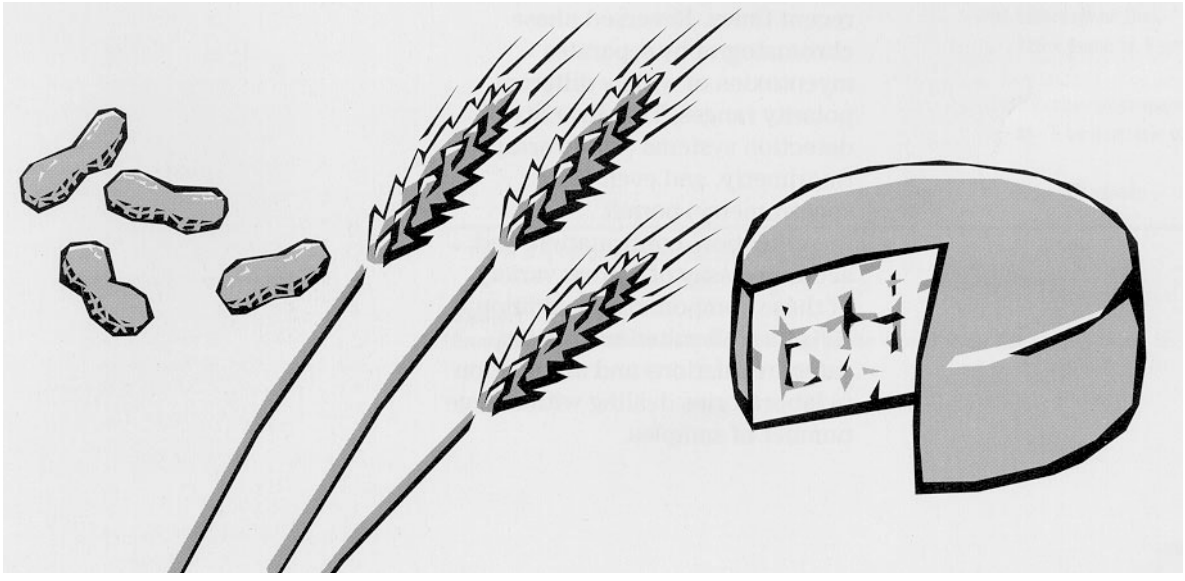
Analysis of Mycotoxins by HPLC with Automated Confirmation by Spectral Library

Application Note

Food Analysis

Rainer Schuster
Gerhard Marx
Michael Rothaupt

This note describes the sample preparation, chromatographic separation and detection of four different types of mycotoxins in food samples. Deciding which approach to adopt for analyzing these depends on the sample matrix and the type of fungus it has been contaminated with. Various professional organizations have proposed a variety of sample preparation methods–those for aflatoxins, ochratoxin A, patulin and zearaleneone are described here. All HPLC separations have been performed on reversed phase material (normal phase chromatography; a diol column can be used for patulin) and monitored with UV-visible absorbance diode-array detection (DAD) and fluorescence detection (FLD) for aflatoxins, ochratoxin and zearalenones or mass spectrometry (MS) for aflatoxins. Most compounds have been identified and confirmed by UV-visible absorbance spectral library search, purity control and by retention time tagging.



Introduction

Mycotoxins are highly toxic compounds produced by fungi. These toxins can contaminate foodstuffs when storage conditions are favorable to fungal growth. Mycotoxin nomenclature very often results from the fungi where the substance was first detected, for example aflatoxins in Aspergillus flavus strains, ochratoxin in Aspergillus ochraceus, patulin in Penicillium and Aspergillus, zearalenone in Fusarium. Most of these mycotoxins have been identified after cases of poisoning in livestock or the population at large. In 1969 more than 100,000 turkeys died of an unknown condition (so-called Turkey X) that was finally traced to peanuts–a component of their feed contaminated with Aspergillus flavus. During the wartime winter of 1940 in the USSR many people died after eating grain poorly stored and highly contaminated with Fusarium toxins such as zearalenone and fusarin C. A similar case occurred in 1965 in South Africa with ochratoxins found in cereals accumulated unmetabolized in animal kidneys.

Aflatoxins are known to be mutagens, teratogens (causing fetal abnormalities) and carcinogens (particularly in cancer of the liver or kidneys). Ochratoxins cause nephropathies in pigs, are teratogenic, and carcinogenic particularly in the liver and kidneys. Zearalenone shows estrogenic effects in sows and poultry, and affects the liver and kidneys. Patuline is a powerful mutagenic and cytotoxic compound. The intake of these mycotoxins over a long period at very low concentrations may be highly dangerous, yet difficult to combat since the small quantities are difficult to trace.

Currently most mycotoxins are still assayed using thin-layer chromatography (TLC), which permits effective compound separation and characterization. Such assay may be performed with satisfactory sensitivity when the compounds to be detected are fluorescent–a fluorodensitometer reads the plates quantitatively and objectively and has become indispensable to the control laboratory. However due to its higher separation power and shorter analysis times, use of HPLC has expanded rapidly in recent times. Reversed phase chromatography separates mycotoxins of widely different polarity ranges. The diversity of detection systems (diode array, fluorimetry, and even mass spectrometry) permit identification, confirmation and accurate assay of a great variety of these compounds. In addition HPLC is well suited to existing safety regulations and automation in laboratories dealing with a large number of samples.

The complexity in composition of processed foodstuffs makes a fixed routine necessary for analysis:

1. sampling protocol that ensures representative data from any one batch
2. extraction of mycotoxins, using mostly chloroform, acetone, or methanol
3. purification of the extract with clean-up methods
4. concentration of the extract
5. qualitative detection and assay of the mycotoxins.

In this paper we describe the analysis of 4 different types of mycotoxins. First we describe the chemical nature and occurrence of these toxins.

Experimental

Table 1 gives a short overview of analysis conditions for the four different mycotoxins aflatoxins, ochratoxin A, zearalenone and patuline.

Compound class	Matrix	Sample preparation	Chromatographic conditions
Aflatoxins G_2, G_2, B_2, B_1, M_2, M_1	Nuts, spices, animal food, milk, dairy products	Extraction §35LMBG	Hypersil ODS 100 x 2.1-mm id, 3 µm particles *HP 799160D-352* Water-methanol-acetonitrile 63:26:11 as isocratic mixture* Flow 0.3 ml/min at 25°C
Ochratoxin A	Cereals, flour, figs	Extraction §35LMBG Acidify with HCI. Extract with toluene. Clean up SiO_2. Elute toluene-CH_3COOH 9: 1	Lichrospher 100 RP18 125 x 4-mm id, 5 µm particles *HP 799250D-564-3* Water with 2 % acetic acid/acetonitrile, 1 : 1* Flow 1 ml/min at 40°C
Zearalenone	Cereals	Extract with toluene. Sep-pak clean up. Elute toluene-acetone 95: 5.	Hypersil ODS 100 x 2.1-mm id, 3 µm particles *HP 799160D-352* Water-methanol-acetonitrile 5:4:1 as isocratic mixture* Flow 0 : 45 ml/min. at 45°C
Patuline	Apple products	Clean-up on Extrelut Silica gel clean up Elute toluene-ethyl acetate 3: 1.	a)Superspher RP 18 125 x 4-mm id, 4 µm particles *HP 799250D-464,* Water-acetonitrile, 95 % to 5 % gradient Flow 0.6 ml/min at 40° C b) Lichrospher Diol 125 x 4-mm id, 5 µm particles Hexane-isopropanl 95:5 as isocratic mixture Flow 0.6 ml/min

* 100 % B is recommended for cleaning the column.

Table 1. Sample preparation and chromatographic conditions for mycotoxins in foodstuffs

Compound types:

Aflatoxins–are chemical derivatives of difurancoumarin (figure 1). Although a number of different aflatoxin metabolites are known, interest is usually focused on the four main aflatoxins B_1, B_2, G_1, G_2 and the so-called milk toxin M_1.

Aflatoxin B_1 is in the majority of cases the most abundant toxin, the most toxic and the most potent carcinogen. Maximum levels for B1 are usually given for the individual compound, 2 ppb in Germany, 5 ppb in France and 1 ppb in Switzerland, for example. United States legislation regulates the aflatoxin content of a contaminated product as the sum of B_1-plus-B_2-plus-G_1-plus-G_2, which may not exceed 20 ppb. Aflatoxins are most often analyzed in nuts, for example peanuts and pistachios, cereals, figs, bread, meat, eggs, butter, milk, margarine, juices, cottonseed products, and cocoa beans. Considering the complexity of these matrices, sample preparation is the most important step for reliable results.

Since fungal growth–and therefore contamination by aflatoxin–is not homogeneous, normal sampling gives mediocre results. The US Department of Agriculture (USDA) tackled the problem by defining a sampling protocol for peanuts which involves as many as eight assays on four samples of more than
1 kg. Sampling is not specified in European countries. At best, a few hundred grams are taken to determine the mean level of aflatoxins in a batch as large as of a couple of thousand tons of grain.

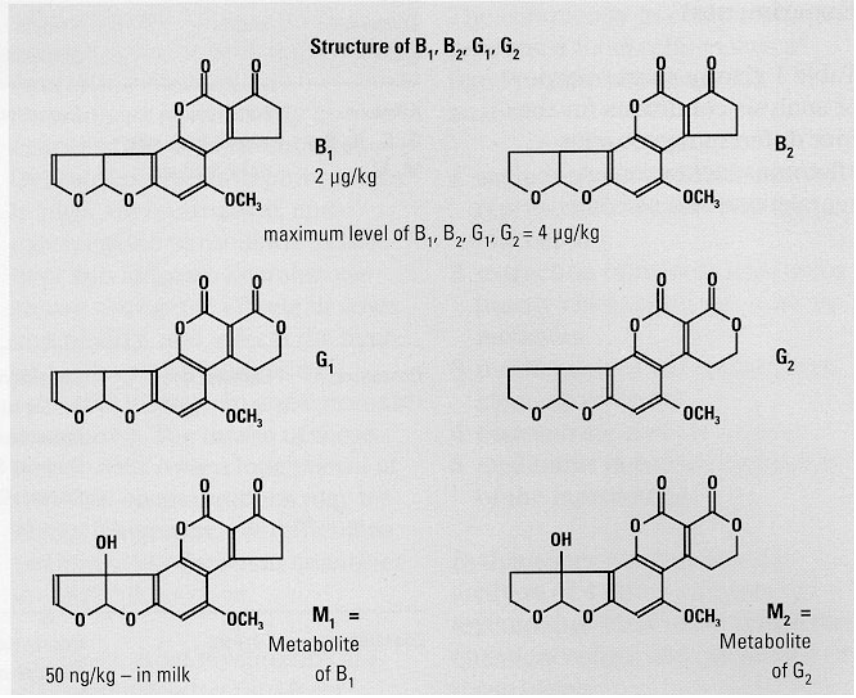


Figure 1. Structure of aflatoxins and their maximum permitted concentrations (given for Germany[1])

Ochratoxins–The mycotoxin ochratoxin A can be produced by different fungi including Aspergillus and Penicillium. Of the ochratoxins A, B, and C, the latter two so far have not been found in naturally contaminated products. Beside nephrotoxicity, ochratoxin A has hepatoxic, teratogenic and carcinogenic properties in the kidneys. Ochratoxin A was found in various foodstuffs. Analysis of more that 900 plant samples show a contamination rate of about 13 %, mostly in barley, oats and wheat. The concentrations of ochratoxin A found varied from 0.1 to 200 µg/kg.[2] A review of results from various countries, covering around 7000 samples, reported that contamination was about 14 %.[3] Ochratoxin A is the primary agent in so-called mycotoxic porcine nephropathy (MPN) a disease prevalent in pigs. The toxicity is a third that of the toxicity of aflatoxin B_1 in rats. The main human intake is assumed to be through the consumption of pork and wholemeal products.

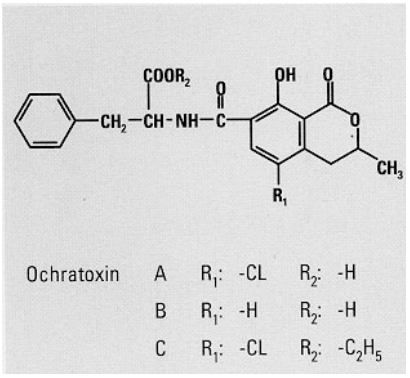


Figure 2. Structure of three common ochratoxins

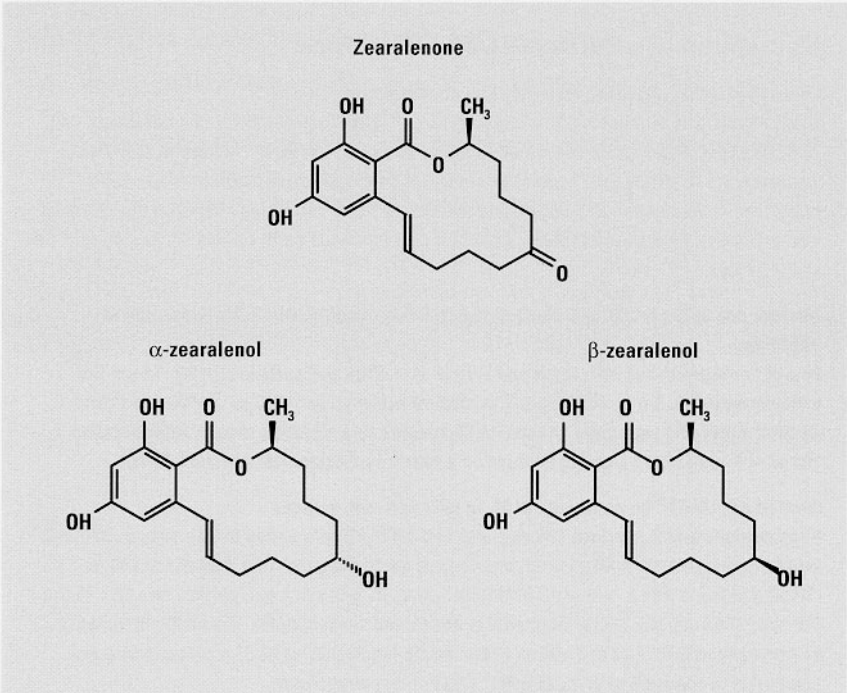


Figure 3. Chemical structures of zearalenone, a–and b-zearalenol and a -zearalanol (Zeranol)

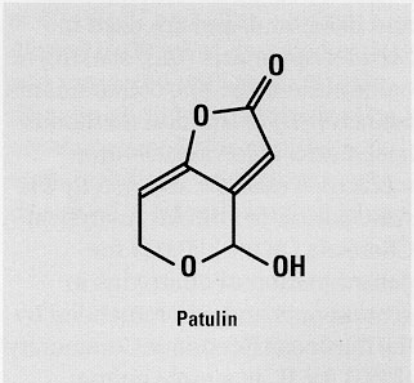


Figure 4. Chemical structure of patulin

Zearalenone–an estrogenic-efficient mycotoxin produced by Fusarium, occurs mainly in a variety of natural products such as corn and other grains. Whereas the acute toxicity of zearalenone is low, its intake is linked to various possible estrogenic disease effects in children.[4] After carcinogenity was determined in rodents, a recent risk assessment resulted in an estimated safe intake of not more than 0.05 µg for each kilogram body weight per day for humans.[12] Use of contaminated animal feed means that these compounds are present in cow's milk as zearalenone and the diastereomer metabolites and b-zearalenol. Another zearalenone derivative is the synthetic a -zearalanol (also known as Zeranol) which is used in some countries for fattening cattle. The recommended limit for zearalanol is 10 µg kg for liver and 2 µg/kg for other meats.[7] Their use as anabolic agents is prohibited in the European Community. investigations of food and animal feedstuffs have shown zearalenone concentrations between 0.001 and 2.0 mg/kg.[5] The highest levels, 1700 mg/kg, were found in silo-corn.[6]

Patulin–unstable in cereals, mainly occurs in fruit, especially apples, is a metabolite of several fungi of Penicillium and Aspergillus. Most of the survey work has been done on apple juices and apple-based products.

Aflatoxin sample preparation

For sample preparation different methods are described in the literature.[8,9,18] Lipid should be eliminated after analyte extraction if lipid content exceeds 5 %. Solvents in which the aflatoxins are insoluble are hexane, petroleum ether, pentane and isooctane, and are used in Soxhlet apparatus (6h), shaking or column clean up. The contaminants branch (CB) extraction method is based on a chloroform-water mixture, a method adopted by the Association of Official Analytical Chemists (AOAC) 20.029 for determination of aflatoxins in ground nuts and recommended by the European Economic Community (EEC) for B_1 in simple animal nutrition foodstuffs.

Whatever extraction method is used the resulting extract still contains, besides the aflatoxins, various impurities (lipids, pigments and so on) requiring an extra clean-up step. Apart from purification by precipitation or by liquid-liquid partition, the most commonly used technique is column adsorption chromatography.

Extraction of aflatoxins with chloroform

CB method (AOAC and EEC) for extraction of aflatoxins

50 g of finely ground sample are mixed with 25 g of diatomaceous earth and moistened with 25 ml of water. This mixture is carefully shaken, diluted in 250 ml of chloroform and shaken vigorously for 30 minutes on a vibration shaker. A 50 ml portion of chloroform extract is collected for purification and assay. The addition of water facilitates chloroform penetration into substrates derived from plants, while the diatomaceous earth retains various substances like pigments.

Method §35 *Lebensmittel und Bedaffspeyenstands Gesetz* (LMBG)[8] for extraction of aflatoxins

20 g of finely ground sample are mixed with 20 g of silica gel, particle size 20–45 μm (for example celit 545, Serva, Germany). This mixture is diluted with 200 ml of chloroform and 20 ml of water and vigorously shaken for 30 minutes on a vibration shaker. After filtration, 100 ml are evaporated close to dryness on a rotary evaporator (temperature 40 °C).

Method §35 LMBG[9] for extraction of M_1 in milk and milk powder

50 ml acetone and 5 g sodium chloride and 1 ml 1 N H_3PO_4 are added to 50 g milk, or 10 $_9$ milk powder homogenized in 40 g water, and shaken for 10 minutes. After addition of 100 ml dichloromethane and a further 10 minutes shaking, 25 g of silica gel, particle size 20–45 μm (for example celit 545, Serva, Germany) is added and shaken again. The dichloromethane/ acetone phase is filtered and 100 ml of the filtrate (equivalent to 33.33 g milk or 6.66 g milk powder) is evaporated to dryness at 40 °C in a rotary evaporator.

Purification of aflatoxins

Method according to AOAC/ EEC and §35 LMBG regulations[8]

A glass column (400 x 30mm) is filled in succession with 5 g sodium sulfate, 10 g of silica gel (63–200 μm, dried at 105 °C for lie), and 15 g anhydrous sodium sulfate topped up with some cotton-wool. The extract to be cleaned is added on top and eluted with 15–20 ml chloroform. Then the column is washed with 150 ml hexane and 150 ml diethylether to remove lipids and other interfering compounds from the aflatoxins. The aflatoxins are eluted with 150 ml of a chloroform/methanol (97: 3) mixture. The eluate is dried down and redissolved in a suitable solvent for assay by HPLC (methanol).

Extraction of aflatoxins from milk, AOAC 26.139

To 25 ml of milk, 10 drops of NH_4OH are added, swirled and diluted by 70 ml acetonitrile. The mixture is shaken for 1 minute and centrifuged for 5 minutes at 1000 rpm. The aqueous alkaline acetonitrile supernatant is transferred and evaporated on a rotary evaporator at 45 °C. The residue is acidified with 15 drops (about 500 μl) of HCl to pH 1.3 and partitioned into methylene chloride on the liquid-liquid extraction column, ChemElut™ (Analytichem, United Kingdom). The dichloromethane is evaporated off by rotary evaporation. After cooling, the residue is redissolved in 1.5 ml of dichloromethane, evaporates and redissolves in 500 μl hexane-dichloromethane (1: 9). Clean-up is performed on a BondElut NH_2 cartridge (Analytichem, United Kingdom) conditioned with hexane-dichloromethane (1: 9).100 μl of the milk extract is transferred to the column; fats are removed with 230 μl hexane-dichloromethane (1: 9) while zearalenone is eluted with 1 ml of methanol. After evaporation of methanol the residue is dissolved in 500 μl of mobile phase.

Extraction of ochratoxins with toluene

According to §35 LMBG Method 15-00-1, AOAC 26.100–26.125

30 ml of 2 M HCl in 50 ml of 0.4 M magnesium chloride solution is added to 20 g of ground and mixed sample. After homogenization, 100 ml toluene is added and shaken vigorously for 60 minutes. The suspension is separated by centrifuge and 50 ml of the toluene supernatant is passed through a preconditioned Sep Pak silica gel column. The column is washed with two 10-ml aliquots of hexane, 10 ml of toluene/acetone (95: 5) and 5 ml of toluene. Ochratoxin A is eluted with two aliquots of 15 ml toluene/acetic acid (9: 1) and dried down at 40 °C. The residue is redissolved in 1 ml of mobile phase and filtered.

Ochratoxin sample preparation

Methylester derivatives of the ochratoxins can also be analyzed. From the extracted sample, 500 µl is evaporated to dryness and redissolved with 1 ml of dichloromethane and 2 ml of 14 % boron trifluoride in methanol. The solution is heated for 15 minutes at 50–60 °C and after cooling diluted in 30 ml of distilled water and extracted with three 10-ml aliquots of dichloromethane. The organic phase is filtered through sodium sulfate, dried down and dissolved in 500 µl of mobile phase.

Zearalenone sample preparation

Thanks to Eppley s technique,[13] zearalenone, aflatoxins and ochratoxin can be simultaneously extracted on Sep-Pak silica cartridges. The sample is added as a toluene extract, washed with toluene and zearalenone is eluted with 10 ml of toluene-acetone (95: 5) mixture.

Patulin sample preparation

Two approaches are documented.[1] Fruit juices can be cleaned on an Extrelut cartridge followed by analyte extraction on a silica gel column with toluene-ethyl acetate (3: 1) before HPLC assay.[14] The analyte can be extracted into ethyl acetate, followed by partition extraction into 1.4 % Na_2CO_3 solution and back into ethyl acetate. After evaporation of the ethyl acetate at 40 °C, the residue is dissolved in methanol-ethyl acetate (9: 1) if it is to be analyzed on a reversed-phase column packing material or in hexane-isopropanol if a diol-phase column packing material is used (details of suitable columns are given in table 1).[18]

Chromatographic separations, peak confirmation and quantification

We used a Hewlett-Packard HP 1090 Series M liquid chromatograph with DR 5 binary solvent-delivery system, variable-volume auto-injector, temperature-controlled column compartment and solvent-preheating device. Mobile phase methanol and acetonitrile were of HPLC reagent quality (Baker, Gross-Gerau, Germany). A diode-array UV-Visible absorbance detector was used together with HPLC^{3D} ChemStation software to automatically quantify the mycotoxins and identify them using spectral libraries. Fluorescent species were detected using an HP 1046A programmable flourescence detector (FLD) under the control of the HPLC^{3D} ChemStation, using lex 265 nm, lem 455 nm for aflatoxins, lex 247 nm, lem 480 nm for ochratoxin A and lex 236 nm, lem 464 nm for zearalenone. Aflatoxins were also determined using mass spectrometry on an HP 5989 MS Engine equipped with negative ion detection and Thermospray options. The electron filament capability was used to provide higher sensitivity. LC eluant passed through a capillary tube and was simultaneously heated to approaching the boiling point. The resulting liquid-vapor was injected into the mass spectrometer where it was ionized and analyzed. The mass spectrometer was controlled and the data were analyzed by the HP 59940A MS ChemStation (HP-UX series).

Results and discussion

Aflatoxin assay by HPLC-DAD and HPLC-FLD

Thin layer chromatography can be replaced by reversed phase HPLC, improving accuracy, and dramatically speeding up the time required to assay, for example B_1 takes three hours by TLC, M_1 four hours. Figure 6 shows a separation of the common aflatoxins M_2 (5 ng), M_1 (10 ng), G_2 (1.5 ng), G_1 (5 ng), B_2 (1.5 ng), B_1 (5 ng) on a reversed phase column (refer to table 1 for conditions).

Flow rate	0.30 ml/min
Mobile phase	Isocratic water–methanol–acetonitrile (63: 26: 11) mixture
Detection:	
Fluorescence	lex 365 m, lem 455 nm
Diode-array	365 nm

Due to the extreme differences in fluorescence yields for B_2 and B_1 respectively G_2 and G_1 (B_2 FLD yield is about 60 times higher than B_1) it can be useful to run both detectors in series. Diode-array detection in addition gives us the UV-visible absorbance spectra dimension for further identification of the aflatoxins. As an alternative to the isocratic run with subsequent 100 % B wash, a gradient analysis from 35 % B (methanol-acetonitrile, 26: 11) to 55 % B in 10 min and 100 % B in 14 min (at 35 °C) might be used. Peaks become much sharper than under isocratic conditions, with higher signal-to-noise, and less polar compounds in the food extract are eluted in this run.

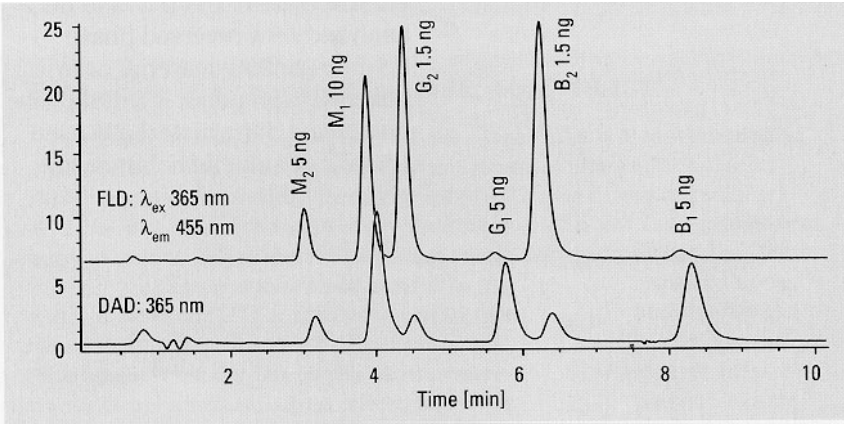


Figure 5. Analysis of the common aflatoxins by fluorescence and diode-array detection

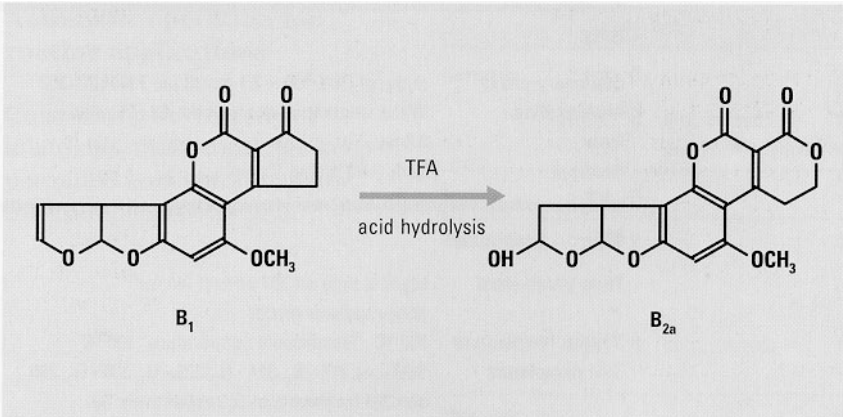


Figure 6. Structure of aflatoxins after hydrolysis with trifluoracetic acid (TFA), from B_1 to B_{2a}

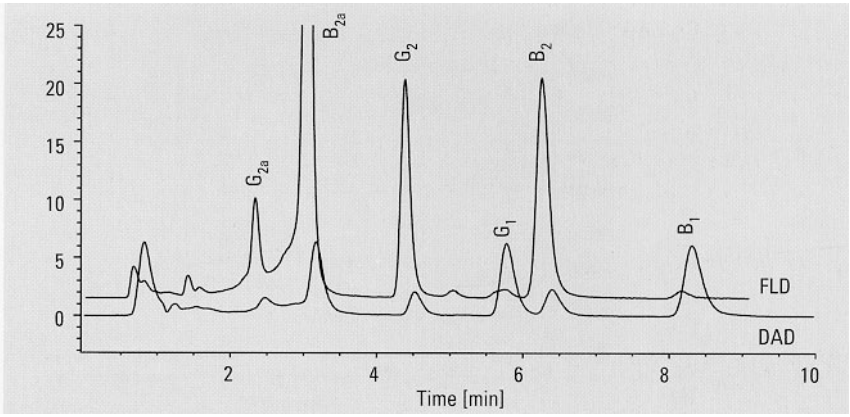


Figure 7. Analysis of the aflatoxins G_2, G_1, B_2, B_1 and G_{2a} and B_{2a} (hemiacetals), with conditions as for figure 1

If fluorescence is used alone it might be desirable to improve the B_1 and G_1 fluorescence yield by hydrating the double bond of the furanic ring (figure 6) with trifluoroacetic acid (TFA) to form the corresponding hemiacetals B_{2a} and G_{2a}. This approach can also be used as a confirmation tool for B_1 and G_1. A separation of the four aflatoxins and the hemiacetals B_{2a} and G_{2a} is shown in figure 7.[17]

Sensitivity of the B_1 can also be improved by formation of an iodine derivative[10] or by modification of the flow cell to a cell filled with fine silica particles.[11]

Aflatoxin assay by LC-MS

For highest sensitivity and selectivity we have investigated the use of mass spectrometry. The aflatoxin standard (not including M_2 and M_1) was diluted fivefold and 1 µl was injected resulting in concentrations of 1 ppm for G_1 and B_1, and 300 ppb for G_2 and B_2 (figure 8). A further dilution of 1: 10 and the 1-µl injection is shown in figure 9. Detection limits for G_1, B_2 and B_1 are less than 50 ppb for this separation. An example of thermospray application is shown in figure 11.

HPLC	
Stationary phase	Hypersil ODS 100 x 2.1 mm, 3 µl 799160D-352
Mobile phase	Water-methanol-acetonitrile 163: 26: 11)
Flow	0.3 ml
Gradient	32 %–60 % B in 10 min
MS	
Tune parameters	Manual tune on 367 adduct ion for polypropylene glycol
Source temperature	250°C Quadropole temperature 120 °C
SIM parameters	SIM ions 312–B_1, 314–B_2, 328–G_1, 330–G_2, 286 and 284 fragments of G_1 respectively G_2
Dwell time	600 msec
Electron multiplier	2500 V On Mode negative
Thermospray stem temperature	95 °C Filament ON

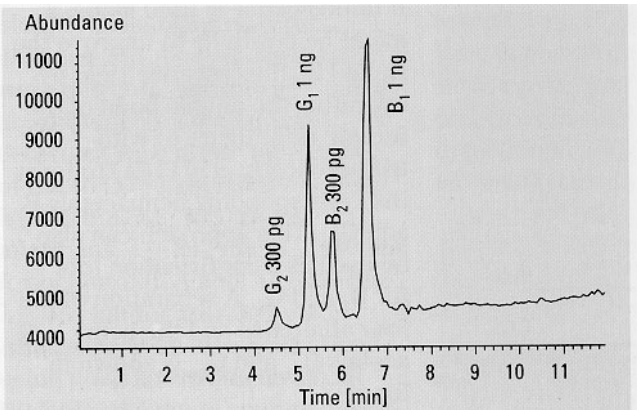


Figure 8. Analysis of G_2, G_1, B_2 and B_1 using HPLC and thermospray mass spectrometry

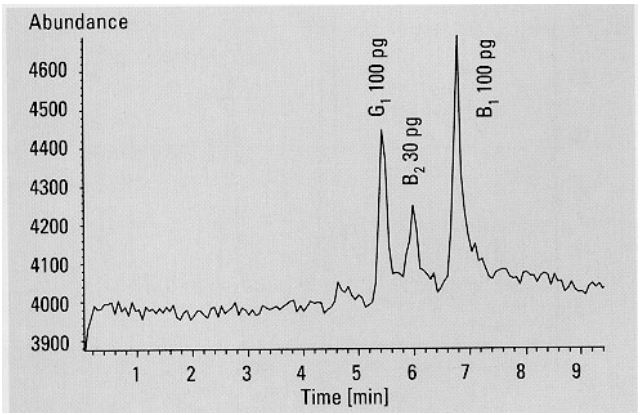


Figure 9. G_1, B_2, and B_1 in the low picogram range at 1-µl injection volume

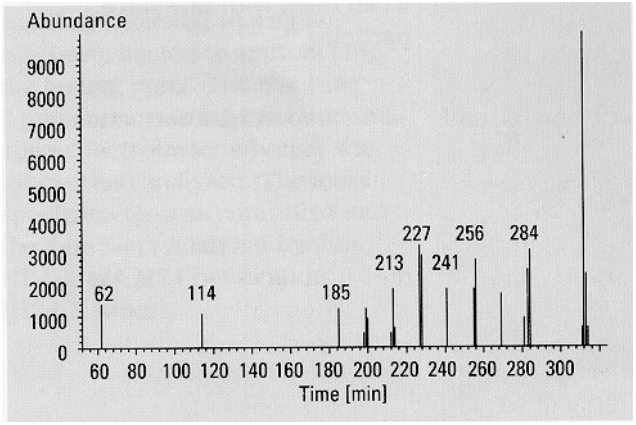


Figure 10. Total ion monitoring spectrum of aflatoxin B_1

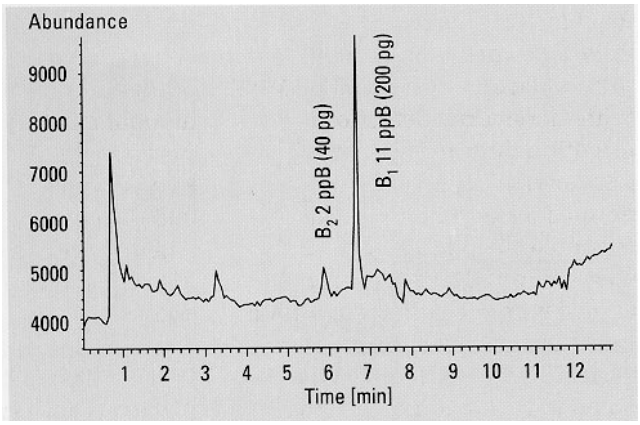


Figure 11. Extract of pistachio nut according to § 35 LMBG with 1-µl injection (see also figure 12 with 2-µl injection volume)

Automatic operation for routine applications

Considering the toxicity of aflatoxins, most countries keep permitted concentrations low, for examples see table 2.

	Aflatoxin B_1	Total aflatoxin	Milk toxin
Germany	2 ppb B_1	4 ppb $\sum\{B_1, B_2, G_1, G_2\}$	50 ppt M_1 milk
	20 ppb B_1 animal feed	50 ppt $\sum\{B_1, B_2, G_1, G_2\}$	
	diet and baby food		
France	5 ppb B_1		
Switzerland	1 ppb B_1	5 ppb $\sum\{B_1, B_2, G_1, G_2\}$	50 ppt M_1 in milk
			20 ppt M_1 baby food
			250 ppt M_1 cheese
	2 ppb B_1 corn, cereals	10 ppt $\sum\{B_1, B_2, G_1, G_2\}$	
		diet and baby food	
USA, FDA		20 ppb $\sum\{B_1, B_2, G_1, G_2\}$	500 ppt M_1 milk
WHO, FAO	5ppb B_1	10 ppb $\sum\{B_1, B_2, G_1, G_2\}$	

Table 2. Limits for Aflatoxins in different countries

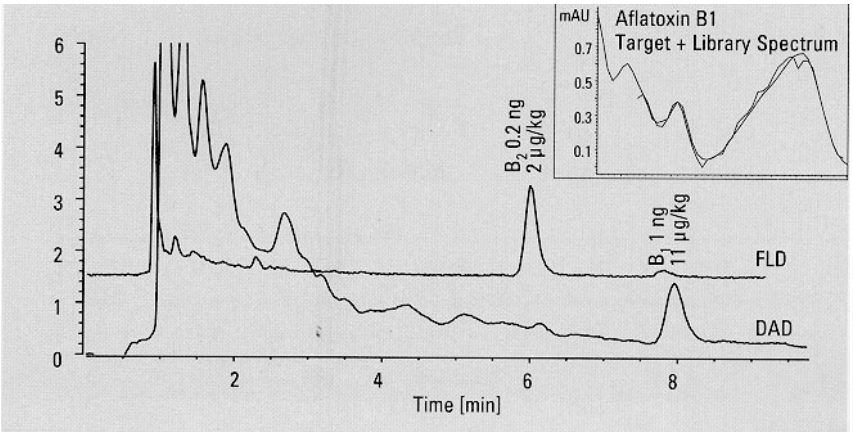


Figure 12. The analysis of a 2-µl injection of pistachio-nut extract (§35 LMBG) detected by FLD and DAD and UV-Spectrum

Sample preparation in the following applications was performed according to §35 LMBG. With diode-array detection, retention time and spectral information can both be incorporated automatically in the report. We created a library of standard mycotoxin spectra tagged with their HPLC retention times. After each run, peak spectra were automatically compared with library spectra, and their purity checked by overlaying several spectra taken in each peak. The customized report prints all this information retention times, chromatogram, library and calibration table, amounts, library search match and purity match factor. The method is fully automatic. Data acquisition and data evaluation including quantification and qualitative identification are performed in one run.

Figure 12 shows a 2-µl injection of pistachio-nut extract detected using FLD and DAD. B_1 is present in less than 1.0 ng (absolute) corresponding to 11 ppb (20 g of material extracted in 500 µl methanol of which 2 µl was injected corresponds to a multiplication factor of 12500 for the ppb value). This is close to the detection limit for fluorescence, while for UV-visible absorbance much lower values are detectable. To determine lower concentrations by FLD, larger injection volumes are needed. Fluorescence has the advantage of high selectivity- and therefore no matrix effects–whereas the diode-array detector shows much higher sensitivity for B_1 and G_1 and can be used for additional confirmation using the automatic library search program. Figure 13 shows the printout of such a search. A report header contains all the important information, such as data file name, the library used, search threshold values, peak purity, while the quantitative report contains the corresponding retention times (from library, calibration table and chromatogram), purity and library match factors and names of the identified compounds.

Report from automatic Library search

***** REPORT *****

Operator Name:	RS	Vial/Inj.No.: 100/1	
Date & Time:	10 Apr 92 4:06 pm		
Data File Name:	FOOD:AFLA-PIST		
Integration File Name:	DATA:AFLA1.I		
Calibration File Name:	FOOD:AFLA-DAD.Q		
Quantitation method:	ESTD	calibrated by Area response	
Sample Info:	PISTATIO NUTS according to $35 LMBG (reduced to 500 µl MeOH)		
Misc. Info:	20g extr. CH2CL2–clean Silicag–HEXANE, ETHER, CHC13		
Method File Name:	AFLA-DAD.M	Wavelength from: 250 to: 400 nm	
Library File Name:	FOOD:AFLATOX.L	Library Threshold: 959	
Reference Spectrum:	8.32 min	Peak Purity Threshold: 950	
Time window from:	5.0 % to: 5.0 %	Smooth Factor: 5	
Dilution Factor: 12.5	Sample Amount: 0.0	Resp.Fact.uncal.peaks: None	

Name	Amount [µg/kg]	Peak-Ret. [min]	Call-Ret. [min]	Lib.-Ret [min]	Purity Match factor	Library	Res.
B1	11.48	A 7.956	7.18	8.18	967	987	6.4
	11.48						

Figure 13. Printout of the report of the analysis in figure 12

A milk sample spiked with 600 ng/l of aflatoxin M_1 was prepared according to § 35 LMBG. 2 µl were injected and detected with FLD and DAD (figure 14). The sub-nanogram amounts of M_1 (0.6 ng) could be detected by DAD and a spectral search for confirmation was performed.

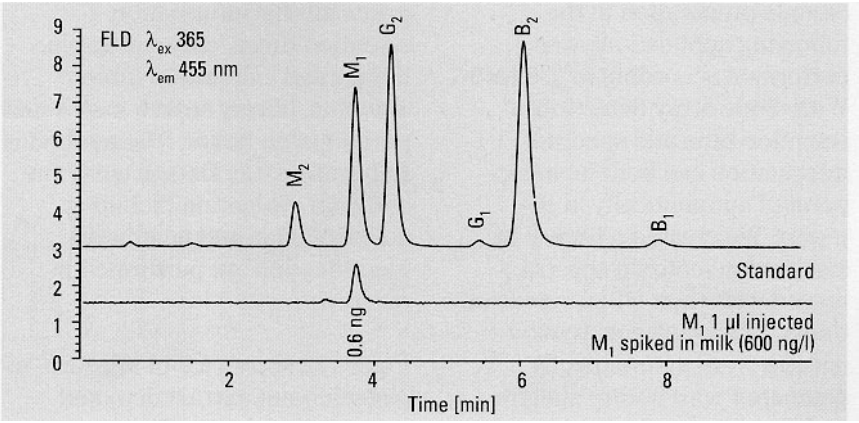


Figure 14. Analysis of spiked milk sample (600 ng/l) using sample preparation § 36 LMBG

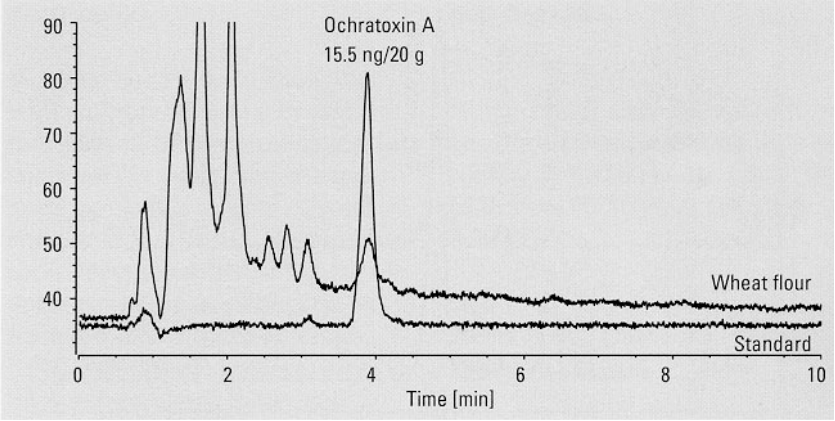


Figure 15. Analysis of ochratoxin A in wheat flour with the corresponding standard

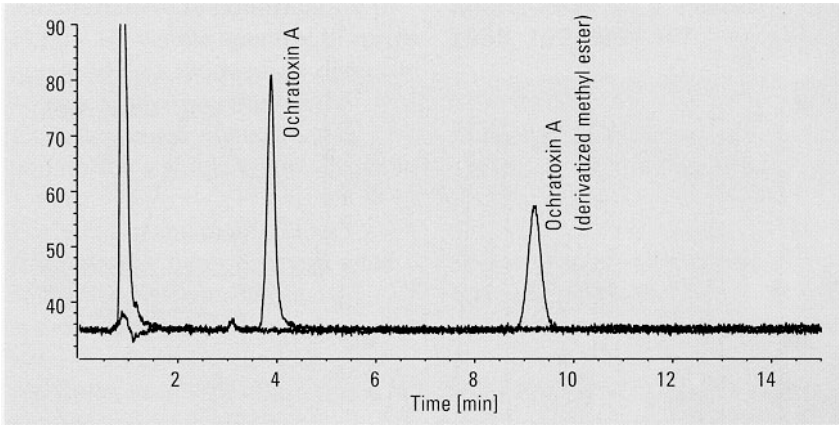


Figure 16. Separation of ochratoxin A and the ochratoxin A methylester derivative (1 ng absolute)

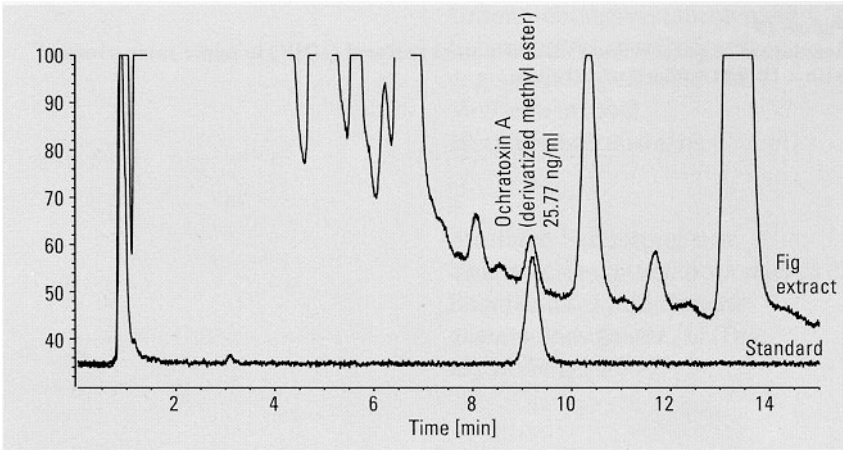


Figure 17. Analysis of a fig extract where ochratoxin A has been derivatized and overlaid with the corresponding methyl ester standard

Ochratoxin A assay by HPLC

Separation was achieved on a reversed phase column (LiChrospher 100 RP 18 125 x 4-mm id, 5 µm particles) with water /2 % acetic acid / acetonitrile (1: 1) and detected at lex 247 nm, lem 480 nm with a fluorescence detector, 20-µl injection volume and 40°C column temperature.

This analysis was confirmed with a derivatization of the mycotoxin to the methyl ester (figure 16).

The analysis also works well in more complicated matrices, for example, figs (figure 17).

Zearalenone assay by HPLC-DAD and HPLC-FLD

Separation was achieved on an Hypersil ODS narrow-bore column (100 x 2.1-mm id, 5-µm particles) using a 50 parts water, 40 parts acetonitrile, 10 parts methanol isocratic mobile phase mixture. DAD detection wavelength was 236 nm with 20 nm bandwidth, fluoresecence detection was at lex 236 nm, lem 464 nm. Figure 18 shows a standard composed of 5 ng a-zearalenol, 2 ng b-zearalenol and 8 ng zearalenone. We recommend the DAD for sensitivity and spectral confirmation, while higher selectivity is given by fluorescence.

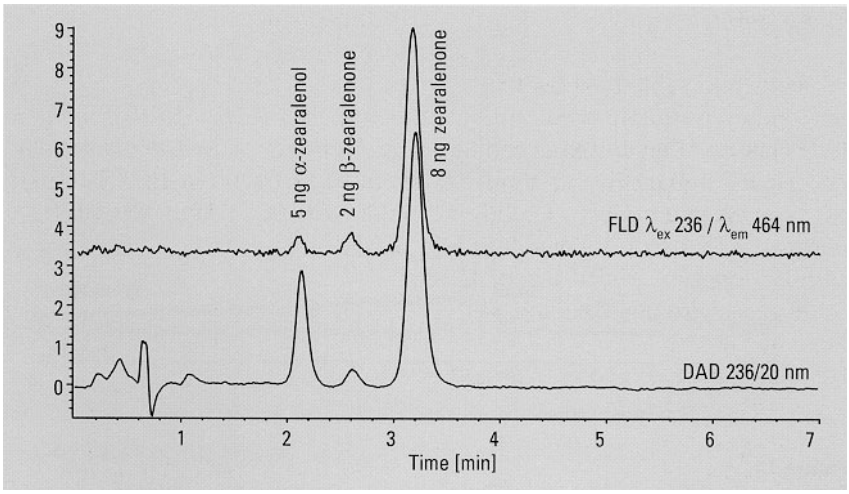


Figure 18. Analysis of zearalenone and its metabolites with FLD and DAD detection

Patulin assay by HPLC-DAD

A major problem for the analysis of patulin in apple products (juice, pies and so on) is the high content of 5-hydroxy methyl furfural (HMF) a compound that elutes close to patulin and absorbs light in the ultraviolet region also. Separation of both HMF and patulin was achieved on a silicagel column and also on a diol column (a reversed phase column based on silica gel with two hydroxyl endings). Figure 18 shows a separation of the compounds in an apple juice sample on a diol column (conditions given in table 1). Patulin was detected at 270 nm with subsequent identification using spectral library search.

With improved reverse phase column materials, separation can be done on Spherisorb RP 18, 5-µm particles using an acetonitrile gradient from 5 % to 100 % at 40 °C.[16]

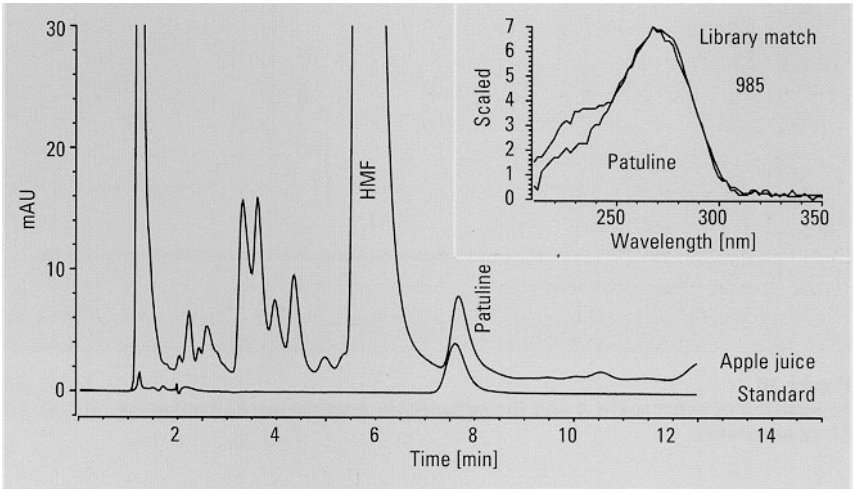


Figure 19. Resolution of patulin and 5-hydro methyl furfural (HMF) in apple juice overlaid with a 10-ng standard of patulin

Conclusion

We have been able to show that with suitable sample preparation four classes of mycotoxins can be successfully quantified at nanogram levels in a variety of solid and liquid foodstuffs. Considering sample complexities, a variety of approaches are possible and we have discussed these at length. HPLC separations were performed on reversed phase materials. Derivatization and subsequent fluorescence detection can improve selectivity for aflatoxins and ochratoxin A, and serve as an additional confirmatory analysis. An alternative to confirmation by FLD—UV-visible spectral libraries acquired on a diode-array detector—can be incorporated in the analytical run and automated, generating a single comprehensive report.

For most of the mycotoxins, fluorescence detection was used for high sensitivity. Mass spectrometry was able to lower detection limits to the low picogram range for aflatoxins, including confirmation via molecular mass.

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Acknowledgments

Martin Greiner, mass spectrometry specialist at Hewlett-Packard's European Customer Support Center, performed the MS work on aflatoxins.

Rainer Schuster is a senior application chemist based at Hewlett-Packard's Waldbronn Analytical Division in Germany.

Dr Gerhard Marx is head of the analysis laboratory at the Landesuntersuchungsamt (State Analytical Services Agency) in Karlsruhe, Germany.

Michael Rothanpt is a marketing development engineer based at Hewlett-Packard's European Marketing Center also in Germany.

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8/93
12-5091-8692

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

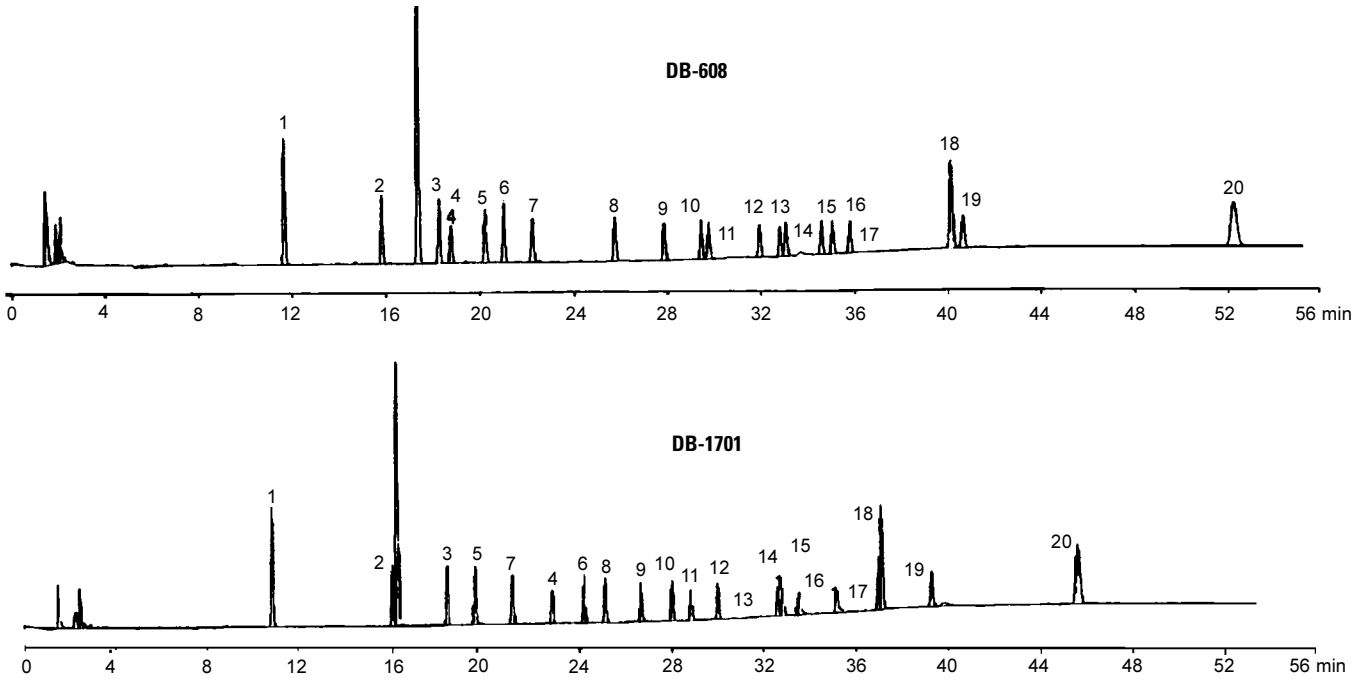

Figure 1. Typical chromatograms of a pesticides standard mix using DB-608 and DB-1701 columns under GC conditions used in environmental testing laboratories. (See Table 2 for peak identification.)

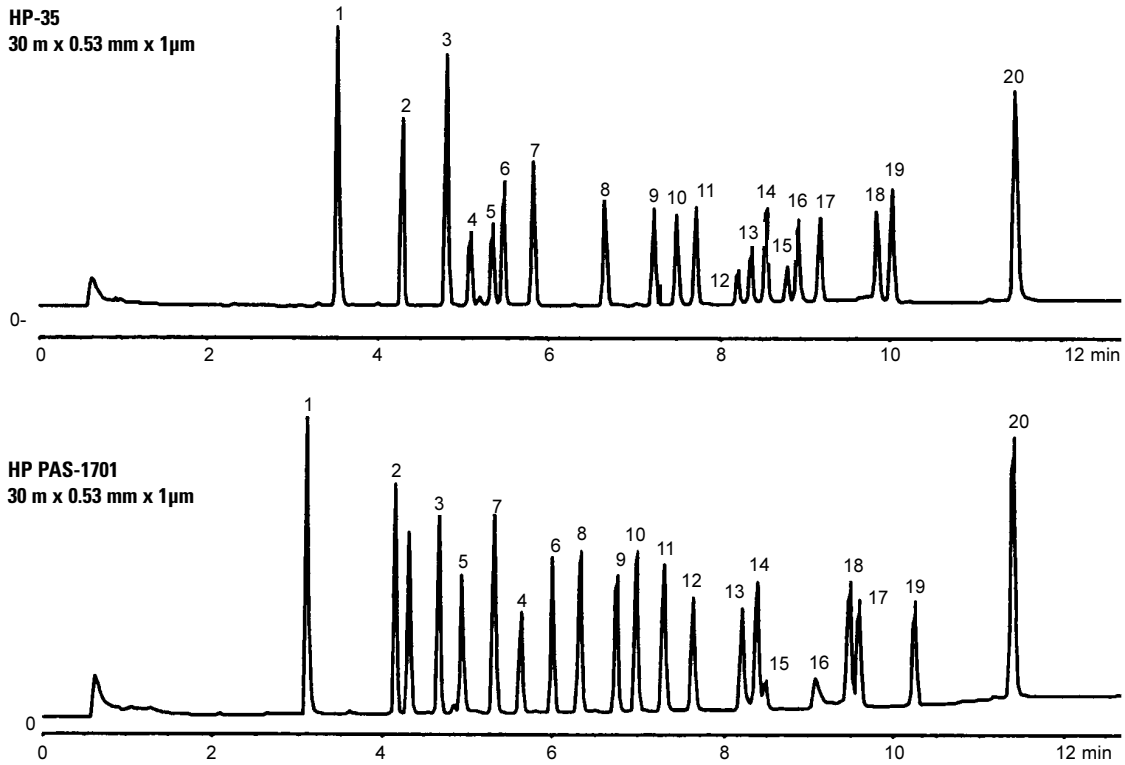

Figure 2. Chromatograms of a pesticides standard mix using HP-35 and HP PAS-1701 columns under the GC conditions listed in Table 1. (See Table 2 for peak identification.)

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

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,

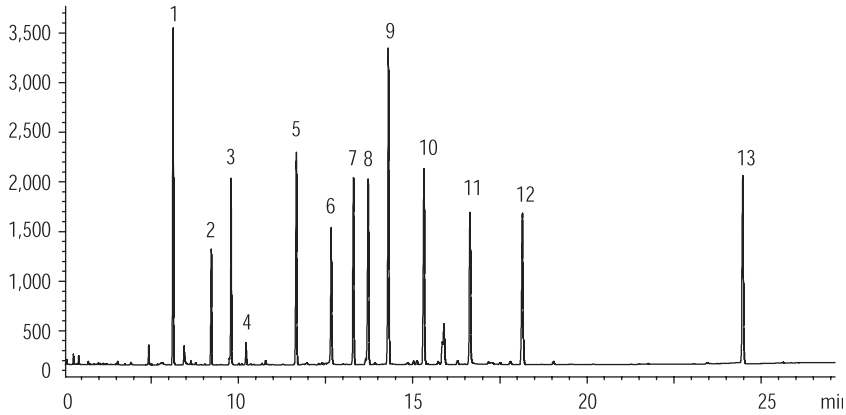

Figure 1. Typical chromatogram of OCPs at 20 or 40 ppb.
See table 1 for conditions. See table 5 for peak identification.

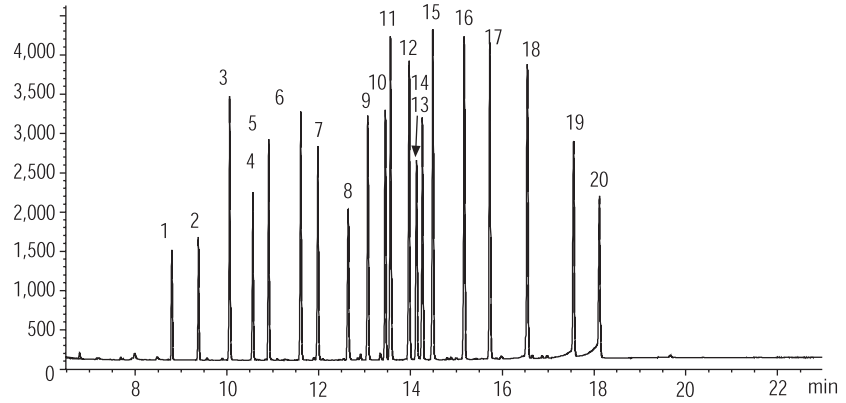

Figure 2. Typical chromatogram of PCB congeners at 50 ppb.
See table 1 for conditions. See table 4 for peak identification.

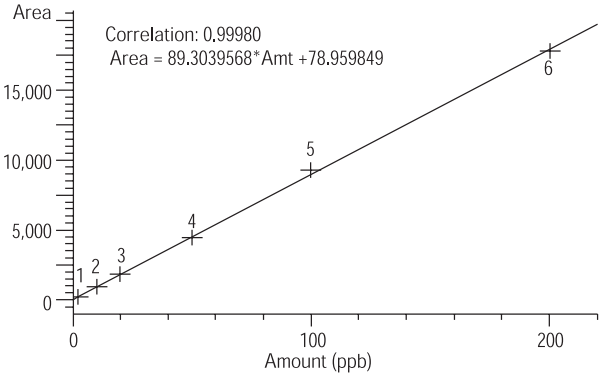

Figure 3. Typical linearity of PCB congener analysis: decachlorobiphenyl from 2-200 ppb.

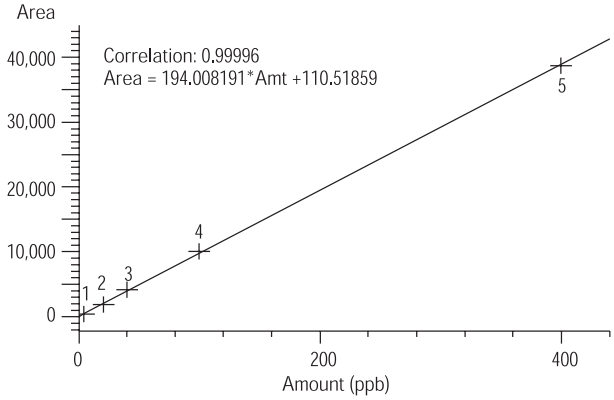

Figure 4. Typical linearity of OCP analysis: 4,4' DDE from 4 to 400 ppb.

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.

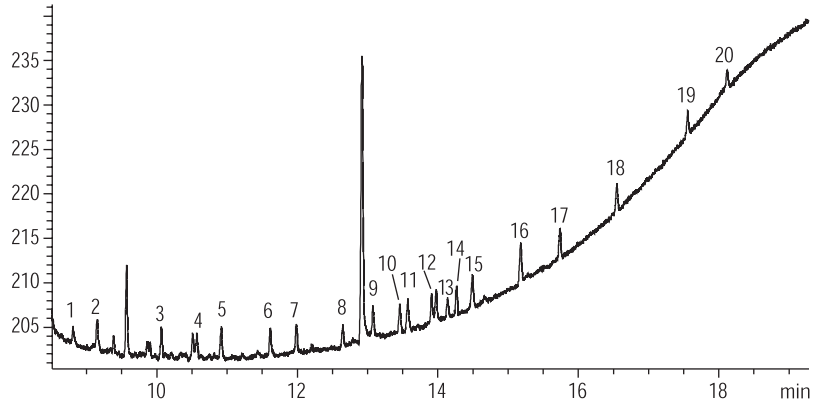

Figure 5. PCB congener mixture at 50 ppt each.
See table 1 for conditions. See table 4 for peak identification.

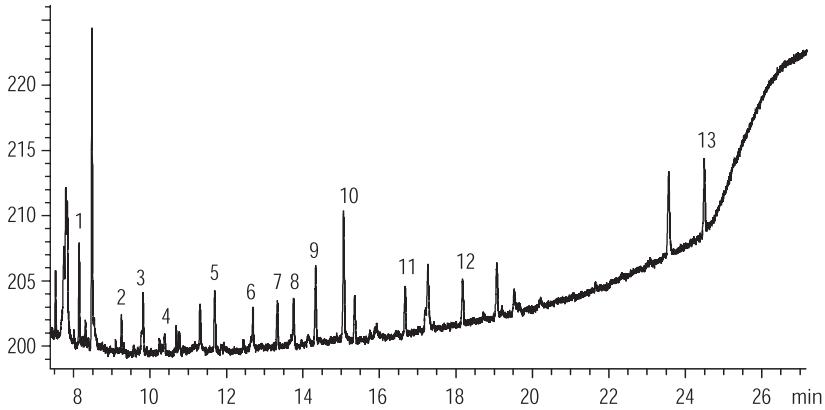

Figure 6. OCP Mixture at 50 to 100 ppt.
See table 1 for conditions. See table 5 for peak identification.

Printed in the USA 3/2000
5965-8556E

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HPLC Analysis of Aflatoxines in Pistachio Nuts using HPLC

Angelika Gratzfeld-Heusgen

Food

Abstract

The following mycotoxins have been analyzed: aflatoxins G_2, G_1, B_2, B_1, M_2, and M1, ochratoxin A, zearalenone, and patuline.

Mycotoxins are highly toxic compounds produced by fungi. They can contaminate food products when storage conditions are favorable to fungal growth. These toxins are of relatively high molecular weight and contain one or more oxygenated alicyclic rings. The analysis of individual mycotoxins and their metabolites is difficult because more than 100 such compounds are known, and any individual toxin is likely to be present in minute concentration in a highly complex organic matrix. Most mycotoxins are assayed with thin-layer chromatography (TLC). However, the higher separation power and shorter analysis time of HPLC has resulted in the increased use of this method. The required detection in the low parts per billion (ppb) range 4,[1, 2, 3] can be performed using suitable sample enrichment and sensitive detection.

Sample preparation

Samples were prepared according to official methods.[2] Different sample preparation and HPLC separation conditions must be used for the different classes of compounds. The table on the next page gives an overview of the conditions for the analysis of mycotoxins in foodstuffs.

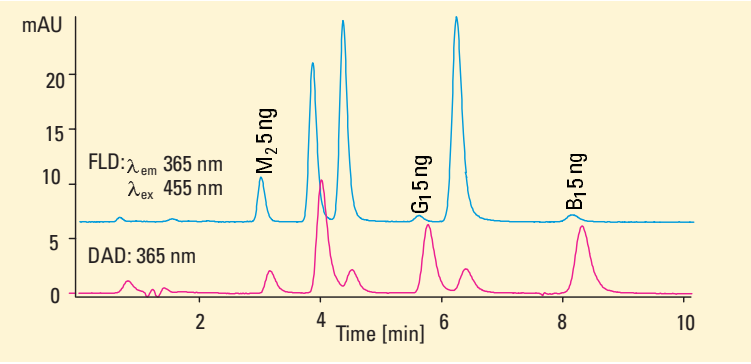

Figure 1
Analysis of aflatoxins with UV and fluorescence detection

Chromatographic conditions

The HPLC method presented here for the analysis of mycotoxins in nuts, spices, animal feed, milk, cereals, flour, figs, and apples is based on reversed-phase chromatography, multisignal UV-visible diode-array detection, and fluorescence detection. UV spectra were evaluated as an additional identification tool.

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HPLC method performance

Limit of detection 1–5 µg/kg

Repeatability
of RT over 10 runs <0.12 %
of areas over 10 runs <1.5 %

Linearity
of UV-visible DAD 1–500 ng
of fluorescence 30 pg to 2 ng

Column class	Matrix	Sample preparation	Chromatographic conditions
Aflatoxins G_2, G_1, B_2, B_1, M_2, M_1	nuts, spices, animal feed, milk, dairy products	• extraction according to Para. 35, LMBG* [4, 5]	Hypersil ODS, 100 × 2.1 mm id, 3-µm particles water/methanol/ACN (63:26:11) as isocratic mixture (100% B is recommended for cleaning the column flow rate: 0.3 ml/min at 25 °C DAD: 365/20 nm Fluorescence detector (FLD): excitation wavelength 365 nm, emission wavelength 455 nm
Ochratoxin A	cereals, flour, figs	• extraction according to Para. 35, LMBG • acidify with HCl • extract with toluene • SiO_2 cleanup elute toluene/acetic acid (9:1)	Lichrospher 100 RP18, 125 × 4 mm id, 5-µm particles water with 2 % acetic acid/ACN (1:1)* flow rate: 1ml/min at 40 °C FLD: excitation wavelength 347 nm, emission wavelength 480 nm
Zearalenone	cereals	• extract with toluene • Sep-pak cleanup • elute toluene/acetone (95:5) • AOAC 985.18:[3] α-zearalenol and zearalenone in corn	Hypersil ODS, 100 × 2.1 mm id, 3-µm particles water/methanol/ACN (5:4:1) as isocratic mixture* flow rate: 0.45 ml/min at 45 °C DAD: 236/20 nm FLD: excitation wavelength 236 nm, emission wavelength 464 nm
Patuline	apple products	• cleanup on Extrelut • silica gel cleanup • elute toluene/ethylacetate (3:1)	Superspher RP18, 125 × 4 mm id, 4-µm particles water 5 %–95 % ACN flow rate: 0.6 ml/min at 40 °C DAD: 270/20 nm or Lichrospher diol, 125 × 4 mm id, 5-µm particles hexane/isopropanol (95:5) as isocratic mixture flow rate: 0.6 ml/min at 30 °C DAD: 270/20 nm

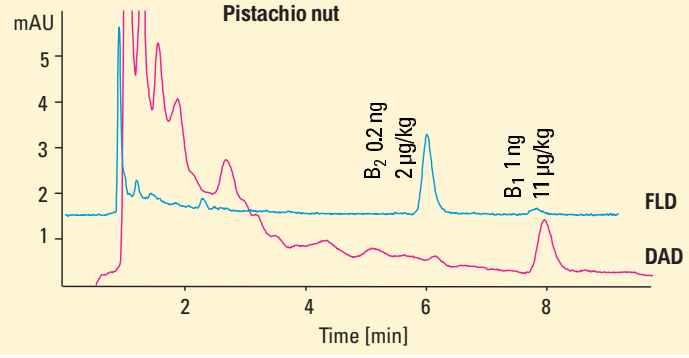

Figure 2
Analysis of aflatoxins in pistachio nuts with UV and fluorescence detection

References

1. R. Schuster, G. Marx, G, M. Rothaupt, "Analysis of mycotoxins by HPLC with automated confirmation by spectral library", *Agilent Application Note* 5091-8692, **1993**.

2. Lebensmittel- und Bedarfsgegenständegesetz, Paragraph 35, Germany.

3. *Official Methods of Analysis, Food Compositions; Additives, Natural Contaminants*, 15th ed; AOAC: Arlington, VA, 1990, Vol. 2.; AOAC Official Method 980.20: aflatoxins in cotton seed products; AOAC Official Method 986.16: Aflatoxins M_1, M_2 in fluid milk; AOAC Official Method 985.18: α-zearalenol.

4. Farrington et. al., "Food Additives and Contaminants, **1991**, Vol. 8, No. 1, 55-64".

5. *Official Methods of Analysis*; Horwitz, W., Ed.; 14th ed.; AOAC: Arlington, VA, **1984**; secs 12.018–12.021.

Equipment

Agilent 1100 Series
- degasser
- isocratic pump
- autosampler
- thermostatted column compartment
- diode array detector,
- fluorescence detector

Agilent ChemStation
+ software

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Released 09/97
Publication Number 5966-0632E

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Analysis of Pesticides in Salad Samples and Spices using HPLC

Rainer Schuster

Food

Abstract

The following compound classes of pesticides have been analyzed: triazines, phenylurea-herbicides, methabenzthiazuron, diquat, paraquat, and mercaptobenzothiazol.

Carbamates and glyphosate also have been analyzed but with different equipment. In most countries, growing concern about the residues of pesticides in food products is evident. Therefore, regulations limiting the concentration of pesticides in foodstuffs have been introduced to protect consumers from contaminated food products. Several methods are used to control these limits. HPLC is recommended for the analysis of low volatile compounds and for compounds that are unstable when heated.

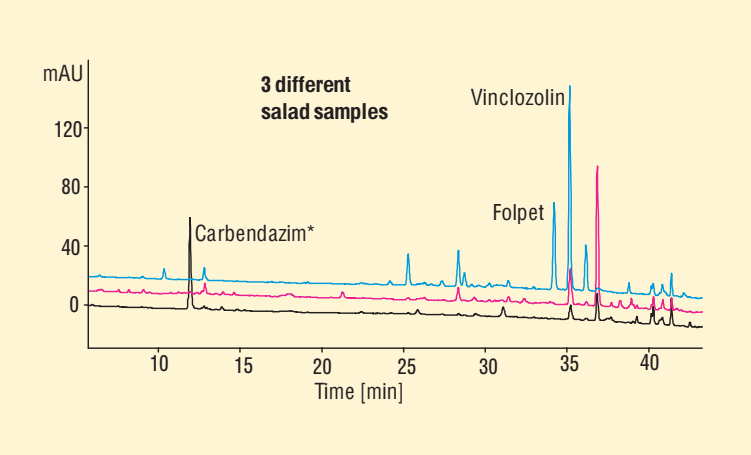

Figure 1
Analysis of pesticide residues in three different salad samples
* Carbendazim has a low recovery rate of only approximately 40 %

Conditions

Column 100 ˘ 3 mm Hypersil BDS, 3 µm
Mobile phase water/ACN (95:5)
Gradient at 10 min 25% ACN
at 26 min 42% ACN; at 34 min 60% ACN
Flushing time 10 min at 100% ACN
Post time 6 min
Flow rate 0.5 ml/min
Oven temp 42 °C
Injection vol 3–10 µl
Detector UV-DAD detection wavelengths 214/15 nm, 230/20 nm, and 245/20 nm reference wavelength 400/80 nm
Sample preparation
Salad was homogenized and then extracted with liquid/liquid extraction. The extract was cleaned with gel permeation chromatography using cyclohexane/ethyl acetate. Spices were prepared according to Specht 22 with gel permeation chromatography.

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Sample preparation

Sample preparation and enrichment depend strongly on the matrix. Drinking water samples, for example, must be extracted using solid-phase extraction, whereas vegetables are extracted with liquid/liquid extraction after homogenization, followed by additional cleaning and sample enrichment.

Chromatographic conditions

The HPLC method presented here was used for the analysis of pesticides in salad samples and spices.[1, 2]

HPLC method performance

Limit of detection
0.01 µg/l

Repeatability
of RT over 10 runs <0.2 %
of areas over 10 runs <1 %

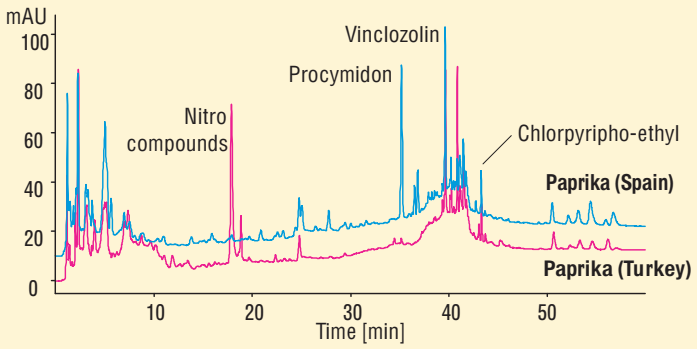
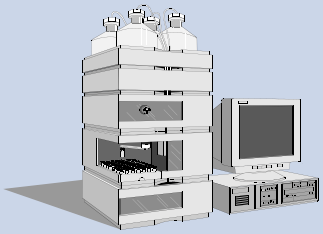


Figure 2
Analysis of pesticide residues in two paprika samples

Equipment

Agilent 1100 Series
- degasser
- quaternary pump
- autosampler
- thermostatted column compartment
- diode array detector, Agilent ChemStation + software



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Released 09/97
Publication Number 5966-0742E

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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

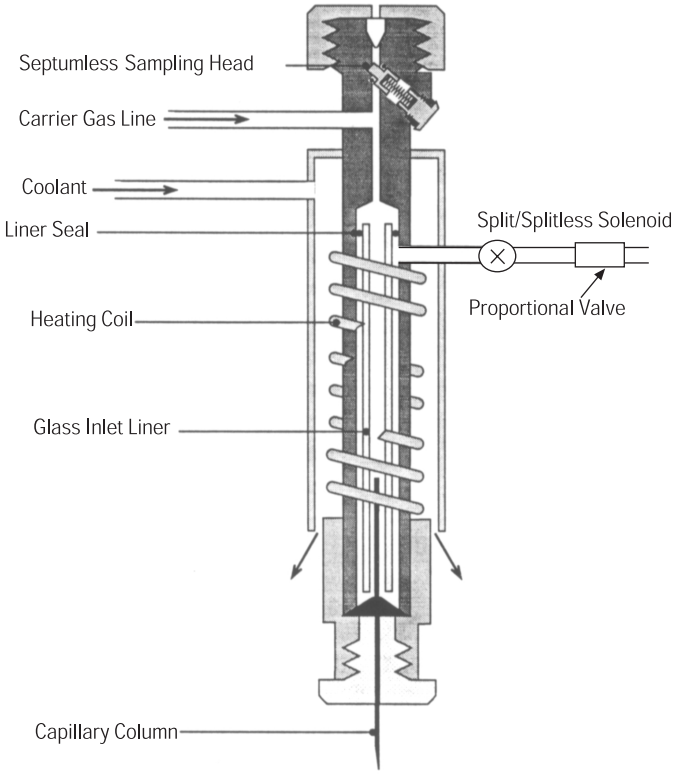
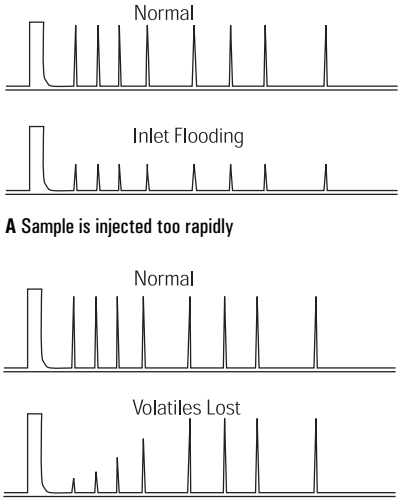


Figure 1. The PTV inlet shown with the septumless head. The inlet is also available with a septum head that may be equipped with a standard septum or a Merlin Microseal. (Figure reproduced with permission of Gerstel GMBH.)

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.



A Sample is injected too rapidly

B Solvent evaporates completely between injections

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).

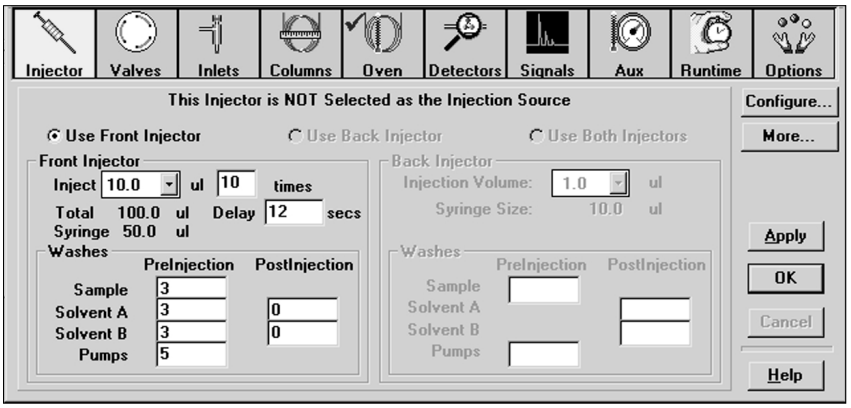


Figure 3. The injector screen from Agilent GC and GC/MS ChemStation software showing the setpoints available for multiple injections. To configure the sampler for multiple injections, set the syringe size, and choose slow injection, click on the Configure button.

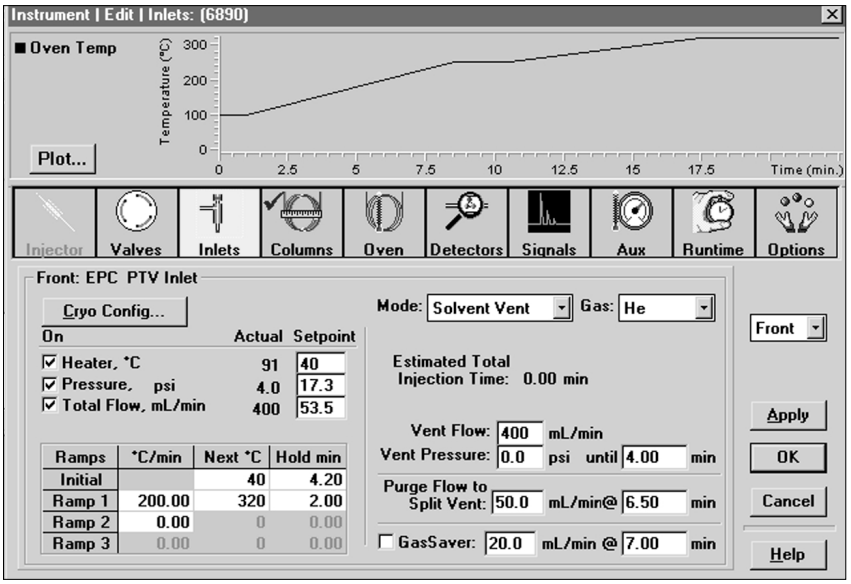


Figure 4. The inlets screen from Agilent GC and GC/MS ChemStation software showing the setpoints available for operation of the PTV inlet in the solvent vent mode.

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.

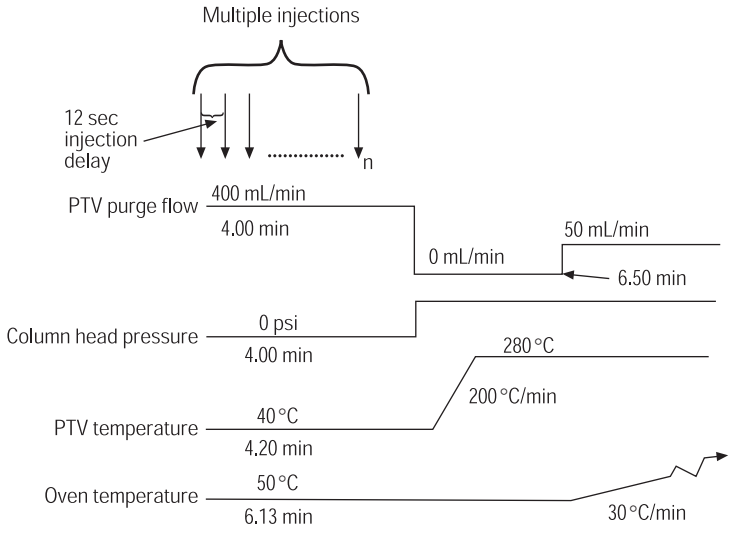
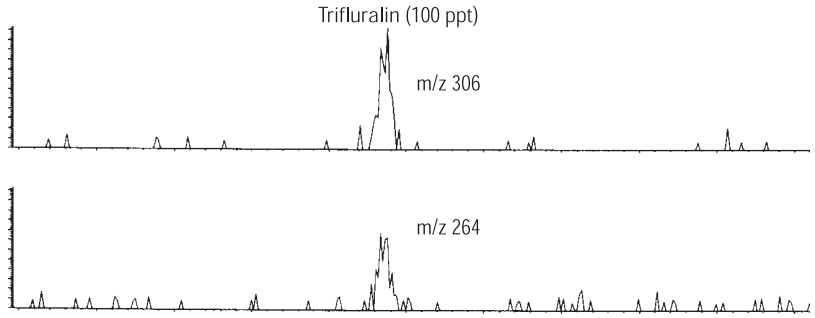


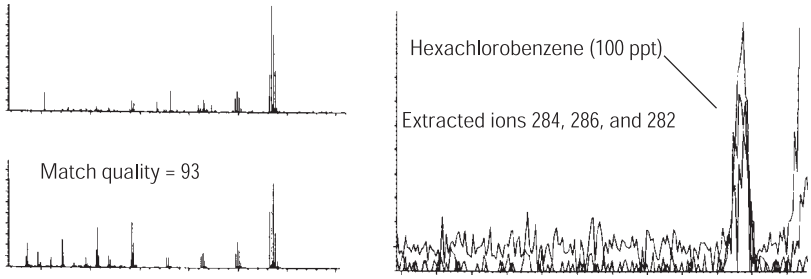
Figure 5. Illustration of the GC and sampler setpoints used for 100-µL injections of pesticide samples. Note that normally, the GC oven hold period would have been at least 6.5 min for this method. A value of 6.13 min pesticide retention times to a data base.

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.)

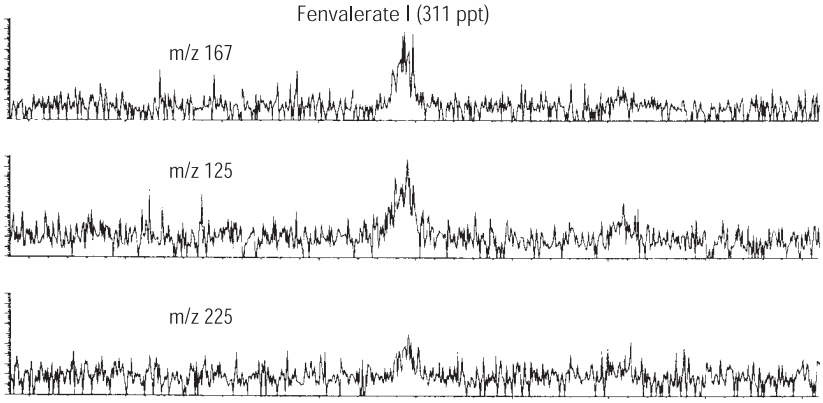


Figure 7. Extracted ion current chromatograms of Fenvalerate I at a concentration of 311 ppt in a pesticide standard. (Ten 10-µL injections were made using the PTV inlet.)

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.

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.

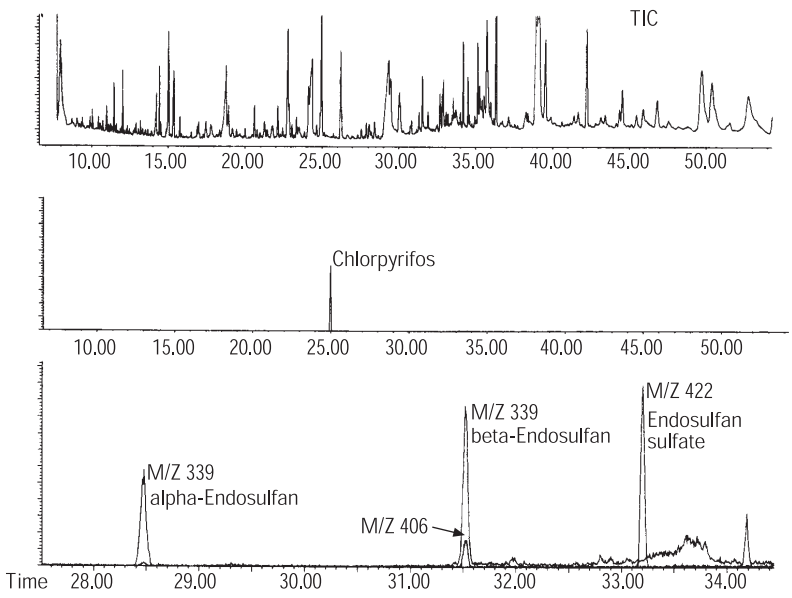


Figure 8. GC/MS Analysis of a bell pepper extract. (Ten 10-µL injections were made using the PTV inlet.) Using LVI, there was sufficient signal to use high mass ions with smaller abundances to achieve greater selectivity.

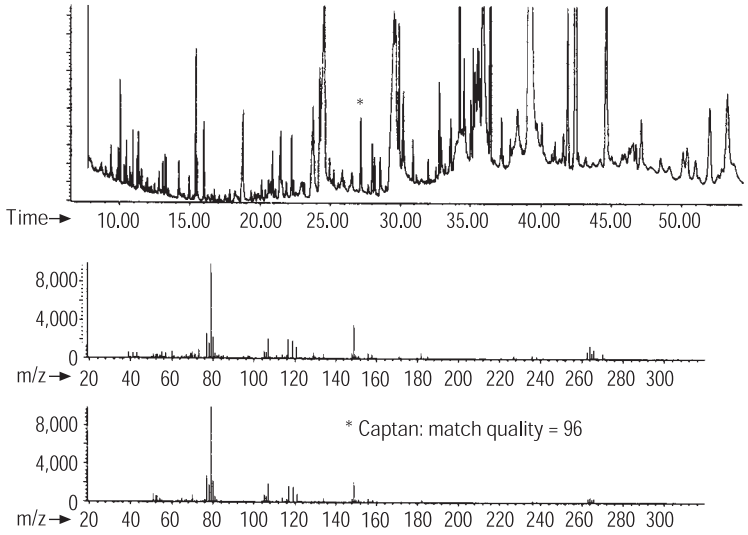


Figure 9. TIC of a pear extract resulting from a 100-µL Injection (10 x 10 µL). Captan was easily detected, and its spectrum gave a library match quality of 96.

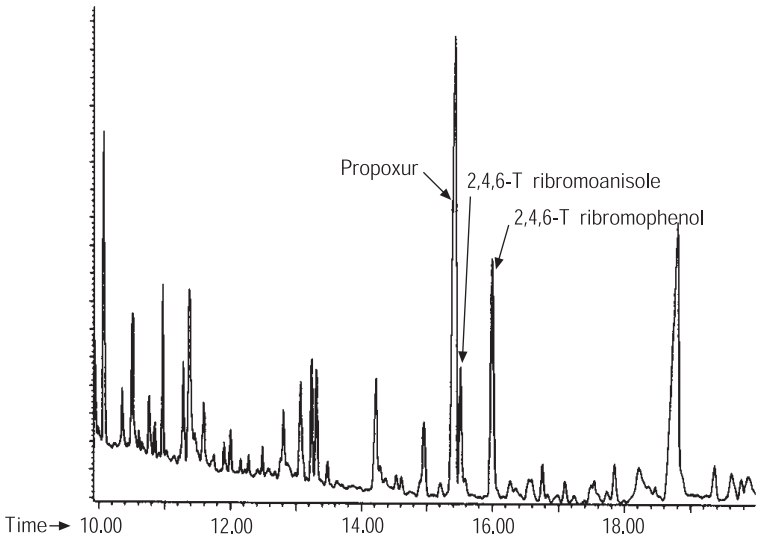


Figure 10. TIC of a pear extract resulting from a 100-µL Injection (10 x 10 µL). Propoxur and two brominated phenolics were easily identified.

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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Analysis of Fumonisin Mycotoxins by LC/MS

Application Brief

Agilent 1100 Series LC/MSD
Foods, Environmental

Friedrich Mandel

Introduction

The fungus *Fusarium*, which is known to infest corn and corn products, produces a group of mycotoxins called fumonisins. The toxicities of the most abundant fumonisins, $B_{1\text{-}3}$, have been extensively studied, and a variety of species-specific toxicities have been published. These compounds may be carcinogenic to humans. Fumonisins are characterized by a 19-carbon aminopolyhydroxyalkyl chain that is diesterified with propane-1-2, 3- tricarboxylic acid. Analogues $B_{1\text{-}3}$ show a difference in the number and position of the hydroxyl groups (Figure 1). Fumonisins B_2 and B_3 have the same molecular weight.

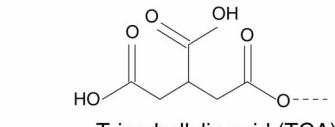
Most analytical methods exclude the detection of one or more of the known fumonisins. Traditional HPLC analysis requires the derivatization of the amino group. In this paper, we show that the Agilent 1100 Series LC/MSD can detect fumonisins without derivatization.

Experimental

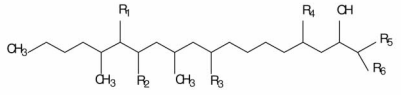
The system comprised of an Agilent 1100 Series binary pump, vacuum degasser, autosampler, thermostated column compartment, diode-array detector (DAD), and LC/MSD. The LC/MSD used electrospray ionization (ESI). Complete system control and data evaluation were done on the Agilent ChemStation for the LC/MSD.

Results and Discussion

The fumonisin analogues were analyzed in scan mode at a high concentration (25 ng) to determine the molecular ion and confirming fragments. The initial conditions showed the molecular ion $[M+H]^+$, but no significant fragment ions. Collision induced dissociation (CID) was used to generate more fragments for structural confirmation. Fumonisin B_2 and B_3, indistinguishable by their spectra, were easily separated chromatographically (Figure 2).



Tricarballylic acid (TCA)



	R1	R2	R3	R4	R5	R6	MW
B1	TCA	TCA	OH	OH	NH2	CH3	721.4
B2	TCA	TCA	H	OH	NH2	CH3	705.4
B3	TCA	TCA	OH	H	NH2	CH3	705.4

Figure 1. Structure of fumonisins.

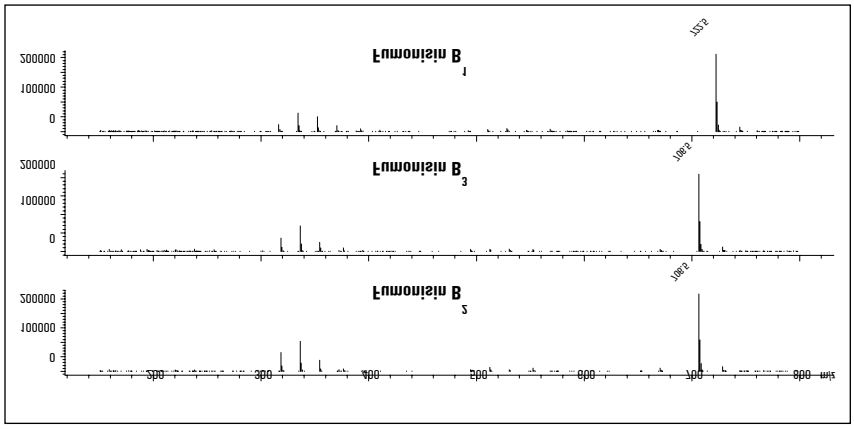


Figure 2. Mass spectra for fumonisin analogues.

The total ion chromatogram (TIC) shows very good sensitivity at 25 ng (Figure 3). To further improve sensitivity, the standards were run in the selected ion monitoring (SIM) mode.

Chromatographic Conditions

Column:	150 x 2.1 mm Zorbax Eclipse XDB, C18, 5 μm
Mobile phase:	A = 5 mM ammonium acetate in water, pH 3 B = acetonitrile
Gradient:	Start with 33% B at 8 min 60% B at 9 min 33% B
Flow rate:	250 μl/min
Injection vol:	5 μl
Column temp:	40°C
Diode-array detector:	Signal 220, 4 nm; reference 550,100 nm

MS Conditions

Source:	ESI
Ion mode:	Positive
Vcap:	4000 V
Nebulizer:	30 psig
Drying gas flow:	10 l/min
Drying gas temp:	350°C
Scan range:	120-820 amu
Step size:	0.1
Peak width:	0.15 min
Time filter:	On
Fragmentor:	Variable 230 V (100-680) 100 V (680-800)

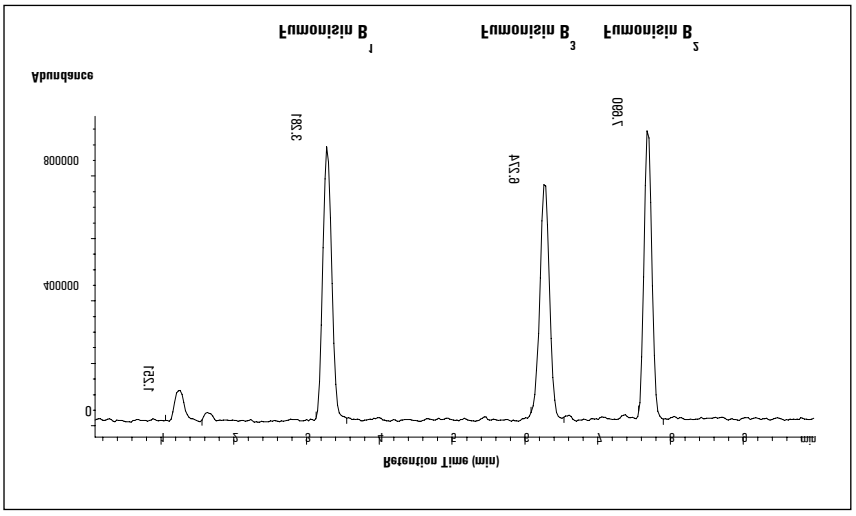


Figure 3. Chromatographic separation of fumonisin analogues at 25 ng.

Figure 4 shows the extracted ion chromatograms for 250 pg of fumonisins in a corn extract. The mass spectra showing the molecular and fragment ions provide high-confidence identification and quantification.

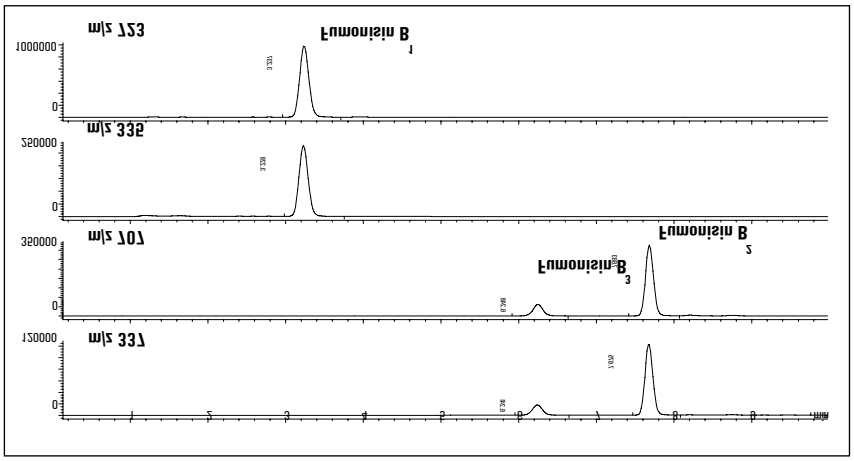


Figure 4. SIM of molecular and fragment ions for fumonisins in spiked corn extract.

Chromatographic Conditions

Column:	150 x 2.1 mm Zorbax Eclipse XDB, C18, 5 μm
Mobile phase:	A = 5 mM ammonium acetate in water, pH 3 B = acetonitrile
Gradient:	Start with 33% B at 8 min 60% B at 9 min 33% B
Flow rate:	250 μl/min
Injection vol:	5 μl
Column temp:	40°C
Diode-array detector:	Signal 220, 4 nm; Reference 550,100 nm

MS Conditions

Source:	ESI
Ion mode:	Positive
Vcap:	4000 V
Nebulizer:	30 psig
Drying gas flow:	10 l/min
Drying gas temp:	350°C
SIM ions:	at 0 min, 334.4, 352.4, 370.4, 722.5 at 5 min, 336.4, 354.4, 706.5
Step size:	0.1
Peak width:	0.15 min
Time filter:	On
Fragmentor:	Variable 230 V (334.5, 352.4, 370.4) 100 V (706.5, 722.5)

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Conclusion

The Agilent 1100 Series LC/MSD is capable of detecting fumonisins at low levels without derivatization. Mass spectrometry allows specific and sensitive detection in complex matrices such as corn extract.

Friedrich Mandel *is an application chemist at Agilent Technologies, Inc.*

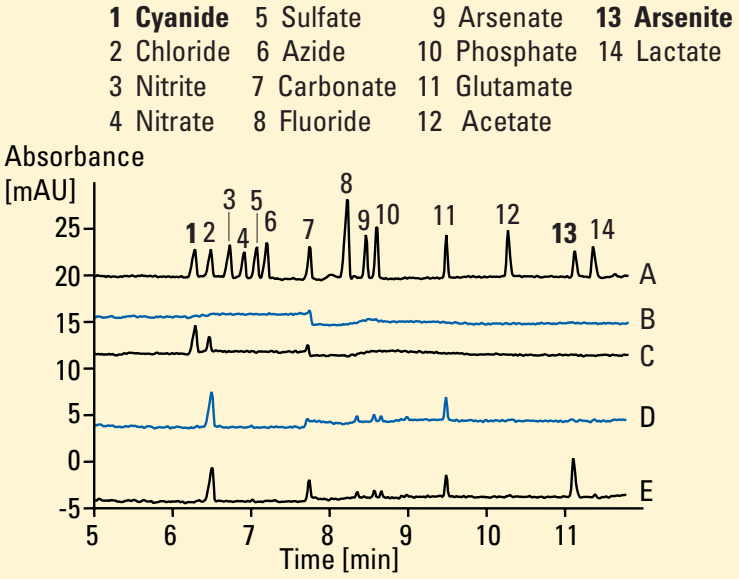
Printed in the U.S.A.
April 2000
(23) 5968-2124E

Analysis of Poisoned Food by Capillary Electrophoresis

Tomoyoshi Soga
and Maria Serwe

Food

Abstract
In cases of poisoning, analytical tools are needed to determine the identity of the toxins quickly and accurately. This enables healthcare professionals to administer appropriate treatment as quickly as possible and helps police to find those responsible. A rapid determination of anionic toxins in adulterated foods and beverages is possible using capillary electrophoresis (CE) with indirect UV detection. Cyanide, arsenite, arsenate, selenate, azide and other anions can be detected within 15 minutes, requiring only minimal sample preparation.



Conditions

Injection
6 s @ 50 mbar
Capillary
fused silica capillary
total length 112.5 cm
effective length 104 cm
internal diameter 50 µm
Buffer
Agilent Basic Anion Buffer
Voltage
-30 kV
Temperature
30 °C
Detection
signal 350/20 nm
reference 275/10 nm

Figure 1
Analysis of cyanide and arsenite in food.
A = anion standard (50 ppm each), B= Oolong tea (1:100 diluted with H_2O), C= Oolong tea as in B, spiked with 100 ppm NaCN, D=curry (1:100 diluted with H_2O, filtered through 0.22 µm filter), E=curry as in D, spiked with 100 ppm $NaAsO_2$

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Experimental

Anion analysis was performed using the Agilent Capillary Electrophoresis system equipped with diode-array detection and computer control via Agilent ChemStation. The analysis is based on the Agilent Forensic Anion Analysis Kit (part number 5064-8208).

Prior to first use, a new capillary was flushed with run buffer for 15 minutes (at 1 bar). Between the analyses the capillary was flushed 2 minutes from the OutHome vial into waste, then 2 minutes from the InHome vial into waste. This procedure avoids baseline fluctuations as a result of buffer depletion. Buffer vials were replaced after 10 runs when using 2 ml vials, after 5 runs, when using 1 ml vials.
Sample preparation consisted simply of dilution with water, or dilution and additional filtration through a 0.22 µm filter, as indicated in figure 1.

Results

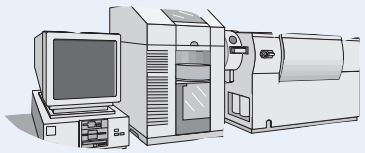
Figure 1 shows the analysis of food spiked with cyanide and arsenite. Depending on the results of this quick analysis, the sample can then undergo a more detailed analysis.

The assay was linear over the range 10–100 ppm with $r^2 > 0.999$. The method detection limit was 5–10 ppm. For the analysis of curry, the repeatability for arsenite (n = 6) was 0.06 % RSD for migration time and 2.7 % RSD for peak area. For cyanide in Oolong tea the respective values were 0.13 % RSD for migration time (n = 10) and 4 % for peak area (sample diluted in 0.01 N NaOH).

Other toxic anions that can be determined are arsenate, azide and selenate (which migrates between azide and carbonate). Compared to ion chromatography (IC), the advantages of CE for this type of analysis are the shorter analysis time and the minimal sample preparation needed for samples with a complex matrix (e.g. curry). Additionally, the analysis of azide and arsenate together with cyanide and arsenite is not possible in one run with IC.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent ChemStation
- Agilent Forensic Anion Analysis Kit



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Released 05/99
Publication Number 5968-5731E

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Fast Screening of Pesticide and Endocrine Disrupters Using the Agilent 6890/5973N GC/MSD System, Part I

Application

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Gas Chromatography
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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.

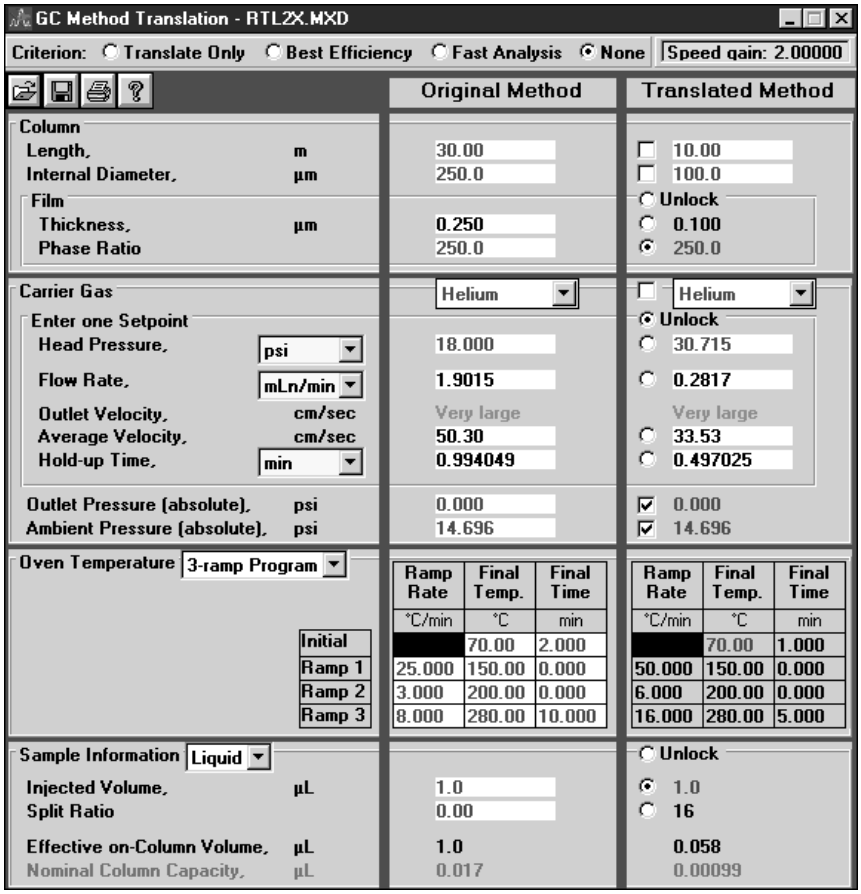

Figure 1. Screen capture showing the method translation (MTL) software data entry used in a twofold speed gain translation.

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.

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.

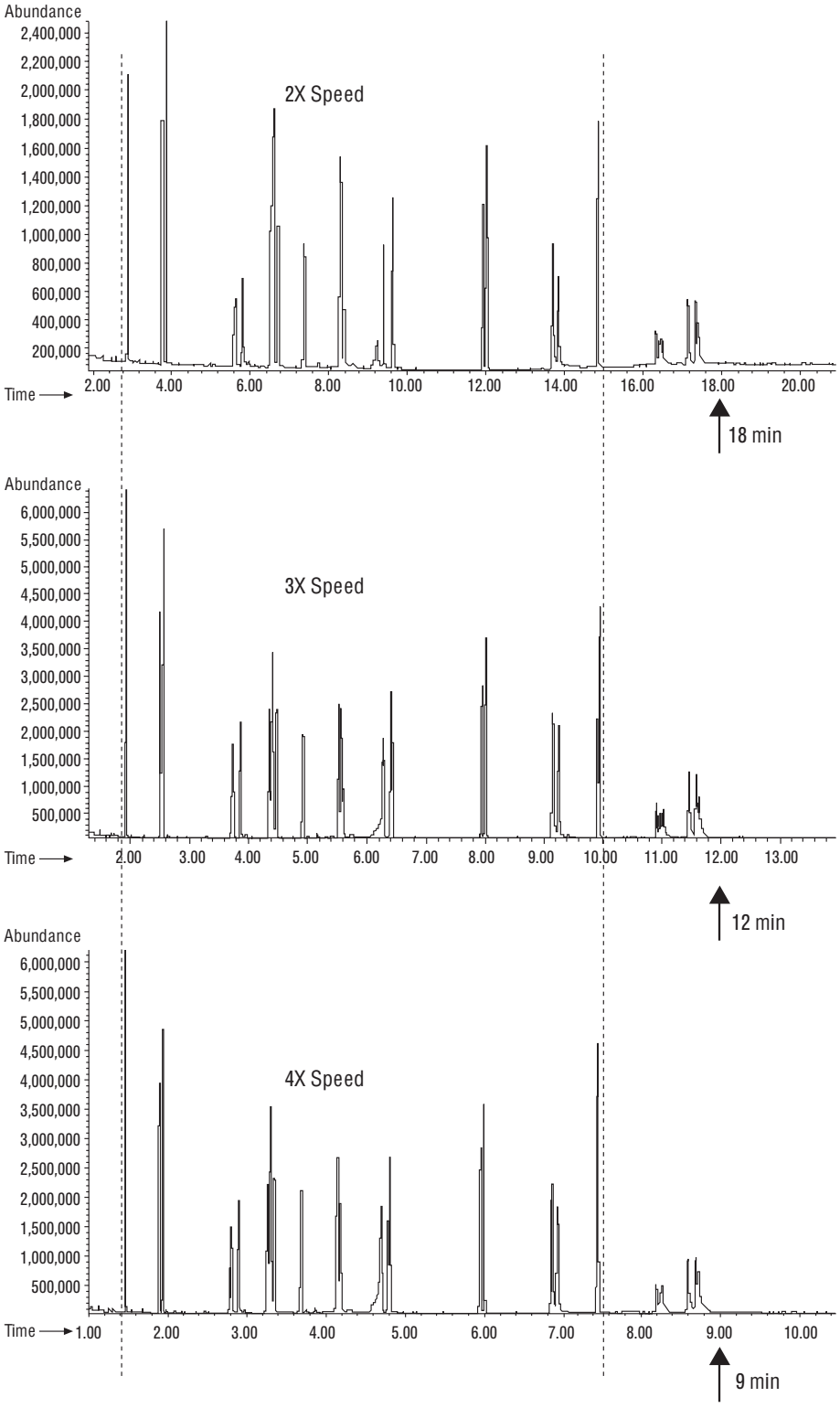

Figure 2. Three TICs of the 2X, 3X, and 4X speedups. The standard analysis (1X) was 42 minutes long. The two vertical lines on the figure are used as references to show the similarity of the TICs.

Printed in USA 2/00
5968-9220E

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Gas Chromatography/Mass Spectrometry Approaches to the Analysis of Acrylamide in Foods

Application

Food Safety

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Abstract

Discovery of acrylamide in cooked foods has required an examination of foods for potential exposure. A classic approach employs extracting acrylamide from the food with water and converting the acrylamide to brominated derivatives. These derivatives are described here in terms of their spectra and response in electron impact and positive chemical ionization. Additionally, a more direct and simple approach involving extraction and direct injection and analysis of acrylamide by positive chemical ionization is described. This screening approach is rapid, robust, and provides low detection limits.

Introduction

The discovery announced in April 2002 by scientists at Sweden's National Food Administration of acrylamide (2-propenamide) in fried and baked foods at levels many times that allowed in water suggested a much higher exposure than previously estimated [1-3]. Acrylamide (Figure 1), a known neurotoxin, is considered a probable human carcinogen. The World Health Organization considers 0.5 µg/L the maximum level for acrylamide in water. However, foods such as french fries, baked potato chips, crisp breads, and other common cooked foods, were found to contain acrylamide between 100 and 1000 µg/kg. Acrylamide was not found in the raw foodstuffs and cooking by boiling produced no detectable levels. Recent work has suggested that acrylamide forms via the Maillard reaction, which occurs when amino acids and sugars (for example, asparagine and sucrose) are heated together [4]. The concern over these relatively high concentrations has led to studies of the occurrence of acrylamide in a wide variety of foods.

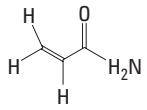


Figure 1 Acrylamide (2-propenamide), CH_2=CHCONH$_2$, 71.08 g/mole, CAS number 79-06-1.

Acrylamide Analytical Methodologies

A wide variety of instrumental approaches have been applied to acrylamide. Recent methods using

liquid chromatography with tandem mass spectrometry (MS-MS) detection have proved useful to approximately 50 µg/kg (ppb) or better using the 72 to 55 *m/z* transition (for example, [5]). This approach has appeared attractive in providing a simple sample preparation strategy. Gas chromatographic methods using MS detection with electron impact (EI) ionization typically suffer from the relatively small size of the molecule and therefore use derivatization. This application note presents alternative gas chromatography/ mass spectrometry (GC/MS) approaches aimed at more rapid screening, as well as the conventional, definitive quantitation via derivatization. These methods are rapid and relatively simple approaches to acrylamide analysis.

Rapid Screening via GC/MS-SIM with Positive Chemical Ionization

EI ionization mass spectrum for acrylamide (Figure 2) reveals very low mass ions; 71, 55, 44 *m/z*. Although there is good intensity at sub-ng levels, the ions are subject to interferences in food samples. The positive chemical ionization (PCI) spectrum achieved with ammonia provides more selective ionization and is of greater utility than EI in food matrices, Figure 3. Ammonia PCI results in two ions; 72 *m/z*, the protonated molecule, $[M+H]^+$, and 89 *m/z* due to the adduct, $[M+NH_4]^+$. PCI provides good selectivity and sensitivity for acrylamide–picogram amounts can be detected.

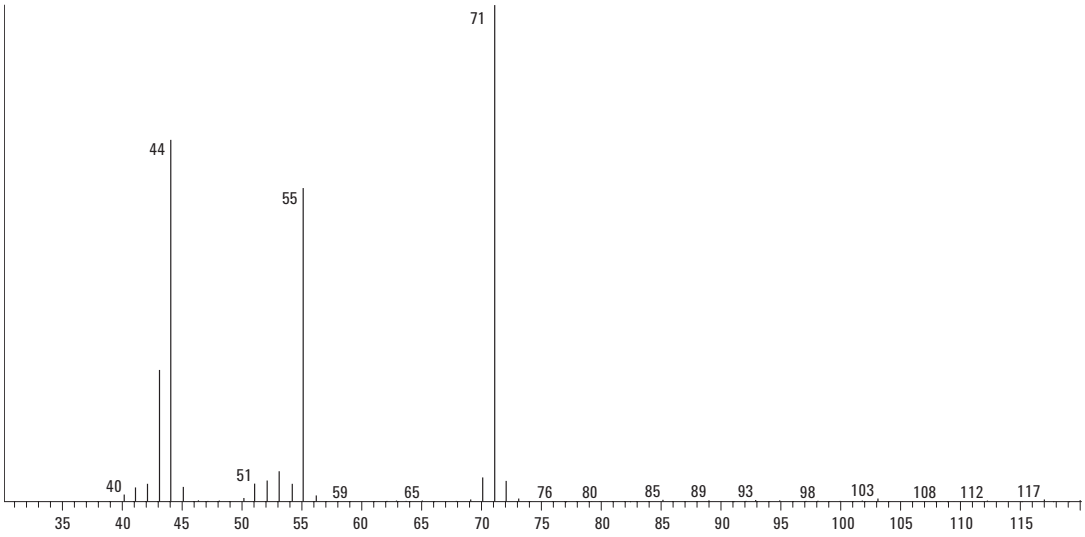


Figure 2 The EI ionization spectrum of acrylamide (40–120 amu).

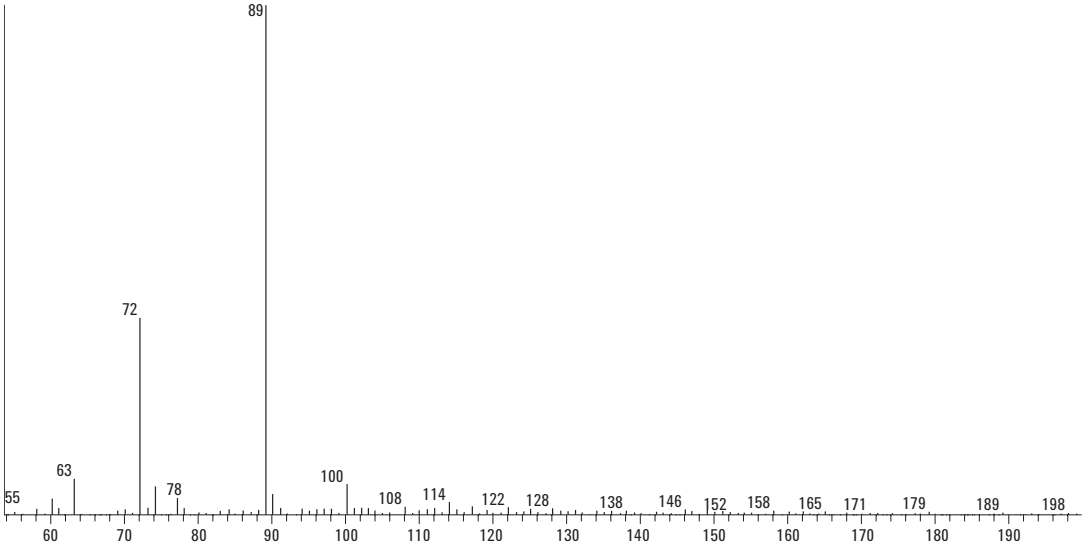


Figure 3 The PCI spectrum of acrylamide with ammonia reagent gas (60–200 amu).

Figure 4 shows a calibration curve from 100 pg to 10 ng collected under the method cited below in the section on Instrumental Parameters.

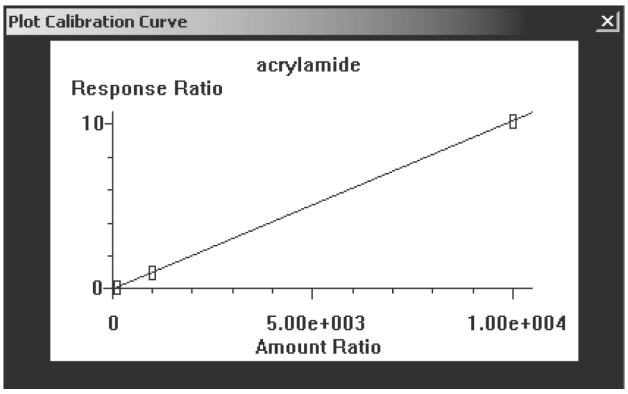


Figure 4 PCI-ammonia SIM calibration curve from 100-picograms to 10 ng ($R^2 = 1.00$).

Screening Sample Preparation

The enhanced specificity obtained through PCI can be used for rapid screening using a very simple and rapid sample cleanup. A food sample is homogenized and pulverized, and 0.4-g subsample is transferred to a centrifuge tube. The sample is extracted with 1 mL of methanol:water (9:1 v/v) solution for 10 minutes using an ultrasonic cleaner. Prior to sonication, 1 µg of labeled $^{13}C_3$-acrylamide is added to the 1-mL solution. After sonication, the sample is centrifuged for about 5 minutes at 8000 rpm. The upper layer is decanted and transferred to a vial for injection and analysis by GC/MS-PCI conditions with selected ion monitoring (SIM). See Table 1 for method parameters for PCI screening of native acrylamide.

Table 1. GC/MS Instrumental Method Parameters for PCI Screening of Native Acrylamide

Inlet parameters		
Liner:		Agilent p/n 5062-3587 Single-taper with glass wool
Temperature:		220 °C
Mode:		Pulsed splitless
Pulse pressure:		30.0 psi
Pulse time:		1.20 min
Purge flow:		50.0 mL/min
Purge time:		1.20 min
Total flow:		54.7 mL/min
Gas saver:		Off
Oven parameters		
Oven maximum:		260 °C
Oven equilibrium time:		0.20 min
Initial temperature:		60 °C
Initial time:		1.00 min
Ramp	Temperature	Time
12 °C/min	230 °C	10.00 min
Run time:		25.17 min
Column parameters		
Capillary column		Agilent 19091X-136 HP-INNOWax
Maximum temperature:		260 °C
Nominal length:		60.0 m
Nominal diameter:		250.00 µm
Nominal film thickness:		0.25 µm
Carrier:		Helium
Mode:		Constant flow 2.0 mL/min
Outlet and pressure:		MSD Vacuum
MSD Parameters		
Solvent delay		7.00 min
Tuning:		PCI Ammonia at 24% (1.2 mL/min)
EM Setting:		PCI Autotune + 400 V
Source temperature:		250 °C
Quad temperature:		150 °C
SIM Parameters		
Resolution:		High
Group ions		Dwell (ms)
72.0		60
75.0		60
89.1		60
92.1		60

Screening Method Results and Discussion

Figure 5 shows the extracted ion chromatograms for a sample of white bread. The baseline shows very little disturbance near the acrylamide analyte due to the selective nature of the PCI with ammonia. The extracted concentration is approximately 34 ng/mL or 85 ng acrylamide per gram white bread. Since acrylamide is formed when amino acids and sugars are heated together, it is logical to suspect the possibility of acrylamide formation in the inlet during injection. To test this possibility, the white bread extract was spiked with 100 ng of acrylamide and reanalyzed. The results calculated 135 ng/mL and suggest that either the relatively low temperature and short duration in the liner due to pressure pulsing mitigate acrylamide formation for this sample or acrylamide formed in the inlet is highly reproducible. This may not be the case in all extracts or under all similar conditions.

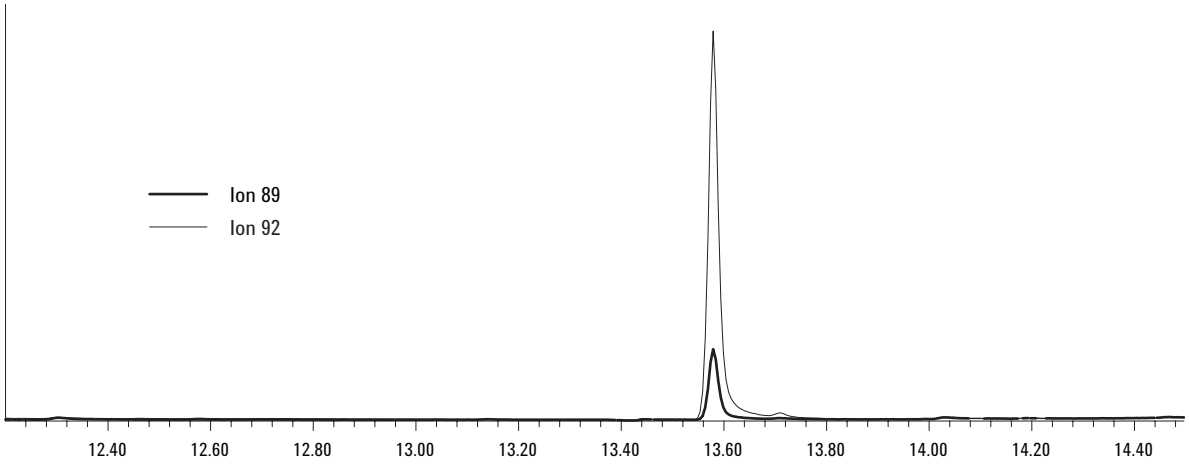
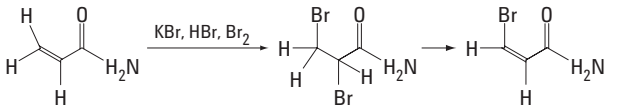


Figure 5. Extracted ion chromatograms for acrylamide (84 ng/g) in sample of white bread.

GC/MS Approaches to Acrylamide Involving Derivatives

Another approach to extraction from foods uses water, in situ derivatization, and liquid-liquid extraction [6, 7]. In this approach acrylamide in a homogenized sample is extracted with (hot) water, 1 g : 10 mL. A strong brominated agent is added and allowed to react. This reaction converts acrylamide to the 2,3-dibromopropionamide. Excess brominating reagent is removed by addition of sodium thiosulfate and the solution centrifuged and/or filtered. The 2,3-dibromopropionamide is extracted by partitioning into ethyl acetate. An option is to further treat this derivative to form a more stable analyte, the 2-bromopropenamide. The overall chemistry is given in Equation 1. Methacrylamide, CH_2=CH(CH_3)$CONH_2$, is frequently used as a recovery surrogate so its behavior is also reported here.



2,3-dibromopropionamide
$C_3H_5Br_2NO$
mol. wt. 230.9 g/mole

2-bromopropenamide
C_3H_4BrNO
mol. wt. 150.0 g/mole

Equation 1

Experimental

Acrylamide and methacrylamide were obtained as neat standards (Sigma-Aldrich Corp) and dissolved in HPLC grade methanol. Labeled acrylamide, 1,2,3-$^{13}C_3$-acrylamide, was obtained at 1 mg/mL methanol (Cambridge Isotope Laboratories, Andover, MA). The brominating reagent solution was made according to the literature [6] with reagent grade KBr, HBr, and bromine water (VWR, San Francisco,CA). Sodium thiosulfate was obtained as a 1-Normal solution (VWR, San Francisco,CA).

Derivatization also followed the literature [6] with addition of 1 mL of brominating reagent to solutions containing acrylamide; over-night derivatization, neutralization by 1-drop 1N sodium thiosulfate and extraction by 1-mL ethyl acetate (pesticide grade, VWR). The dibromo-derivatives were directly injected. The mono-bromo-derivatives were generated by addition of triethylamine.

Instrumental conditions for the dibromopropionamide and bromopropenamide are cited in Tables 2 and 3. All data was collected using 2-µL injections.

Table 2. GC/MS Instrumental Method Parameters for Dibromopropionamide (Dibromo-Derivative of Acrylamide) in EI and PCI with Methane and Ammonia

Inlet parameters		
Liner:		Agilent p/n5181-3315 double-taper
Temperature:		250 °C
Mode:		Pulsed splitless
Pulse pressure:		30.0 psi
Pulse time:		1.20 min
Purge flow:		50.0 mL/min
Purge time:		1.20 min
Total flow:		54.7 mL/min
Gas saver:		On
Oven parameters		
Oven maximum:		325 °C
Oven equilibrium time:		0.50 min
Initial temperature:		50 °C
Initial time:		1.00 min
Ramp	Temperature	Time
45 °C/min	300 °C	2.00 min
Run time:		8.56 min
Column parameters		
Capillary column		Agilent 122-3832 DB-35 ms
Maximum temperature:		340 °C
Nominal length:		30 m
Nominal diameter:		250 µm
Nominal film thickness:		0.25 µm
Carrier:		Helium
Mode:		Constant flow 1.2 mL/min
Outlet and pressure:		MSD Vacuum
MSD Parameters for EI and PCI		
Solvent delay		5.00 min
EI Parameters		
EI Tuning:		Autotune
EM Setting:		Autotune + 400 V
Source temperature:		230 °C
Quad temperature:		150 °C
EI SIM parameters		
Resolution:		Low

(Continued)

Table 2. GC/MS Instrumental Method Parameters for Dibromopropionamide (Dibromo-Derivative of Acrylamide) in EI and PCI with Methane and Ammonia (Continued)

Group ions	Dwell (ms)
2,3-dibromopropionamide	Acrylamide analyte
149.9	10 ms
151.9	10 ms
106.0	10 ms
$^{13}C_3$-2,3-dibromopropionamide	Internal standard
152.9	10 ms
154.9	10 ms
109.9	10 ms
2,3-dibromo-2-methylpropionamide	Ancillary surrogate
120.0	10 ms
122.0	10 ms
164.0	10 ms
166.0	10 ms
PCI Parameters	
PCI Tuning:	PCI Autotune
EM Setting:	PCI Autotune + 400 V
Source temperature:	250 °C
Quad temperature:	150 °C
PCI SIM Parameters	
Methane reagent gas:	MFC 20% (1.0 mL/min)
Resolution:	Low
Group ions	Dwell (ms)
2,3-dibromopropionamide	Acrylamide analyte
231.9	10 ms
233.9	10 ms
149.9	10 ms
151.9	10 ms
$^{13}C_3$-2,3-dibromopropionamide	Internal standard
234.9	10 ms
236.9	10 ms
2,3-dibromo-2-methylpropionamide	Ancillary surrogate
245.9	10 ms
247.9	10 ms
Ammonia reagent gas:	MFC 20% (1.0 mL/min)
Resolution:	Low
Group ions	Dwell (ms)
2,3-dibromopropionamide	Acrylamide analyte
248.9	10 ms
246.9	10 ms
250.9	10 ms
$^{13}C_3$-2,3-dibromopropionamide	Internal standard
251.9	10 ms
249.9	10 ms
253.9	10 ms
2,3-dibromo-2-methylpropionamide	Ancillary surrogate
262.9	10 ms
260.9	10 ms
264.9	10 ms

Table 3. GC/MS Instrumental Method Parameters for 2-bromopropenamide (Monobromo-Derivative of Acrylamide) in EI

Inlet parameters	
Liner:	Agilent p/n 5062-3587 Single-taper with glass wool
Temperature:	250 °C
Mode:	Pulsed splitless
Pulse pressure:	30.0 psi
Pulse time:	1.20 min
Purge flow:	50.0 mL/min
Purge time:	1.20 min
Total flow:	54.7 mL/min
Gas saver:	Off
Oven parameters	
Oven maximum:	325 °C
Oven equilibrium time:	0.50 min
Initial temperature:	50 °C
Initial time:	1.00 min
Column parameters	
Capillary column	Agilent 122-5533 DB-5MS

Ramp	Temperature	Time
25 °C/min	140 °C	0.00 min
45 °C/min	300 °C	1.50 min
Run time:		9.66 min

Maximum temperature:	350 °C
Nominal length:	30.0 m
Nominal diameter:	250 µm
Nominal film thickness:	1.00 µm
Carrier:	Helium
Mode:	Constant flow
1.2 mL/min	
Outlet and pressure:	MSD Vacuum
MSD Parameters for EI and PCI	
Solvent delay	5.00 min
EI Parameters	
EI Tuning:	Autotune
EM setting:	Autotune + 400V
Source temperature:	230 °C
Quad temperature:	150 °C
EI SIM Parameters	
Resolution:	Low
Group ions	Dwell (ms)
2-bromopropenamide	Native acrylamide
148.9	20 ms
150.9	20 ms
105.9	20 ms
$^{13}C_3$-2-bromopropenamide	Internal standard
151.95	20 ms
153.95	20 ms
2,3-dibromo-2-methylpropionamide	Ancillary surrogate
120.0	10 ms
122.0	10 ms
164.0	10 ms
166.0	10 ms

Results and Discussion

EI Ionization

Figures 6 and 7 show the EI mass spectrum of the 2,3-dibromopropionamide and the 2-bromopropenamide, respectively. Note the similar spectra for the two brominated acrylamide derivatives. In EI, the 2,3-dibromopropionamide loses bromide to generate the C_3H_5ONBr ion that shows the isotopic abundance expected from a monobrominated species. The addition of the triethylamine (base) leads to loss of HBr in solution, generating the monobrominated species C_3H_4ONBr which contains one less hydrogen than the dibromo-derivative and appears as the molecular ion in EI. The spectra share a common C_2H_3Br ion that accounts for the fragments at 105.9 and 107.9 *m/z*. Note that use of the $^{13}C_3$-acrylamide as an internal standard prohibits use of the 107.9 ion in acrylamide quantitation due this C_2H_3Br fragment. The dibromo-derivative shows greater response than the monobrominated compound and lacks the 149 fragment which is subject to interferences from phthalates which are ubiquitous in solvents and food packaging. Both compounds demonstrate good linearity over the range of 10 to 500 pg/µL in EI-SIM as shown in Figures 8 and 9, but better EI detection and elution at a higher oven temperature makes the dibromo-derivative more attractive than the monobromo-derivative. However, it has long been known that the 2,3-dibromopropionamide breaks down in the injection port to form the 2-bromopropenamide. The fraction converted is a function of the injection port activity hence the use of the double-tapered liner for the dibromopropionamide analysis as opposed to the single-tapered liner with wool for the bromopropenamide. Use of the ^{13}C-labeled surrogate is necessary to correct for the degradation of the dibromo-derivative but the methacrylamide surrogate may correct fairly well for recoveries of the mono-brominated acrylamide. Because of this and citations of its use in the literature, the EI spectrum for the brominated methacrylamide is shown in Figure 10 and ions are presented in the acquisition method tables. As the 2,3-dibromo-2-methylpropionamide, this surrogate elutes just prior to the 2,3-dibromopropioamide and much later than the 2-brompropenamide on the GC programs cited.

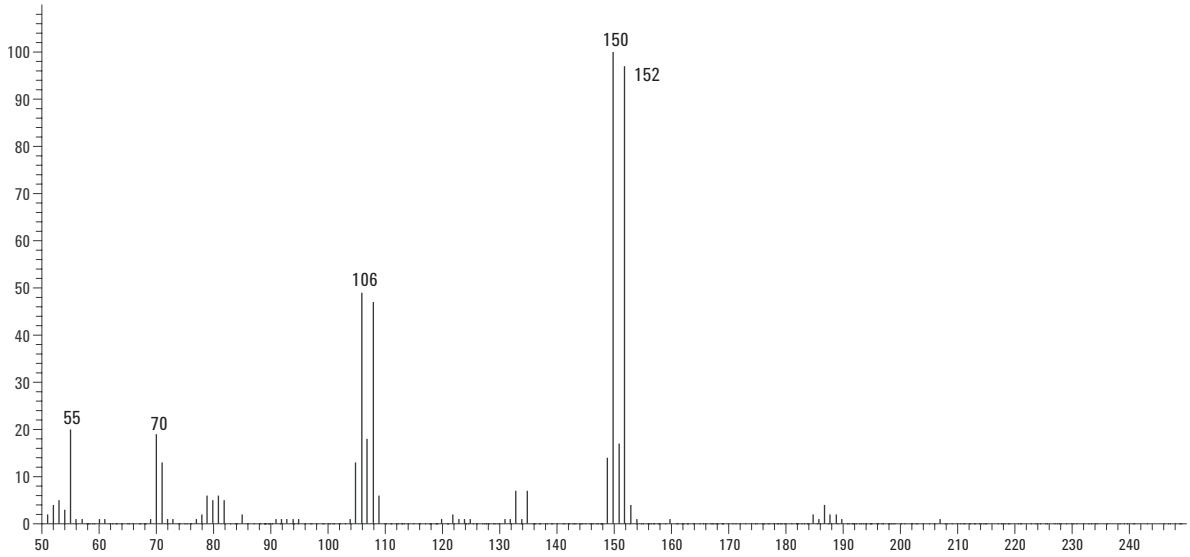


Figure 6. EI ionization spectrum of 2,3-dibromopropionamide.

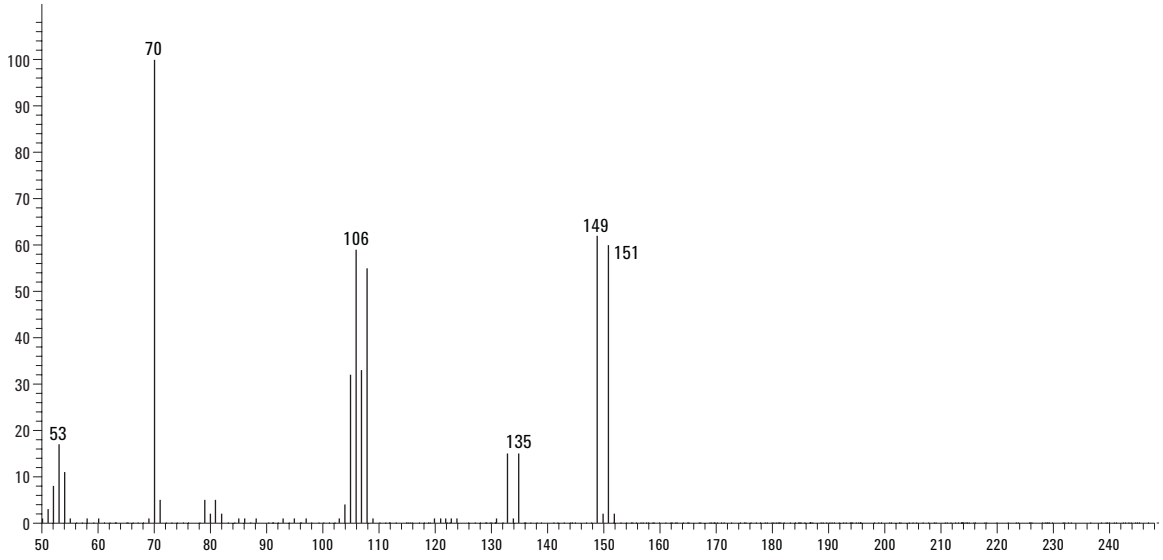


Figure 7. EI ionization spectrum of 2-bromopropenamide.

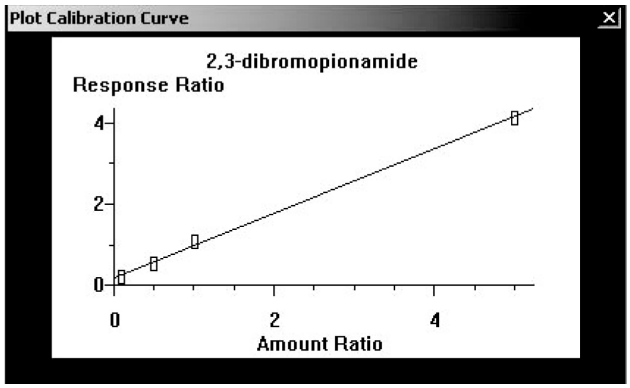


Figure 8. Calibration Curve plot for 2,3-dibromopropionamide from 10 to 500 pg/µL ($R^2 = 0.998$).

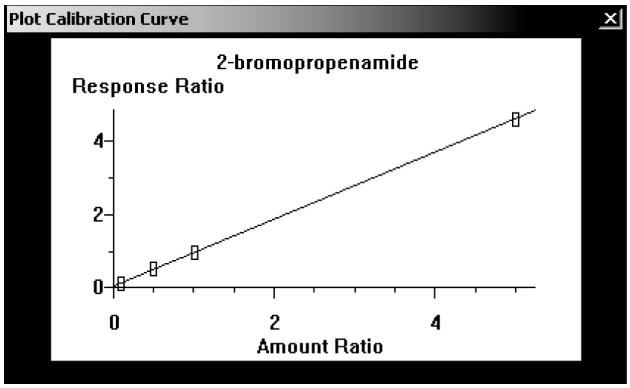


Figure 9. Calibration Curve plot for 2-bromopropenamide from 10 to 500 pg/µL ($R^2 = 0.999$).

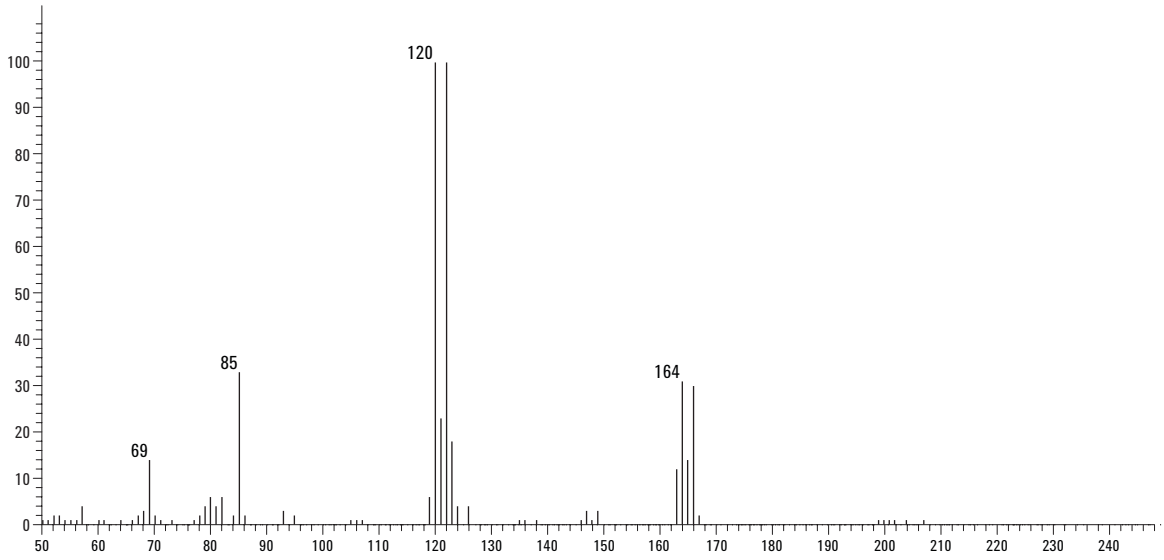


Figure 10. EI ionization spectrum of the alternative, methacrylamide surrogate, 2,3-dibromo-2-methylpropionamide.

PCI

The 2,3-dibromopropionamide spectra in PCI with methane and ammonia reagent gas are shown in Figures 11 and 12. In methane, the highest mass fragment is due to $[M+H]^+$ and in ammonia, $[M+NH_4]^+$. Response with methane is higher than with ammonia and would make a good choice in acrylamide quantitation in samples, if background for that particular food are not an issue. Calibration is similar to that in EI between 10 and 500 pg/µL for both methane and ammonia (R^2 >0.998). It is important that the lower mass fragments that occur in methane and ammonia PCI, *m/z* 72 and 89, respectively, are not used in SIM quantitation. These intense fragments apparently originate through elimination of Br_2 and do not coincide with the cited ions.

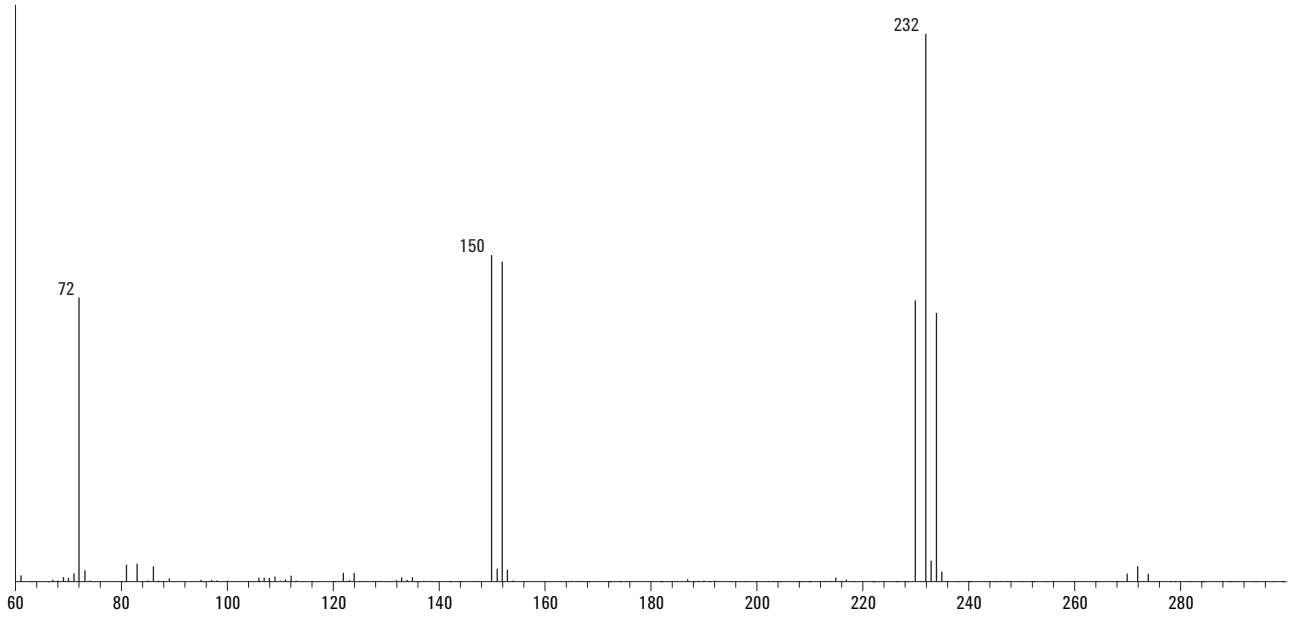


Figure 11. The PCI spectrum of 2,3-dibromopropionamide with methane reagent gas (60–300 amu).

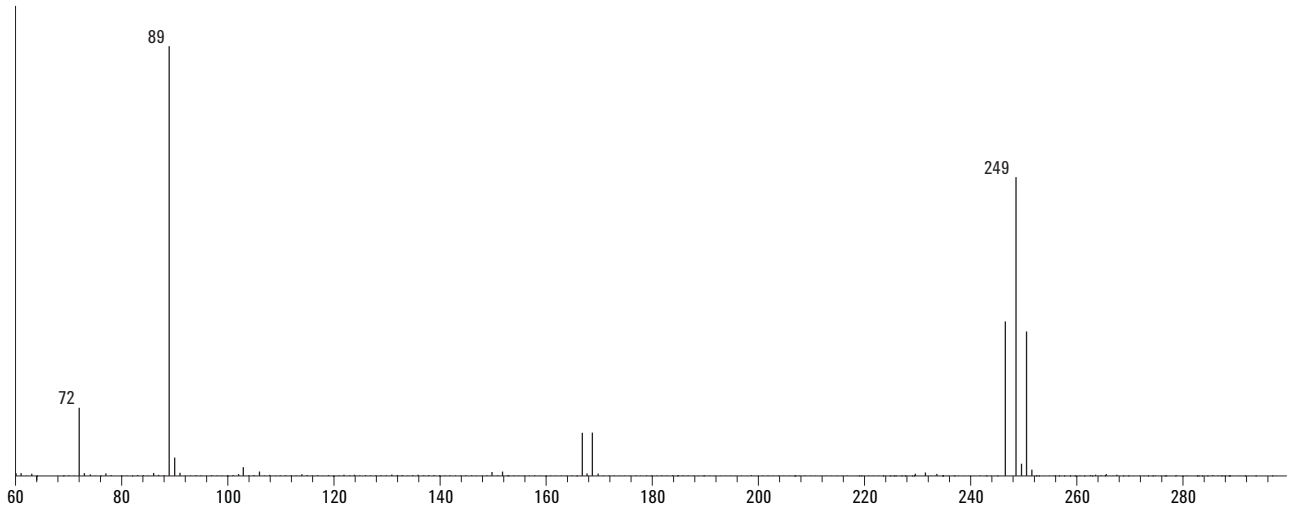


Figure 12. The PCI spectrum of 2,3-dibromopropionamide with ammonia reagent gas (60–300 amu).

Similar to the situation in EI, PCI response of the 2,3-dibromopropionamide exceeds that of the 2-bromopropenamide under either reagent gas. Spectra for this analyte using methane and ammonia are presented in Figures 13 and 14. Highest mass fragments for 2-bromopropenamide also are due to $[M+H]^+$ in methane and in ammonia, $[M+NH_4]^+$. For completeness, the spectra are also included for the brominated methacrylamide surrogate, Figure 15 and 16.

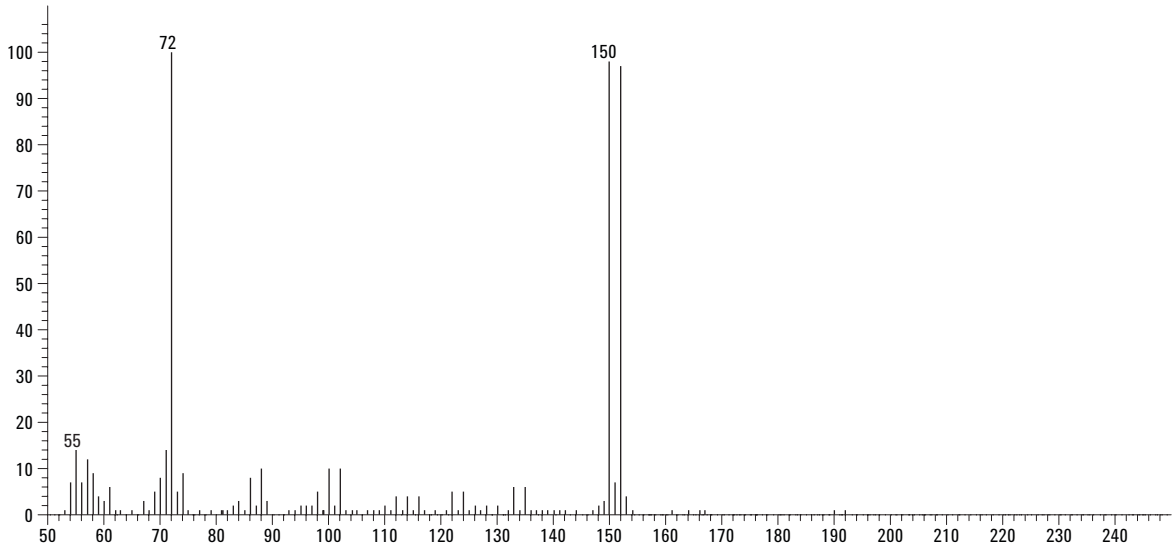


Figure. 13 The PCI spectrum of 2-bromopropenamide with methane reagent gas (50–250 amu).

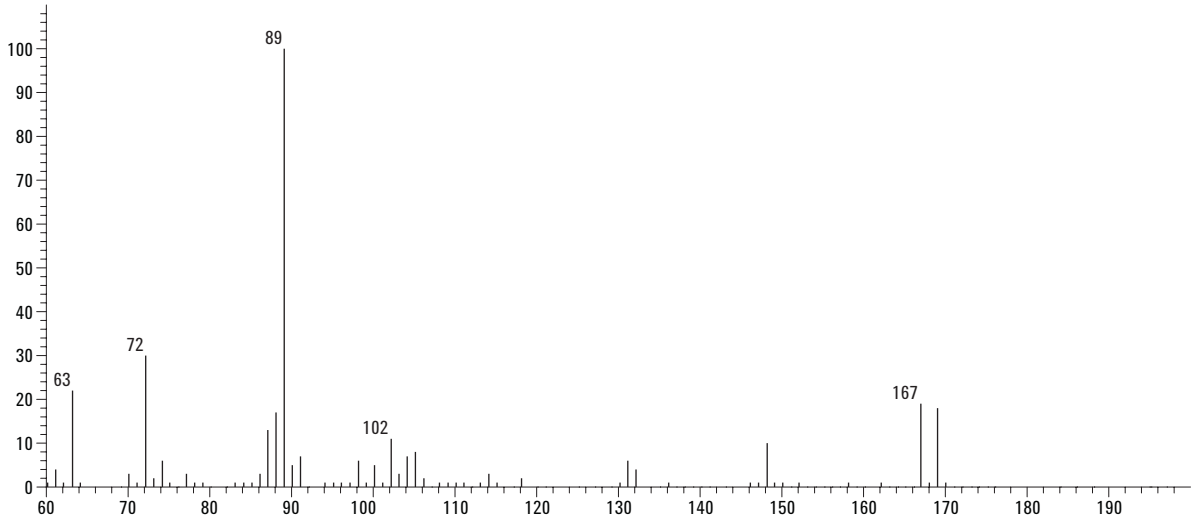


Figure 14. The PCI spectrum of 2-bromopropenamide with ammonia reagent gas (60–200 amu).

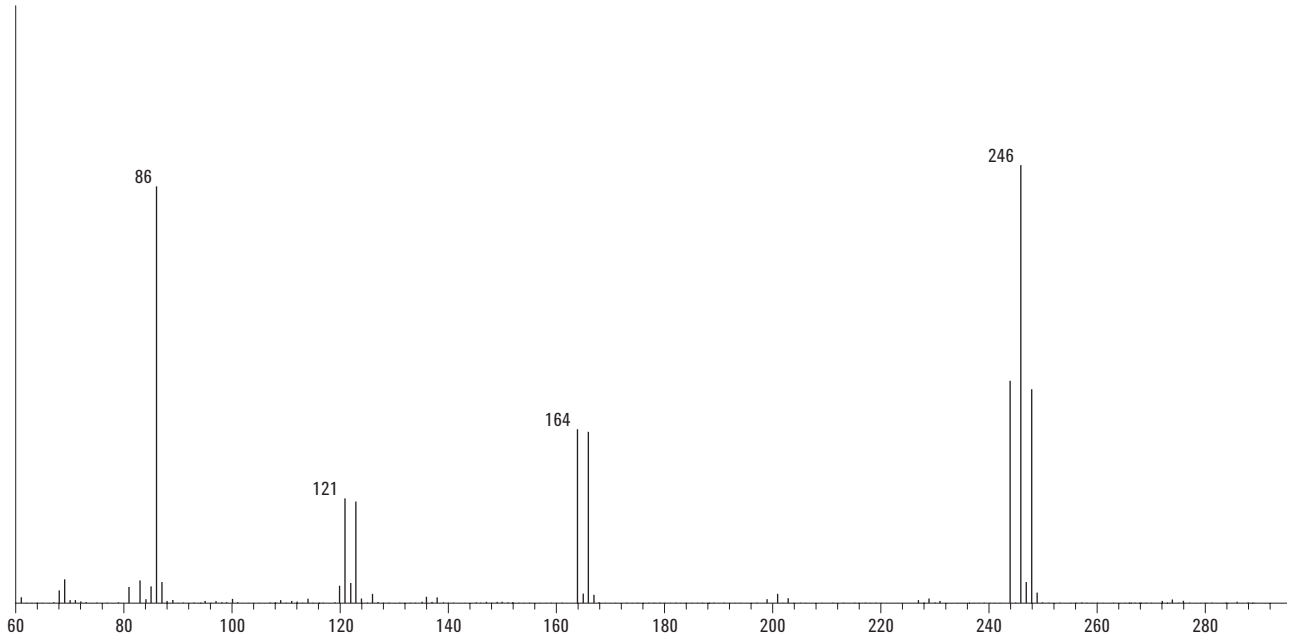


Figure 15. The PCI spectrum of 2,3-dibromo-2-methylpropionamide (the methacrylamide derivative) with methane reagent gas (60–300 amu).

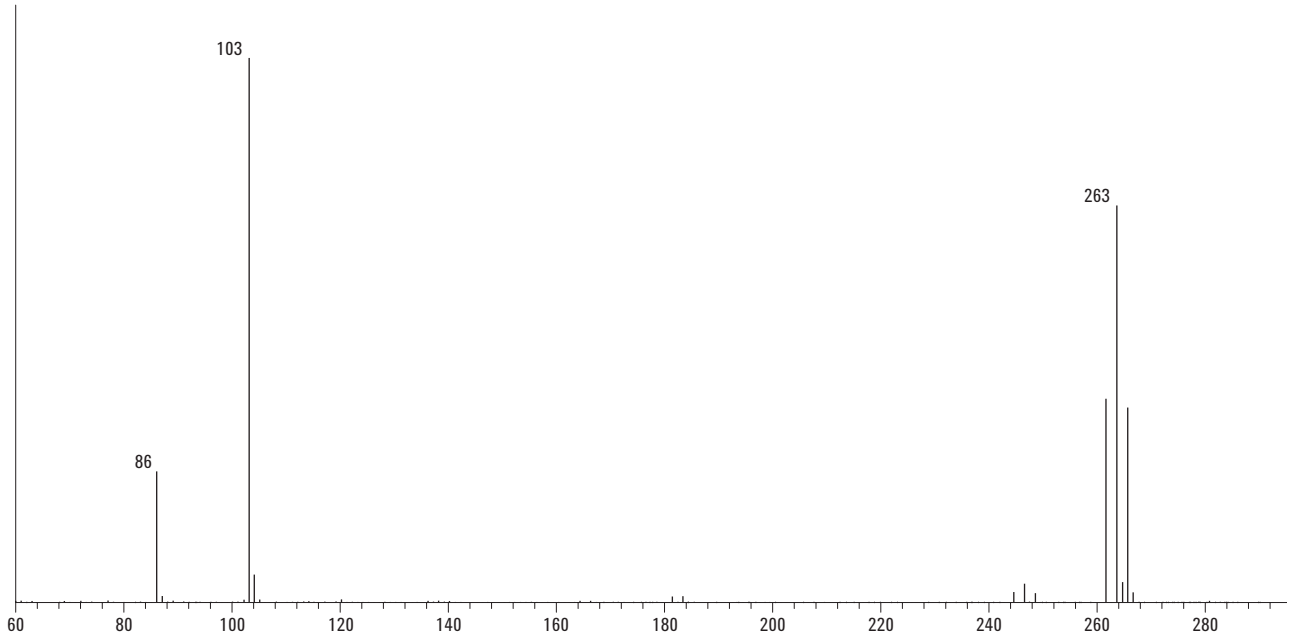


Figure 16. The PCI spectrum of 2,3-dibromo-2-methylpropionamide with ammonia reagent gas.

Conclusions

Since acrylamide was found in a wide range of foodstuffs, a variety of approaches were presented here. The rapid screening approach for native acrylamide using PCI provides a direct and simple method for sensitive detection and quantitation. For approaches using the brominated derivatives, the dibromopropionamide shows superior opportunities for detection and quantitation relative to the 2-bromopropenamide. If, for a particular food product, there are problems in EI, PCI will provide a worthwhile approach for exploration. Methane reagent gas provides about twice the response of ammonia. The degradation of the dibromopropionamide can and must be accounted for by an appropriate labeled internal standard. The methacrylamide surrogate also may be useful for recovery calculations. Data collected on potato chips, and not presented here, suggests this is the case.

References

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Printed in the USA
January 15, 2004
5989-0602EN

Agilent Technologies

A Blind Study of Pesticide Residues in Spiked and Unspiked Fruit Extracts Using Deconvolution Reporting Software

Application

Food

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Abstract

The Agilent Technologies mass selective detector (MSD) coupled with deconvolution reporting software (DRS) provides additional powerful data processing capabilities to the MSD ChemStation software. Reviewing full scan gas chromatography/mass spectrometry data for the confirmation of pesticide residues can be a labor-intensive and time-consuming process requiring great skill and concentration by an experienced analyst. The DRS is able to process a complex food extract total ion chromatogram in about 1 minute, whereas an experienced analyst may take more than 30 minutes to achieve the same quality result. Extensive data shown in this report supports the high confidence level that an analyst can have in results rapidly produced by the DRS.

Introduction

Typical mass spectral pesticide residue analysis requires finding target ions and meeting qualifier ion ratios. It is sometimes very difficult to confirm target compounds from high matrix background because the matrix affects the ion ratios of the target compounds or complicates the spectrum with additional ions. To be certain of the results, background subtraction and manual integration are often practiced. It is, therefore, a time-consuming process to confirm target compounds in a dirty matrix. It can take an experienced analyst 15 to 30 minutes to review/confirm one data file.

Two powerful gas chromatography/mass spectrometry (GC/MS) techniques - Retention Time Locking (RTL) and deconvolution were combined to create a quantitation and screening tool that can identify 567 pesticides and endocrine disrupters from a single run in 1–2 minutes. The Agilent Technologies GC/MSD-DRS provides the additional functionality to the MSD ChemStation.

Experimental

DRS Overview

A detailed overview of the DRS is given in an application note 5989-1157EN [1], available for download at www.agilent.com/chem. The operating principles of the DRS appear in Figure 1.

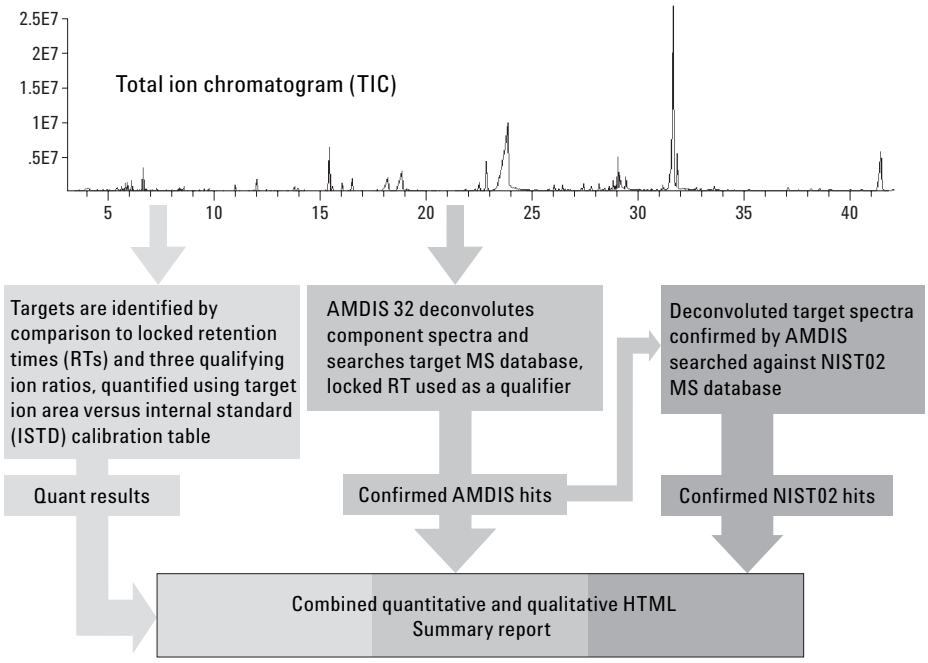

Figure 1. Schematic diagram summarizing the GC/MS DRS.

The quantitation capabilities of the MSD ChemStation are combined with the deconvolution power of the industry standard AMDIS program from NIST. AMDIS is able to separate spectra of interest from dirty matrix spectra present in samples analyzed for pesticides. A third level of confidence is obtained by sending the deconvoluted spectra for library searches of the NIST02 145,000 compound library. A comprehensive report is produced in about 1 minute.

Samples

Six samples of fruit extracts, supplied in 90/10 iso-octane/toluene solvent were received for analysis by GC/MS. The samples were prepared by an accredited food pesticide laboratory based in Scandinavia. Three of the samples were spiked with a number of pesticides at varying concentration levels. Although the range of concentrations of the pesticides in each sample was given, **neither the actual number of pesticides** spiked into each control sample **nor the identities were** supplied. Details of the samples appear in Table 1. The other three samples were 'real', unspiked extracts.

Table 1. Sample Details for Blind Study

Sample number	Matrix extracted	Number of pesticides	Concn range (mg/Kg)	Comments
1	Orange	20–40	0.02–0.20	Control sample - spiked
2	Lettuce	20–40	0.02–0.20	Control sample - spiked
3	Apple	20–40	0.01–0.20	Control sample - spiked
4	Grapes	2–4	0.1–1.0	Real sample
5	Orange	2–4	0.2–5.0	Real sample
6	Apple	2–4	0.05–2.0	Real sample

Instrumentation

The samples were analyzed by full-scan GC/MS using the analytical conditions given in Table 2. Data processing and reporting were performed using the default settings provided with the DRS.

Table 2. RTL GC/MS Analysis Conditions for Fruit Extract Samples

Gas chromatograph	**Agilent 6890N**
Column	30 m x 0.25 mm id x 0.25 µm HP-5MS (p/n 19091S-433)
Carrier gas	Helium
Flow rate	1.9 mL/min at 70 °C
Head pressure	18 psig, constant pressure mode Method RTLocked to methyl chlorpyrifos at 16.593 min
Injector type	PTV, septumless head
Injector temperature (°C), hold time (min), and ramp rate (°C/min)	90 °C (0.3 min) - 1720 °C/min - 250 °C
Vent time	0.2 min
Vent flow	30 mL/min
Vent pressure	0 psig
Purge flow	60 mL/min
Purge time	1.0 min
Syringe volume	50 µL
Injection volume	15 µL
Liner	Empty multibaffle
Oven program: temperature (°C), hold time (min), and ramp rate (°C/min)	70(2)-25-150(0)-3-200(0)-8-280(10)
MSD	**Agilent 5973 inert**
MS interface	280 °C
MS source	230 °C
MS quad	150 °C
Detection mode	EI, Scan 40–550 amu
EM voltage	ATUNE value

Results

The results for the three spiked extracts appear in Table 3 - note that the details of which pesticides were added to the spiked samples were not supplied until after the results were shown to the customer. Those pesticides confirmed by the DRS, are shown lightly shaded. The analytes, shown darkly shaded, are not present in the Agilent RTL Pesticides database. Analyte entries left unshaded were not confirmed.

Table 3. MSD-DRS Results for Three Spiked Fruit Extract Samples

	Sample 1: Control-orange, spiked		Sample 2: Control-lettuce, spiked		Sample 3: Control- apple, spiked	
	Pesticide	Added mg/kg	Pesticide	Added mg/kg	Pesticide	Added mg/kg
1	Methamidofos*	0.10	Diphenylamine	0.10	Mevinphos	0.05
2	Dichlorvos*	0.10	HCB	0.02	Trichlorfon	0.05
3	Acephate*	0.10	Lindane (HCH-gamma)	0.04	Heptenophos	0.02
4	Omethoate	0.10	Diazinon	0.04	Tecnazene	0.01
5	Propachlor	0.20	Chlortalonil	0.04	HCH alpha	0.01
6	Chlorprofam	0.10	Vinclozolin	0.04	HCH beta	0.02
7	Monocrotophos	0.10	Carbaryl	0.20	Dichloran	0.05
8	Dimethoate	0.04	Metalaxyl	0.10	Pyrimethanil	0.02
9	Quintozene	0.02	Pirimiphos-methyl	0.10	Etrimphos	0.02
10	Parathion-methyl	0.10	Malathion	0.10	Ethiofencarb	0.10
11	Dichlofluanid	0.10	Chlorpyrifos	0.10	Metribuzin	0.05
12	Fenpropimorph	0.10	Cyprodinil	0.04	Toclophos methyl	0.01
13	Triadimefon	0.04	Penconazole	0.04	Linuron	0.05
14	Thiabendazole	0.10	Captan	0.10	Aldrin	0.02
15	Tolylfluanid	0.04	Folpet**	0.10	Diethofencarb	0.02
16	Mecarbam	0.10	Procymidone	0.04	Trichloronate	0.02
17	Methidation	0.10	Endosulfan-a	0.04	Triadimenol	0.05
18	Vamidothion	0.10	pp-DDE	0.04	Disulfoton sulfoxide	0.20
19	Imazalil	0.10	Bupirimate	0.04	Disulfoton sulfone	0.02
20	Myclobutanil	0.10	Endosulfan-b	0.04	Fluazinam	0.05
21	Kresoxim methyl	0.10	Aclonifen	0.04	Chlorbenzilate	0.05
22	Tebuconazole	0.10	Ethion	0.04	Oxadixyl	0.05
23	Phosmet	0.10	Triazophos	0.04	Benalaxyl	0.05
24	Fenpropathrin	0.04	Endosulfan-sulfate	0.04	Dicofol	0.05
25	Tetradifon	0.04	Iprodione	0.04	Fenazaquin	0.02
26	Azinphos-methyl	0.10	Bromopropylate	0.10	Pyrazophos	0.05
27	Fenarimol	0.10	Methoxychlor	0.10	Acrinathrin	0.02
28	Azinpfos-ethyl	0.10	Phosalone	0.10	Bitertanol	0.05
29	Prochloraz	0.10	Lambda-Cyhalothrin	0.04	Cyfluthrin beta	0.05
30	Flucythrinate	0.10	Permethrin	0.10	Alpha cypermethrin	0.05
31	Esfenvalerate	0.04	Cypermethrin	0.10		
32	Azoxystrobin	0.04	Fenvalerate	0.04		
33			Deltamethrin	0.10		

* See Discussion item 1.

** See Discussion item 2.

The results for the three 'real' extracts appear in Table 4. Those pesticides confirmed by the DRS are shown lightly shaded. The darkly-shaded analytes are not present in the Agilent RTL Pesticides database. Analyte entries left unshaded were not confirmed. Analytes with an associated concentration were confirmed as present by the customer using NPD/ECD. Lightly-shaded analytes without a concentration label were detected and confirmed by the DRS, but not by the customer.

Table 4. MSD-DRS Results for Three 'Real' Fruit Extract Samples

Sample 4: Grapes
0.68 mg/Kg Captan
0.21 mg/Kg Cyprodinil
0.27 mg/Kg Fludioxinil
Diphenylamine
Sample 5: Orange
2.5 mg/Kg Imazalil
0.25 mg/Kg Medidathion
3.0 mg/Kg Thiabendazole
Sample 6: Apple
0.86 mg/Kg Diphenylamine
0.05 mg/Kg Chlorpyrifos
0.79 mg/Kg Thiabendazole
Dimethoate
Ethoxyquin
Methyl parathion
Endosulfan sulfate
Propargite

Discussion

1. Control - Orange spiked extract

This control sample was spiked with 32 pesticides at levels ranging between 0.02 and 0.10 mg/kg. Twenty-six pesticides were detected and confirmed by the DRS software, two were not reported since they are not present in the Agilent RTL Pesticide database and four were not detected. The spiking was done to the raw matrix, not to a matrix extract. For the polar pesticides (methamidofos and acephate), the recovery was in the 20%–30% range as confirmed by NPD/ECD. Therefore, that explains why these pesticides were not detected by DRS.

2. Control - Lettuce spiked extract

This control sample was spiked with 33 pesticides at levels ranging between 0.02 and 0.20 mg/kg. Twenty-nine pesticides were detected and confirmed by the DRS software, three were not reported since they are not present in the Agilent RTL Pesticide database and one was not detected. The one undetected analyte, (Folpet, marked with two asterisks in Table 3), was detected and confirmed if a higher sensitivity setting was used in the AMDIS deconvolution program.

3. Control - Apple spiked extract

This control sample was spiked with 30 pesticides at levels ranging between 0.01 and 0.20 mg/kg. Twenty-two pesticides were detected and confirmed by the DRS software, six were not reported since they are not present in the Agilent RTL Pesticide database and two were not detected.

Overall, of the 95 spiked analytes in the three control samples, 93% of the pesticides present in the Agilent RTL Pesticide database were detected and confirmed by full-scan library searching of the deconvoluted mass spectra.

4. 'Real' Grape extract

The customer had detected and confirmed three pesticide residues in the Grape extract sample - Captan, Cyprodinil, and Fludioxinil. Of these three analytes, Captan was confirmed by the DRS and Cyprodinil and Fludioxinil are not entries in the Agilent RTL Pesticide database. However, DRS also confirmed an additional pesticide residue - Diphenylamine, which was not reported by the customer.

5. 'Real' Orange extract

The customer had detected and confirmed three pesticide residues in the Orange extract sample - Imazilil, Methidathion, and Thiabendazole. All three of these pesticides were confirmed by the DRS software and no other analytes were confirmed.

6. 'Real' Apple extract

The customer had detected and confirmed three pesticide residues in the Apple extract sample - Diphenylamine, Chlorpyriphos, and Thiabendazole. All three of these pesticides were confirmed by the DRS. In addition, the DRS also confirmed the presence of five additional pesticide residues - Dimethoate, Ethoxyquin, Methyl Parathion, Endosulfan Sulfate, and Progargite. These five pesticides had not been reported by the customer.

Conclusions

The Agilent Technologies MSD-DRS provides additional powerful data processing capabilities to the MSD ChemStation software. Reviewing full scan GC/MS data for the confirmation of pesticide residues can be a labor-intensive and time consuming process requiring great skill and concentration by an experienced analyst.

The DRS is able to process a complex food extract TIC in the order of 1 minute, whereas an experienced analyst may take more than 30 minutes to achieve the same quality result. The DRS software was proven to report the lowest number of false positives and false negatives in the shortest time period.

In scan mode, the detection limit is not as low as in selected ion monitoring (SIM) mode; however, any prior knowledge of the target analytes (retention times or characteristic ions) is not required for the DRS.

The extensive data shown in this report, run under totally blind conditions, shows the high degree of confidence that an analyst can have in the results produced by the DRS in minutes.

Reference

1. Philip L. Wylie, Michael J. Szelewski, Chin-Kai Meng, and Christopher P. Sandy, "Comprehensive Pesticide Screening by GC/MSD Using Deconvolution Reporting Software", Agilent Technologies, publication 5989-1157EN, www.agilent.com/chem

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Printed in the USA
October 5, 2004
5989-1654EN

New Tools for Rapid Pesticide Analysis in High Matrix Samples

Application

Food Analysis

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Abstract

Recent developments in GC/MS hardware and software make it possible to analyze samples with high levels of matrix contamination much faster than ever before. New tools such as mass spectral deconvolution, reliable and inert effluent splitters, and column backflushing capabilities can be combined to produce large time savings. By accelerating the chromatographic run, post-run bakeout, and data interpretation steps, analysis times can be shortened by at least three-fold versus conventional methods. These tools are especially useful in analyses with high levels of matrix background, such as the inspection of the food supply for contaminants. In addition to monitoring for pesticide residues, the threat of terrorism has recently raised concerns over deliberate contamination of food with other toxic materials. This article describes a GC/MS system for the rapid screening of foodstuffs for chemical contaminants with a special emphasis on pesticides, organophosphorus, and organosulfur compounds.

Introduction

Techniques for decreasing the analysis time of gas chromatography (GC) methods have been developed in recent years.

Tools like Method Translation [1] have made it straightforward to reduce analysis time by a known factor and maintain the exact relative elution order of the analytes. The use of appropriate shorter and smaller diameter columns can maintain the same resolution while achieving a much shorter analysis time.

One application area where this approach has met difficulty, however, is the gas chromatography/mass spectrometry (GC/MS) analysis of pesticides in complex matrices like food. This application requires that speed-up schemes maintain column capacity in order to handle the large matrix peaks and achieve low detection limits for analytes. Since chromatography is governed by the triangle of speed, resolution, and capacity, resolution must be sacrificed to increase speed at the same capacity. The problem is that chromatographic resolution is also needed to confirm the identity of any target analytes detected in the presence of interferences from the sample. In this note, the reduction in chromatographic resolution in faster analysis is more than adequately compensated for by use of spectral deconvolution [2] and simultaneous element-selective detection for the confirmation step.

The system consists of a GC/MS with a dual-wavelength flame photometric detector (DFPD) for the simultaneous collection of phosphorus, sulfur, and mass spectral data.

The GC column effluent is split between the two detectors in the ratio of 2:1 in favor of the mass selective detector (MSD). The system is retention-time locked to the Agilent pesticide library [1] scaled to threefold faster times, which contains the

retention times and spectra for 567 pesticides used worldwide. Samples are analyzed with MS in full-scan electron-impact ionization (EI) mode. The combination of precise retention times, elemental, and mass spectral data is used to screen for specific target compounds. The flame photometric detector (FPD) data also highlights any non-target, P- or S-containing compounds for identification by MS.

The MS data is screened using the standard quantitation software based on retention time (RT), ion ratios, and spectral cross correlation. The MS data is also processed using spectral deconvolution software, which greatly reduces spectral interferences from the matrix. The deconvoluted spectra are then searched against a table of targets. Any hits are confirmed by searching against the main NIST library. This process is automated by the Agilent Deconvolution Reporting Software (DRS), also providing significant time savings in data interpretation.

The system described here uses column backflushing, a technique used to save large amounts of time with complex samples. Backflushing is done with the splitter hardware. This technique removes heavy residues from the column much faster and at lower temperatures than the conventional bakeout step at the end of the run. This reduces MS source contamination by preventing the higher levels of column bleed and heavy matrix components from entering the MSD. It also increases the column lifetime.

The approach used thus reduces analysis time in three major ways: shortening the chromatographic run time; automating data interpretation; and reducing bakeout time. Other notable advantages are the ability to change columns and/or inlet liners without venting the MSD, and a reduced need for MS source cleaning.

System Configuration

The system configuration used is shown in Figure 1. Key components are:

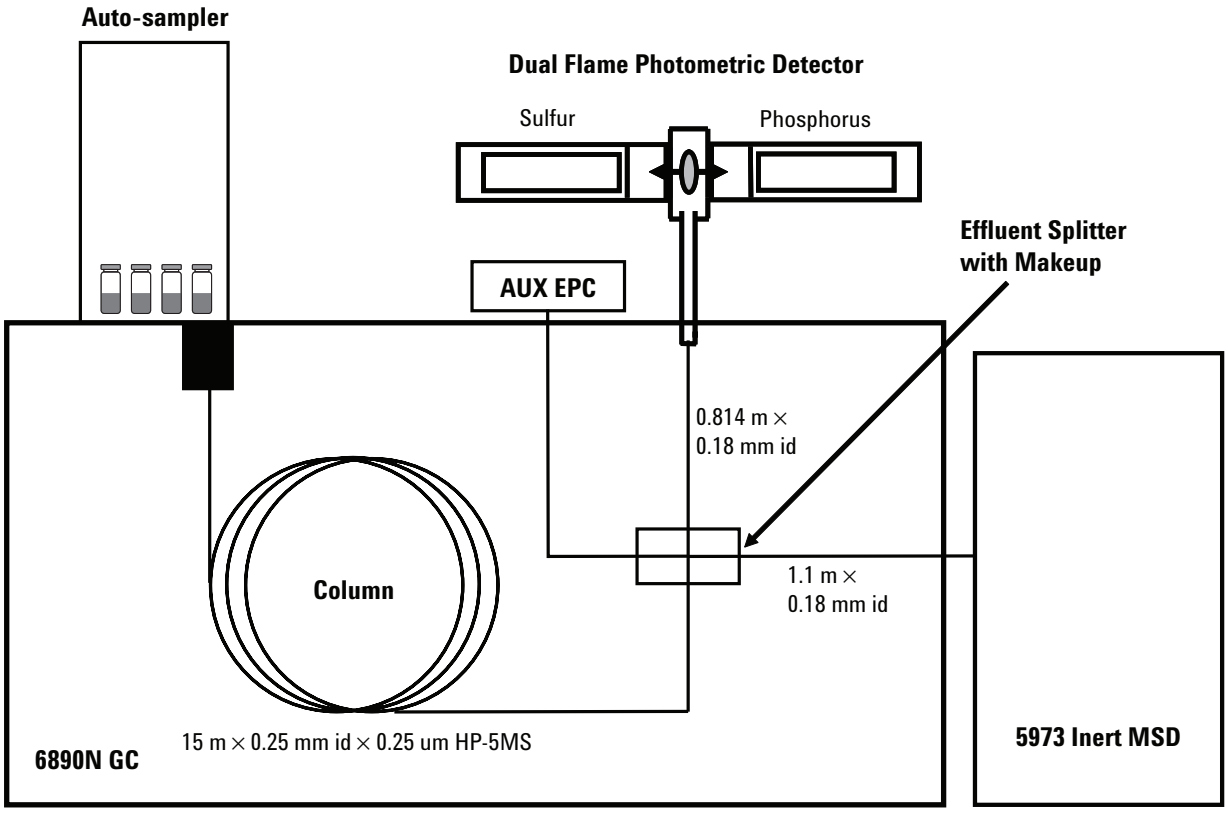


Figure 1. System configuration.

Key Components

Fast Oven With the 6890N 220V oven (option 002), the pesticide analysis method can be run precisely 3 times faster (14 min) using a 15 m HP-MS column. If the 220V GC is further equipped with SP1 2310-0236 (puts MSD interface in back of oven under rear injection port) and the G2646-60500 oven insert accessory (reduces oven volume twofold), the speed can be increased to 4.8 times faster (9 min). The cool-down time of the oven is also reduced.

Dual FPD 6890N Option 241 is a single flame photometric detector with two optical detection channels, one for sulfur and one for phosphorus. The signals from the DFPD are collected, stored, and processed by the MS ChemStation simultaneously with the MS data. The FPD data can be used in several ways. Nontarget organophosphorus compounds like new pesticides or designer nerve agents are highlighted. The presence of an element at the retention time of an identified compound can be used to support confirmation of identity. The response on the FPD can be used for quantitative or semi-quantitative analysis, especially for situations where no calibration standard is available for an identified analyte.

Microfluidic Splitter 6890N Option 889 uses diffusion bonded plate technology combined with metal column ferrules to make an inert, easy-to-use, leak-free, high-temperature column effluent splitter. The splitter uses Auxillary (Aux) electronic pneumatics control (EPC) for constant pressure makeup (6890N Option 301). The Aux EPC makeup can be pressure programmed at the end of the run to higher pressure, while at the same time the inlet pressure is lowered to near ambient. This causes the flow in the column to reverse direction, backflushing heavy materials out the split vent of the inlet. The Aux EPC also allows column changing and maintainance without venting the MSD. When the column fitting is removed from the splitter, helium from the makeup supply purges the fitting, preventing air from entering the MSD. If the column is attached to the splitter but removed from the inlet, helium flows backwards through the column and out the inlet end. Inlet maintainance or column headtrimming can be done without cooling and venting the MSD and air is not introduced into a hot source.

MSD System The 5973N Inert with Performance Electronics and performance turbo (G2579A) EI MSD is used. This configuration provides faster full scan rates while maintaining sensitivity. The scan rates are compatible with the narrower peaks generated by fast chromatography. The performance turbo pump is required to handle the higher flows associated with fast chromatography and backflushing.

DRS Software (G1716AA) Spectral deconvolution of the MS data allows identification of analytes in the presence of overlapped matrix peaks. This significantly reduces chromatographic resolution requirements, allowing much shorter analysis times. DRS utilizes the AMDIS deconvolution program from NIST, originally developed for trace chemical weapons detection in complex samples. DRS presents the analyst with three distinct levels of compound identification: (1) ChemStation, based on retention time and four ion agreement; (2) AMDIS, based on “cleaned spectra” full ion matching and locked retention time; (3) NIST02 search using a >147,000 compound library.

Instrument Operating Parameters

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

Split injection was used to match the amount of matrix to the column capacity. Citrus oils cause retention shifts if excess sample is injected. Splitless injection could be used for samples with significantly less matrix. 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.

The 6890 220V oven was needed for the ramps described in Tables 1 and 2. This oven program is necessary for the precise 3× speed increase of the RTLocked pesticide database.

The 15-m HP-5ms column has the same phase ratio as the 30 m column traditionally used for the 1× method. This shorter column allows a flow rate for a 3× precisely scaled faster method. The outlet is listed as “unspecified” because the column connects to the splitter. The splitter pressure is operated at a constant 3.8 psig using an auxillary EPC module.

The 5973 inert Performance Electronics data acquisition sampling rate was set to 1, which is faster than the typical setting of 2. Signal-to-noise is improved over previous systems at faster sampling rates. More data points allows for easier integration and better deconvolution to compensate for the loss in resolution using a shorter column.

The microfluidic splitter parameters are chosen to provide the desired split ratio between detectors

while meeting the flow requirements of the detectors used. A primary consideration with the current system is to make sure that the flow to the MSD does not exceed ~4 mL/min while collecting analyte data. It was also desired to split the effluent 2:1 in favor of the MSD. These parameters were entered into the spreadsheet calculator (included with the splitter), which calculated the lengths and diameters of the detector restrictors

Table 1. Gas Chromatograph and Mass Spectrometer Operating Parameters

GC	Agilent Technologies 6890		
Inlet	EPC Split/Splitless		
Mode	Split, 1.0 µL injected		
Inlet temp	250 °C		
Pressure	23.84 psi		
Split ratio	10:1		
Split flow	44.1 mL/min		
Total flow	48.1 mL/min		
Gas saver	Off		
Gas type	Helium		
Inlet Liner	Siltek Cyclosplitter, 4 mm id, Restek part number 20706-214.1		
Oven	220V		
Oven ramp	°C/min	Next °C	Hold min
Initial		70	0.67
Ramp 1	75	150	0.00
Ramp 2	9	200	0.00
Ramp 3	24	280	3.33 (end of pesticide ramp)
Ramp 4	50	320	50.0 (end of oil elution)
Total run time	13.96 min to elute pesticides		
Total run time	64.76 min to elute heavy components from citrus oils		
Equilibration time	0.5 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP-5MS, p/n 19091S-431		
Length	15.0 m		
Diameter	0.25 mm		
Film thickness	0.25 µm		
Mode	Constant Pressure = 23.84 psi		
Inlet	Front		
Outlet	Unspecified		
Outlet pressure	3.8 psi (aux pressure to splitter)		
Back Detector (FPD)			
Temperature	250 °C		
Hydrogen flow	75.0 mL/min		
Oxidizer flow	100.0 mL/min		
Oxidizer gas type	Air		
Mode	Constant makeup flow		
Makeup flow	60.0 mL/min		
Makeup gas type	Nitrogen		
Flame	On		
Lit offset	5.00		
Photo multiplier	On		

Table 1. Gas Chromatograph and Mass Spectrometer Operating Parameters (Continued)

Signal 1		Signal 2	
Data rate	5 Hz	Data rate:	5 Hz
Type	Back detector	Type:	Front detector
Save data	On	Save data:	On
Zero	0.0 (Off)	Zero:	0.0 (Off)
Range	0	Range:	0
Fast Peaks	Off	Fast Peaks:	Off
Attenuation	0	Attenuation:	0

AUX Pressure 5	
Description	
Gas type	Helium
Initial pressure	3.80 psi
Initial time	0.00 min (this value will follow oven ramp)
MSD	Agilent Technologies 5973 Inert Performance Electronics
Tune file	Atune.U
Mode	Scan
Solvent delay	1.00 min
EM voltage	Atune voltage
Low mass	45 amu
High mass	450 amu
Threshold	0
Sampling	1
Scans/sec	6.68
Quad temp	150 °C
Source temp	230 °C
Transfer line temp	280 °C
Splitter	Agilent 6890N Option 889
Split ratio	2:1 MSD:DFPD
MSD restrictor	1.1 m × 0.18 mm id deactivated fused silica tubing
DFPD restrictor	0.81 m × 0.18 mm id deactivated fused silica tubing

Backflush Instrument Operating Parameters

Instrument operating conditions for backflushing are shown in Table 2. These are starting parameters and will have to be optimized depending on sample matrix. Conditions listed here are only those that are different from the Table 1 conditions.

Instead of baking the column at 320 °C for 50 min, the heavy matrix material is backflushed through the column inlet, out the split vent. This is accomplished in 5 min at 300 °C, saving 45 min of run time, preserving column life, and shortening cool down time.

The column inlet pressure is reduced to 1.1 psi by using the ramped pressure feature of the EPC. At the end of the backflush time, it is ramped back to initial conditions.

Simultaneous with ramping the inlet pressure down to 1.1 psig, the Aux EPC splitter pressure is ramped up to 23 psig. This increase in pressure at the column outlet, along with the decrease in inlet pressure, backflushes the column. The backflush pressure for the Aux EPC is set to limit the flow to the MSD to < 8 mL/min. This is calculated using the Agilent Flow Calculation software program, also supplied with the splitter kit. The calculator is used to find the pressure which gives the desired flow through the MSD splitter restrictor (1.1 m × 0.18 mm id) at the backflushing temperature 300 °C. The result was 23.6 psig which would produce a flow of 8 mL/min, so 23 psig was used.

The backflush time is determined empirically and depends on the sample matrix. The process is to try a backflush run followed by a blank run with the conventional long-hold to see if the heavy materials

are completely removed. If not, the process is repeated with a longer backflush time. As a very rough guide, start with a backflush time of five void times for the backwards flow. Using the Agilent Flow Calculation software with the “inlet pressure” at 23 psig, the “outlet pressure” at 1.1 psig, and the temperature at 300 °C, the hold-up time (void time) is 0.423 min. The rough guide says that the column should be backflushed for 2.12 min. This works for removing most heavies, but 5 min is required in this case to remove them all.

At the end of the backflush time, the Aux EPC is ramped back to initial conditions.

The MSD is time-programmed to collect data over the time range of 1 to 13.96 min. All of the pesticides elute during this time range.

Table 2. Backflush Gas Chromatograph and Mass Spectrometer Operating Parameters. Use Table 1 Conditions Except for These Differences

Backflush Oven Conditions			
Oven ramp	°C/min	Next °C	Hold min
Initial		70	0.67
Ramp 1	75	150	0.00
Ramp 2	9	200	0.00
Ramp 3	24	280	3.33 (end of pesticide ramp)
Ramp 4	50	300	5.40 (end of oil backflush)
Total run time	13.96 min to elute pesticides		
Total run time	19.76 min to elute heavy components from citrus oils		
Backflush Column Conditions			
Mode	Ramped Pressure		
Press ramp	psi/min	Next psi	Hold min
Initial		23.84	13.96 (end of pesticide ramp)
Ramp 1	99	1.1	5.57 (backflush time)
Ramp 2	99	23.84	0.00
Backflush AUX 5 Conditions			
Press ramp	psi/min	Next psi	Hold min
Initial		3.8	13.96 (end of pesticide ramp)
Ramp 1	99	23.0	5.61 (backflush time)
Ramp 2	99	3.8	0.00
Backflush MSD Conditions			
Timed MS detector entries			
Time (min)	State (MS on/off)		
13.96	Off		

Results

A mixture of 25 pesticides was run at 1×, 3×, and 4.8× speeds. The Total Ion Chromatograms (TICs) are shown in Figure 2.

There is some loss in resolution, but the loss is limited because the shorter columns are run at flows closer to the optimum. The resolution losses are mitigated by using the faster scanning capabilities of the performance electronics together with DRS.

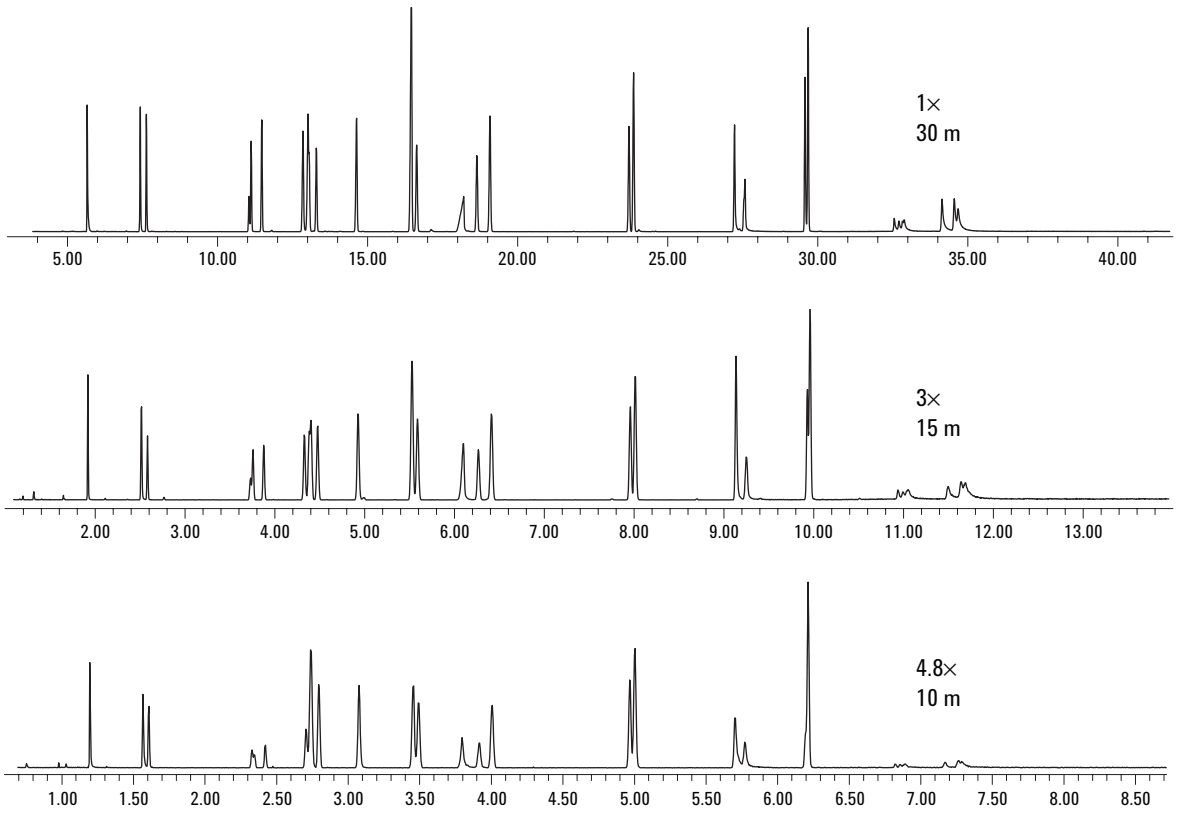


Figure 2. TICs of pesticide test mix at three different scaled speeds. All columns have the same phase ratio.

A neat lemon oil was analyzed using the 3× speed conditions. The TIC is shown in Figure 3 with the DFPD phosphorus (P) and sulfur (S) data channels. The ChemStation software can simultaneously acquire two signals of GC data with the MSD data. The pesticides elute within the 14 min window shown, but the matrix continues to elute for an additional 50 min at 320 °C.

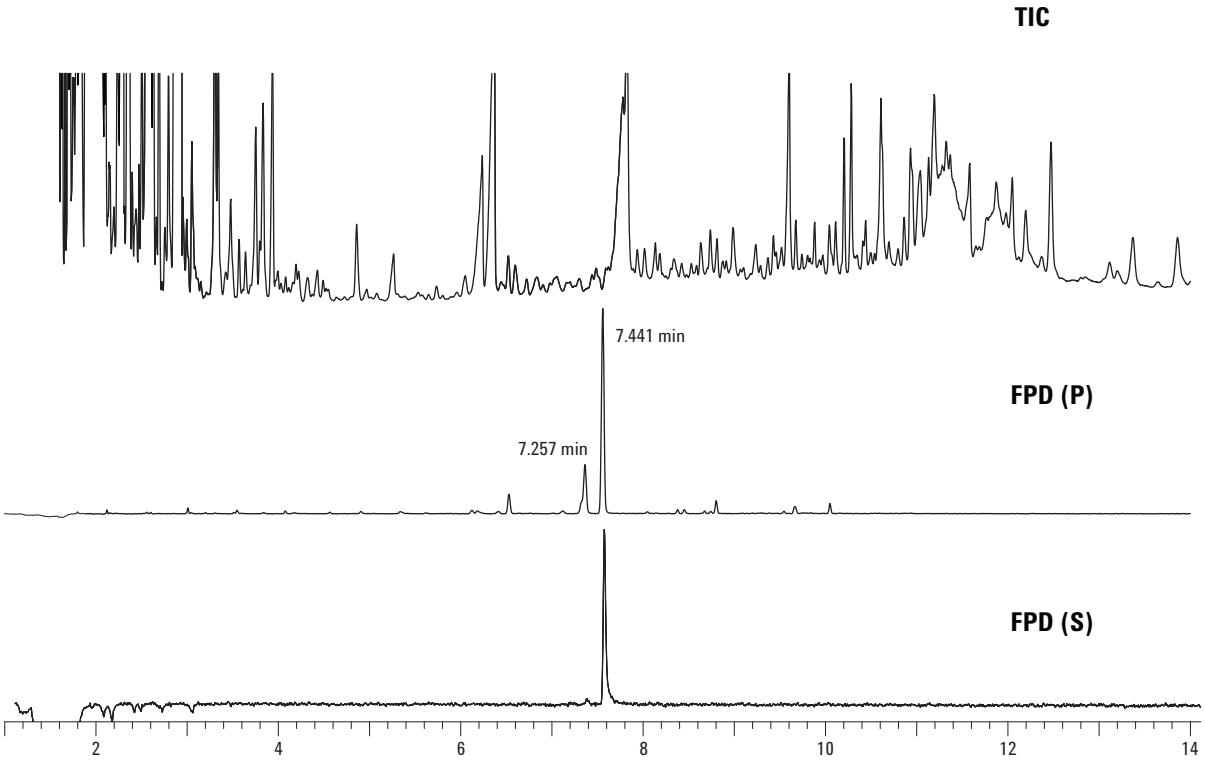


Figure 3. MS and DFPD data from lemon oil analyzed with 3x method.

The P and S chromatograms indicate the possible presence of numerous pesticides. The largest P peak, 7.441 min, also contains S. A PBM reverse Library search identified the peak as Methidathion ($C_6H_{11}N_2O_4PS_3$) with a match quality of 45. It was also identified using a target ion and three qualifier ions.

The raw apex spectrum of the P peak at 7.257 min is shown in the top of Figure 4. No match was found for a P-containing compound in the top 20 library search results. It was also not identified by the ChemStation target and qualifier ion criteria due to out-of-range ratios.

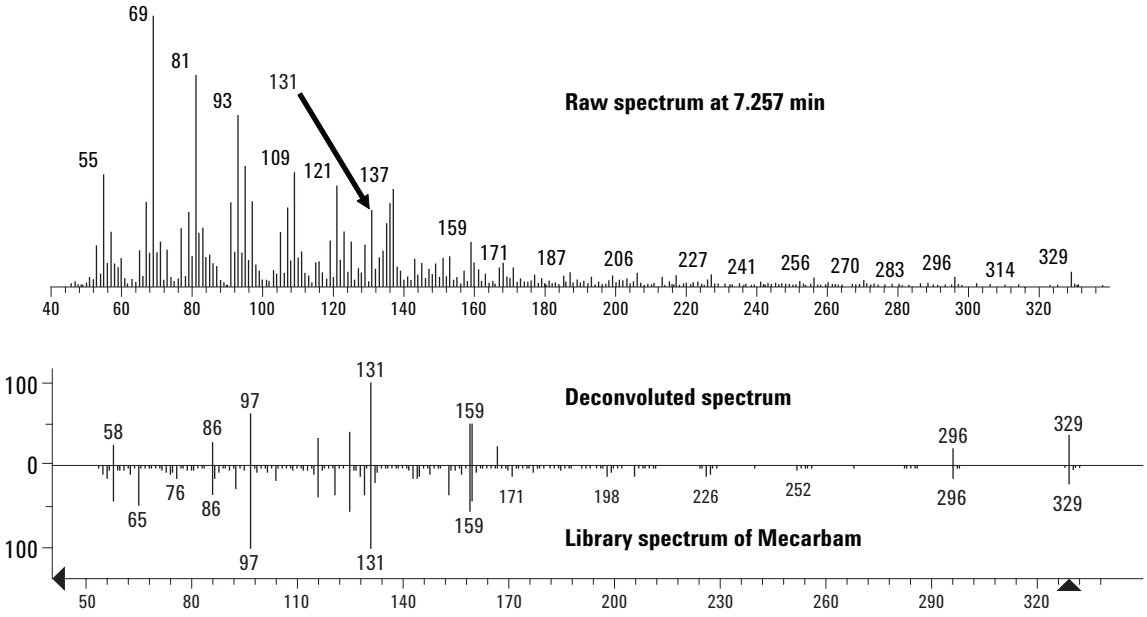
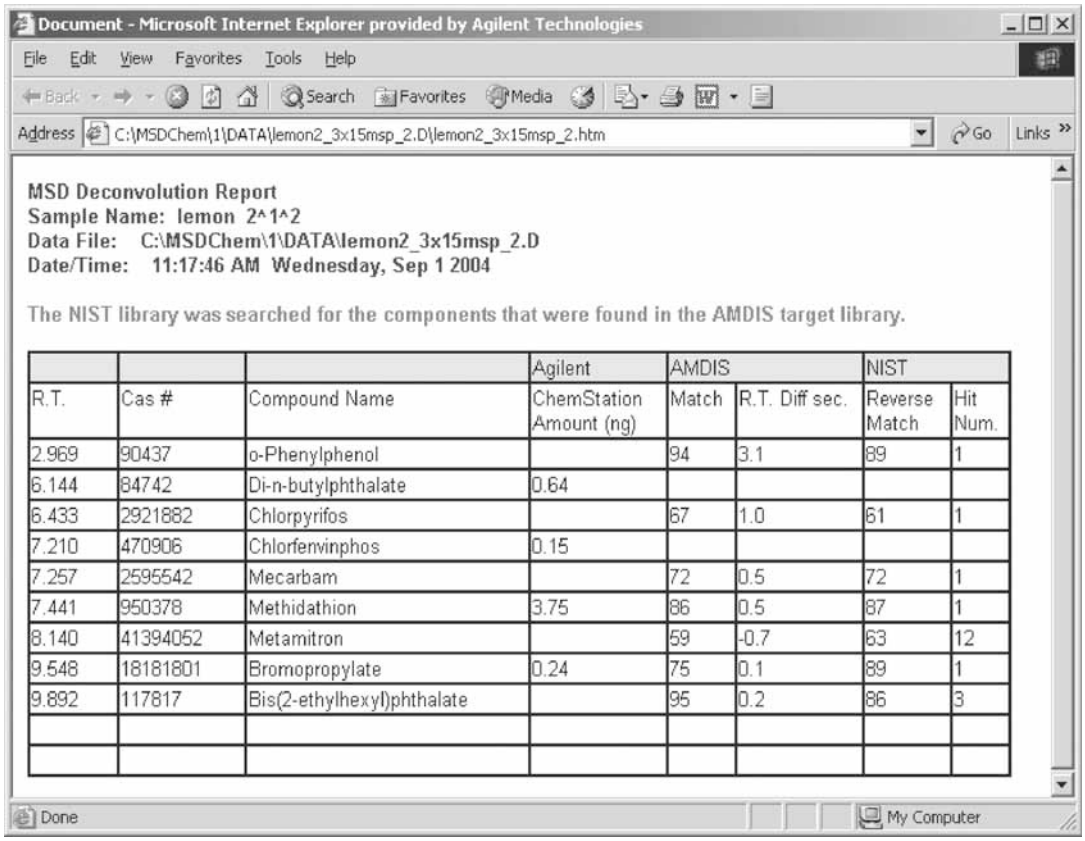


Figure 4. Top - Raw mass spectrum of peak at 7.257 min in lemon oil. Bottom - Deconvoluted spectrum of 7.257 min peak compared to NIST02 library spectrum of Mecarbam.

The DRS report is shown in Figure 5. The peak at 7.257 min is clearly identified as Mecarbam by the DRS software. The deconvoluted spectrum has a match factor of 72, compared to both the Agilent Pesticide AMDIS database and the NIST02 library. Additionally, the DRS report shows the elution time to be only 0.5 s from expected. Further confirmation is the presence of P with S barely visible. The deconvoluted spectrum for the peak at 7.257 min is shown at the bottom of Figure 4, together with the NIST library spectrum of Mecarbam.

The DRS report displays the amount for compounds found by the normal ChemStation quant process. The compounds must be properly calibrated to have a meaningful value. In this lemon oil, the ChemStation found four compounds. The AMDIS portion of DRS found two of the same compounds and an additional five compounds missed by the ChemStation. The NIST02 library search confirmed all of the compounds found by AMDIS using the NIST02 >147,000 compound library. The DRS results show good match quality at the locked retention times for seven target compounds.

No single software package can produce this same confidence level in compound identification. An experienced analyst would take 1–4 hours to process this complex sample manually with each of the three software packages used by DRS. DRS produced this report is less than two minutes.



			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
2.969	90437	o-Phenylphenol		94	3.1	89	1
6.144	84742	Di-n-butylphthalate	0.64				
6.433	2921882	Chlorpyrifos		67	1.0	61	1
7.210	470906	Chlorfenvinphos	0.15				
7.257	2595542	Mecarbam		72	0.5	72	1
7.441	950378	Methidathion	3.75	86	0.5	87	1
8.140	41394052	Metamitron		59	-0.7	63	12
9.548	18181801	Bromopropylate	0.24	75	0.1	89	1
9.892	117817	Bis(2-ethylhexyl)phthalate		95	0.2	86	3

Figure 5. DRS Report for lemon oil.

Backflushing

Citrus oils contain significant amounts of material that elute after the last pesticide. This requires a 150-min hold at 320 °C to elute all of the heavy material with a 1× method. The total run time for the 1× method is therefore 195 min, as shown in Table 3.

Table 3. Method Run Time Comparison

Column	30 m	15 m	10 m
Speed	1×	3×	4.8×
Run time	min	min	min
Pesticides	42	14	8.75
No backflush matrix	195	65	40.6
With backflush matrix	n/a	20	12.5

The 3× method requires a 50-min hold at 320 °C, as shown at the top in Figure 6, resulting in a 65-min run time. With backflushing, all of this heavy material is removed in 5 min at 300 °C, as shown in the bottom of Figure 6. This is a 9-fold reduction in analysis time compared to the 1× method.

4.8x Method

Using the 220V oven, SP1 2310-0236, and oven insert accessory, the method can be scaled to 4.8× faster, as shown in Table 3. There is a practical limit to the amount of matrix that can be tolerated with the reduced resolution using a 10-m column. However, for matrices less complex than a citrus oil, the 4.8× method can be successfully used to save even more time. The Performance Electronics allows running at faster scan speeds while maintaining signal/noise ratio. Sufficient points across a peak are maintained even with faster chromatography.

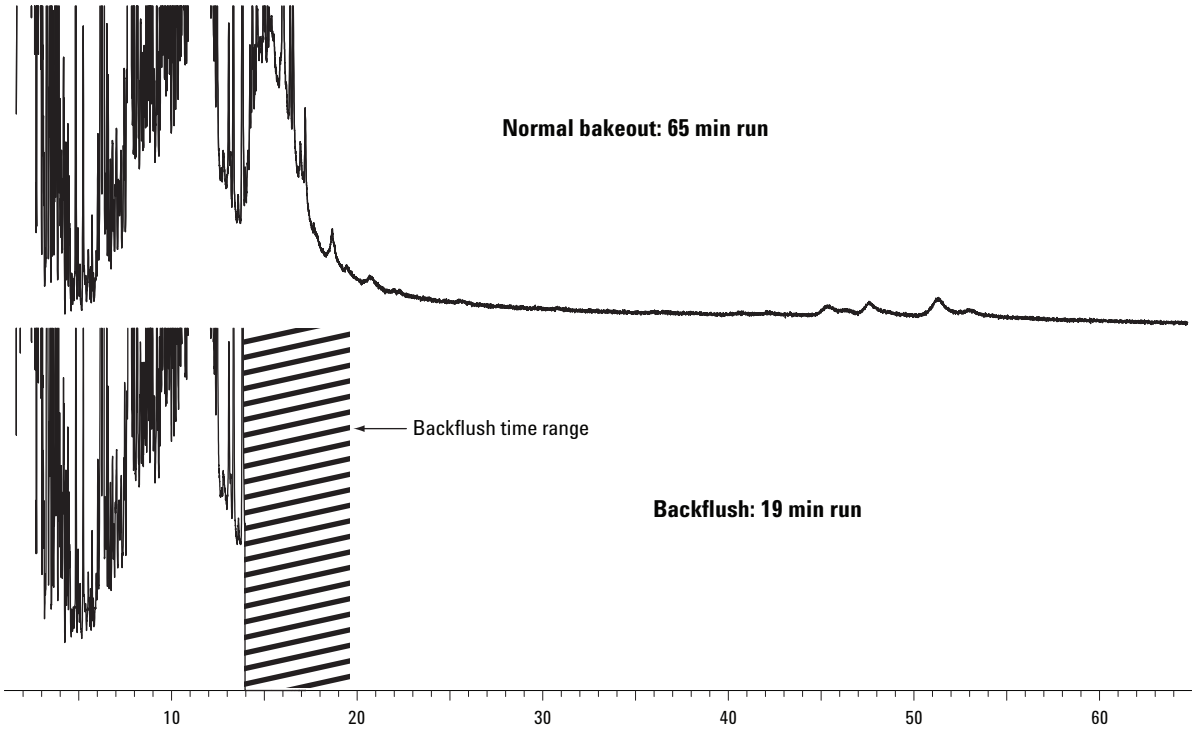


Figure 6. Top - 3× lemon oil analysis with 50 min bakeout at 320 °C. Bottom - 3× lemon oil analysis with 5 min backflush at 300 °C.

Conclusions

New tools are available for the analysis of pesticides in complex matrices. These tools can be combined to significantly reduce analysis and data processing time.

- Fast oven - programming rates needed for faster runs
- Shorter column - 3× faster runs with sufficient resolution
- Microfluidic splitter - confidence in results using selective detection simultaneous with MS data
- Backflush - additional 3× reduction in run time with lower column temperatures for extended lifetime
- Performance Electronics - maintain signal/noise at faster sampling rates
- DRS - three levels of target analyte identification in less than two minutes

Using the above tools, the run time for analysis of lemon oil was reduced from 195 minutes to 20 minutes (nine-fold). DRS reduced the data analysis from hours to minutes.

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2. P. Wylie, M. Szelewski, and C. K. Meng “Comprehensive Pesticide Screening by GC/MSD using Deconvolution Reporting Software” Agilent Technologies publication 5989-1157EN www.agilent.com/chem

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Printed in the USA
October 13, 2004
5989-1716EN

Agilent Technologies

Identification and Isolation of DSP-Toxins Using a Combined LC/MS-System for Analytical and Semipreparative Work

Application

Food Safety

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Abstract

The configuration and operation of a combined liquid chromatography/mass spectrometry (LC/MS) system to identify and isolate DSP-toxins is described. In the analytical mode, okadaic acid (OA) and dinophysistoxin-1 (DTX-1) are more selectively and sensitively monitored when compared to LC with fluorescence detection. With less sample preparation, the detection limits are decreased by a factor of 3–5, depending on the matrix. In semipreparative mode, OA and DTX-1 could be isolated from crude extracts of *Prorocentrum lima* algae using mass-based fraction collection with a purity >98%. Due to this method, reference standards of DSP toxins are now commercially available.

Introduction

Diarrheic shellfish poisoning (DSP) is a gastrointestinal syndrome that occurs in humans after the consumption of bivalve mollusks such as scallops, mussels, clams and oysters. The symptoms include abdominal pain, vomiting, nausea, headache, diarrhea, chills, and fever. DSP toxins can be classified in three groups: the okadaic acid (OA) group involving OA and the dinophysistoxins (DTXs), the pectenotoxin group (PTXs) and the yessotoxin group (YTXs).

Inside the OA group, OA and DTX-1 are the main toxins responsible for DSP outbreaks. The outbreaks led to the establishment of control programs for marine biotoxins in many countries. In Germany residues of DSP toxins in mussels are controlled at present under the regulation of the Fischhygiene-Verordnung of 8th June 2000. This Order requires the testing of shellfish for the presence of toxins by means of animal tests (mouse bioassays) or by chemical analytical procedures [1, 2]. Liquid chromatography with fluorescence detection is an established technique, but it requires the derivatization of the not naturally fluorescent DSP toxins. Using LC/MS coupled with electrospray ionization (ESI) more sensitive and selective results are attainable with less sample preparation.

The greatest problem regarding the analytical methods for monitoring DSP toxins is the availability of pure reference material. The DSP toxins OA and DTX-1 can be isolated from crude extracts of *Prorocentrum lima* algae (see Figure 1) using mass-based fraction collection in semipreparative mode. The present work describes the configuration, setup, and operation of a combined LC/MS system for analytical and semipreparative work.

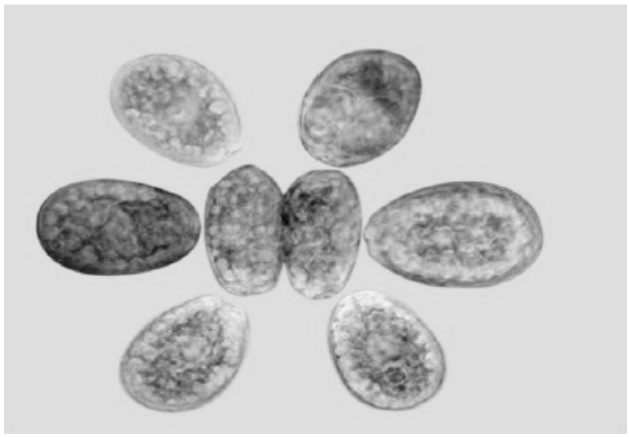
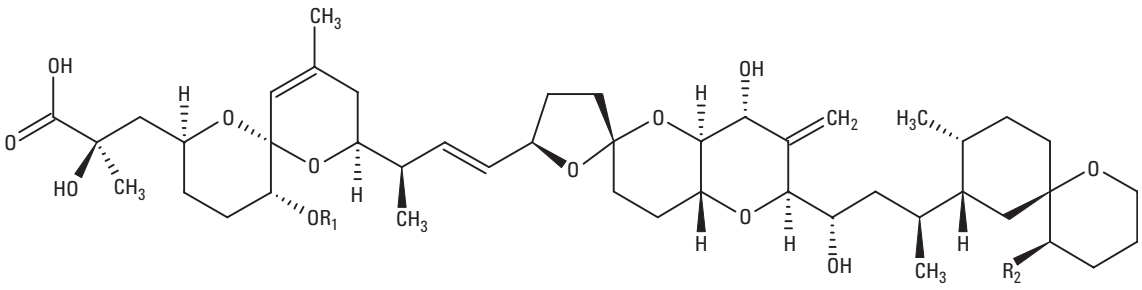


Figure 1. ***Prorocentrum lima*** **algae under the microscope.**

Experimental

The DSP toxins shown in Figure 2 were analyzed in this work. The analyses were conducted in two modes: Analytical and Semipreparative.



OA: $R_1 = H, R_2 = H$ $C_{44}H_{68}O_{13}$
DTX-1: $R_1 = H, R_2 = CH_3$ $C_{45}H_{70}O_{13}$

Chemical and physical properties: Polyether structure, Carboxylic acid
Lipophilic, and no chromophore

Figure 2. DSP toxins.

LC/MS Method Details - Analytical

LC Conditions

Instrument:	Agilent 1100 HPLC (Quaternary pump)
Column:	150 × 3.0 mm ZORBAX SB-C18, 5 µm
Mobile phase:	A Water (0.1% Formic acid) B Methanol
Gradient:	20% B at 0 min 20% B at 5 min 80% B at 20 min
Stop time:	28 min;
Post time:	4 min
Flow rate:	0.6 mL/min
Injection vol:	10 µL

MS Conditions

Instrument:	Agilent LC/MSD
Source:	Positive/Negative switching ESI
Drying gas flow rate	12 L/min
Nebulizer:	60 psig
Drying gas temp:	350 °C
V_{cap}:	3000 V (positive and negative)

LC/MS Method Details - Semipreparative

LC Conditions

Instrument 1:	Agilent 1100 HPLC (Quaternary pump)
Column:	50 × 9.4 mm ZORBAX SB-C18, 5 µm
Mobile phase:	A Water (0.1% Formic acid) B Methanol
Gradient:	20% B at 0 min 20% B at 5 min 80% B at 20 min
Stop time:	28 min
Post time:	4 min
Flow rate:	7.0 mL/min
Injection vol:	100 µL (250 µL using Multiple Draw Mode)
Instrument 2:	Agilent 1100 HPLC (Isocratic pump) for makeup flow
Flow rate:	0.8 mL/min (50% H_2O + 50% MeOH + 0.1% Formic acid)
Active splitter:	Split ratio 271:1

MS Conditions

Instrument:	Agilent LC/MSD
Source:	Negative ESI
Drying gas flow:	12 L/min
Nebulizer:	60 psig
Drying gas temp:	350 °C
V_{cap}:	3000 V (positive)
MSD Fraction Collection Setup	
FC Mode:	Use method target mass; Adducts: $(M–H)^-$

Results and Discussion

Analytical Work

In the analytical mode of the LC/MS system (Figure 3) the DSP toxins were monitored using ESI with positive/negative mode switching. The positive ion mode is four times more sensitive than the negative ion mode (Figure 4). Mass spectra for OA and DTX-1 show a sodiated molecular ion instead of a protonated molecular ion, and characteristic fragment ions $[M+H – nH_2O]^+$, where n = 1–4, formed by a sequential loss of water. In negative ion mode only the $[M–H]^-$ ion is detected. LC/MS provided a more selective and sensitive method for monitoring DSP toxins in comparison to LC with fluorescence detection (Figure 5), by a factor of 3–5.

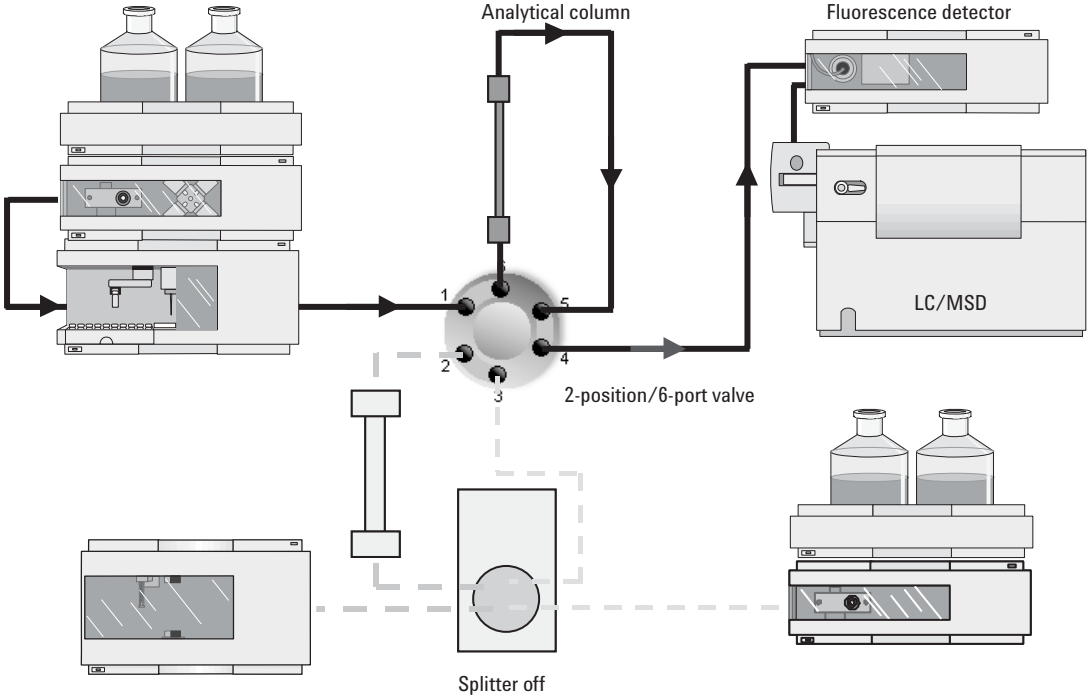


Figure 3. System diagram (analytical work).

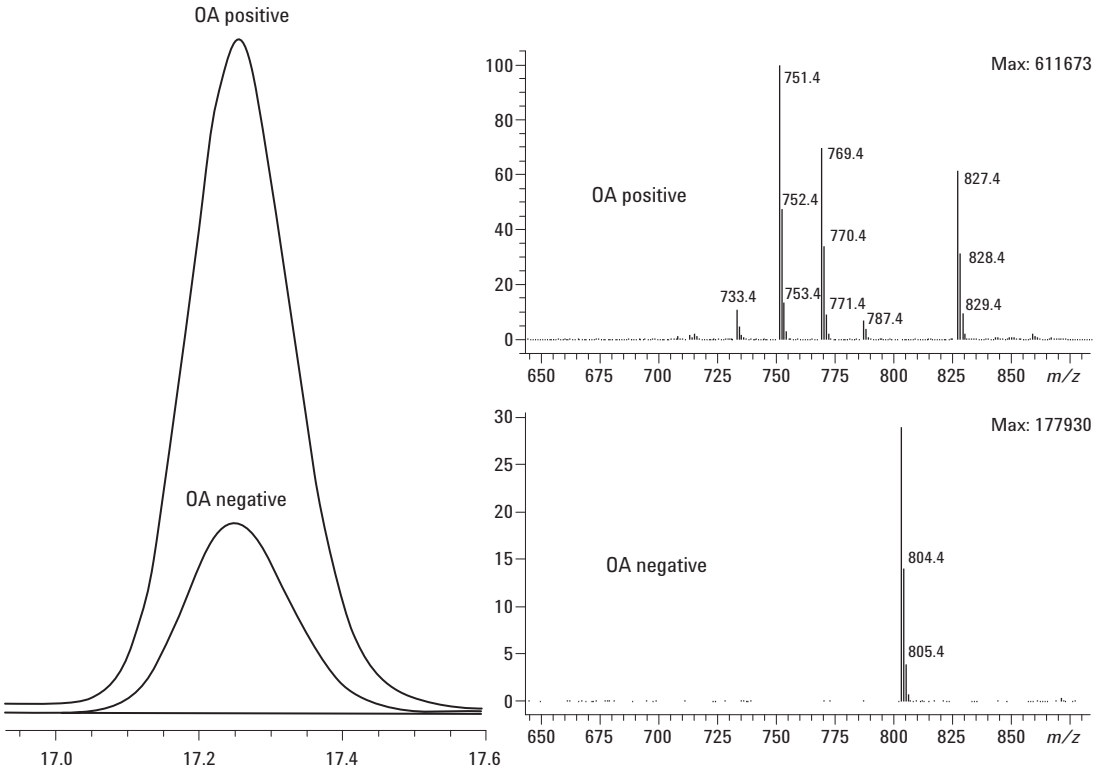


Figure 4. LC/MS analysis of OA.

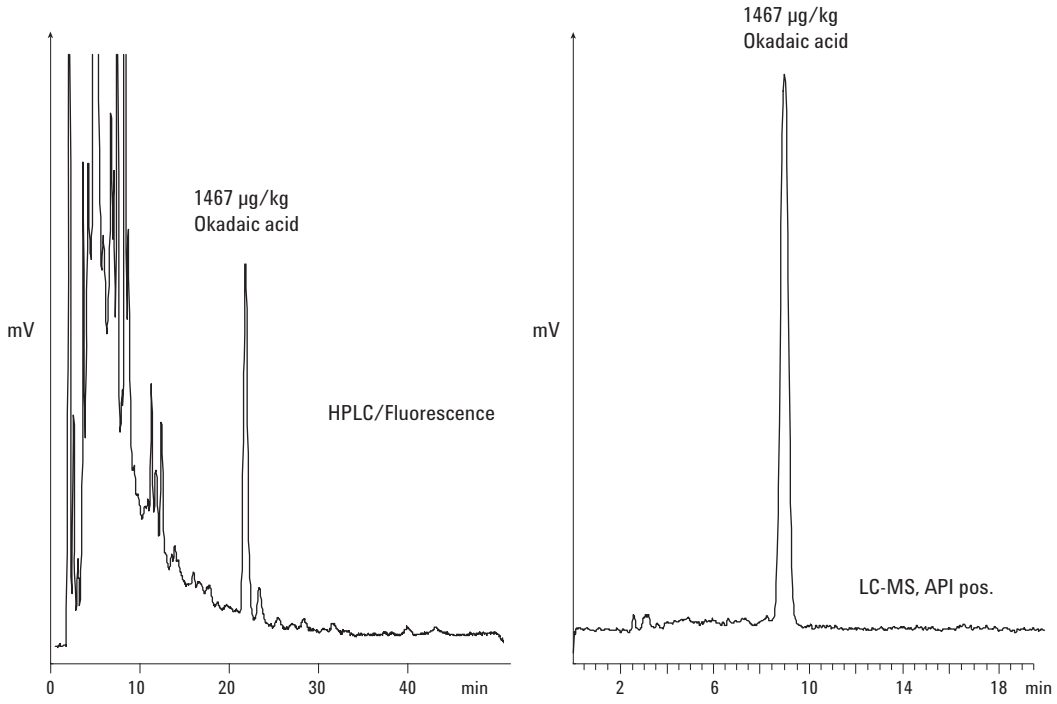


Figure 5. Comparative analysis of DSP toxins in shellfish.

Semipreparative Work

The reference standards could be obtained by switching the system to semipreparative mode (Figure 6). The valve is switched to position 2.

The main flow now goes to the semipreparative column and then through the splitter to fraction collector (AS). The make-up flow goes through the splitter where it picks up some of the compound from the main flow and goes to the MS-detector (MSD).

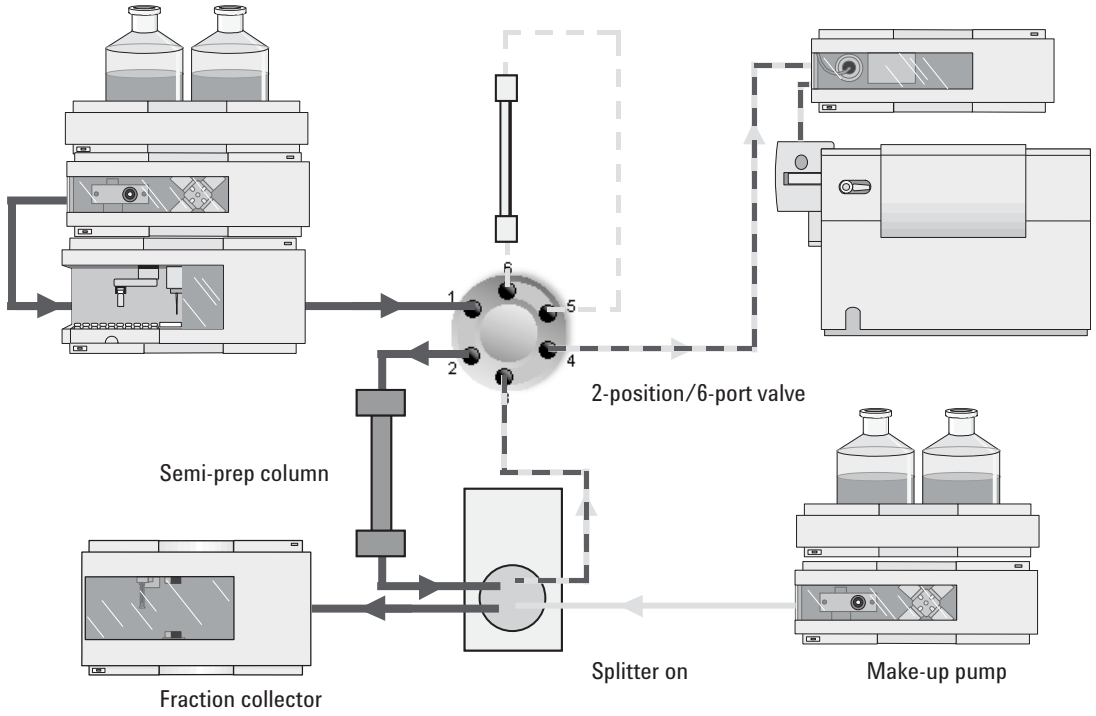


Figure 6. System diagram (semipreparative work).

Besides OA and DTX-1, a new OA-toxin with similar mass spectral properties could be isolated from crude extracts of *Prorocentrum lima* algae using mass-based fraction collection (Figure 7). The mass-based fraction collection of a methanolic extract of *Prorocentrum lima* algae results in three fractions: OA, DTX-1 and an unknown toxin. From MS^n experiments it can be determined that the molecular structure of the unknown toxin must be very similar to those of OA and DTX-1.

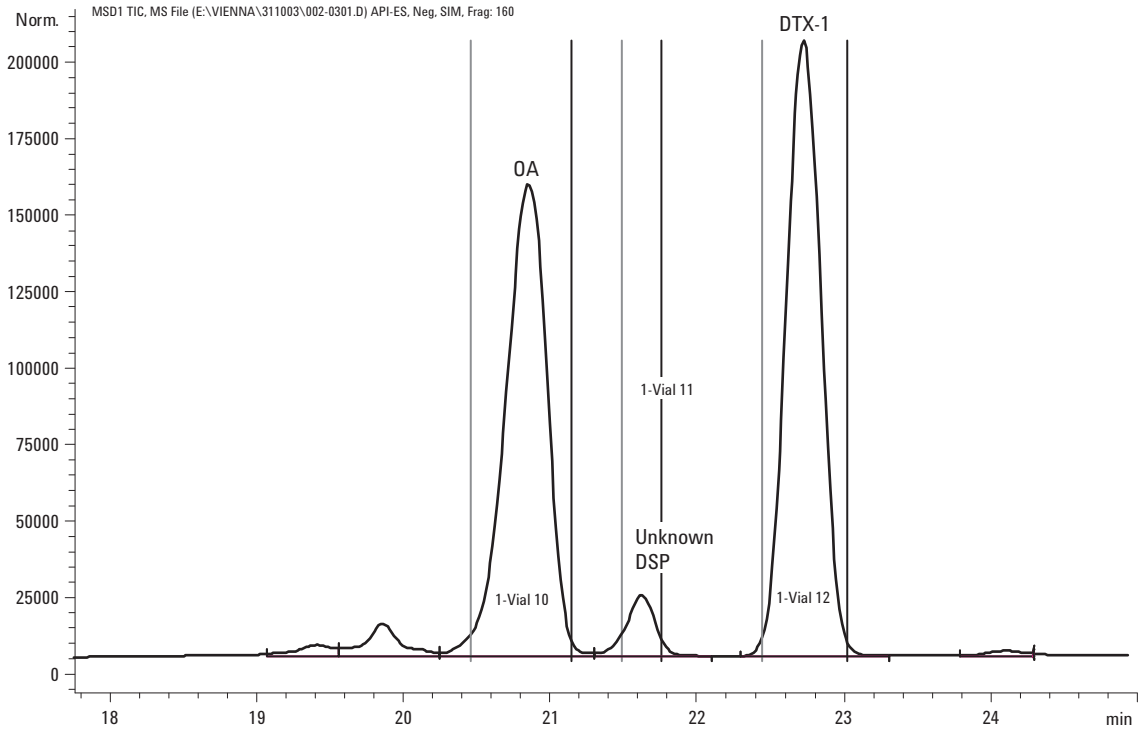


Figure 7. Mass-based fraction collection of DSP toxins.

Because of the low concentration, the target compounds had to be collected from multiple injections of the same sample, a process usually referred to as pooling (Figure 8). Reanalysis of the collected fractions gave results for purity >98%. This method is robust (Figure 9) and has now resulted in making reference standards of DSP toxins commercially available.

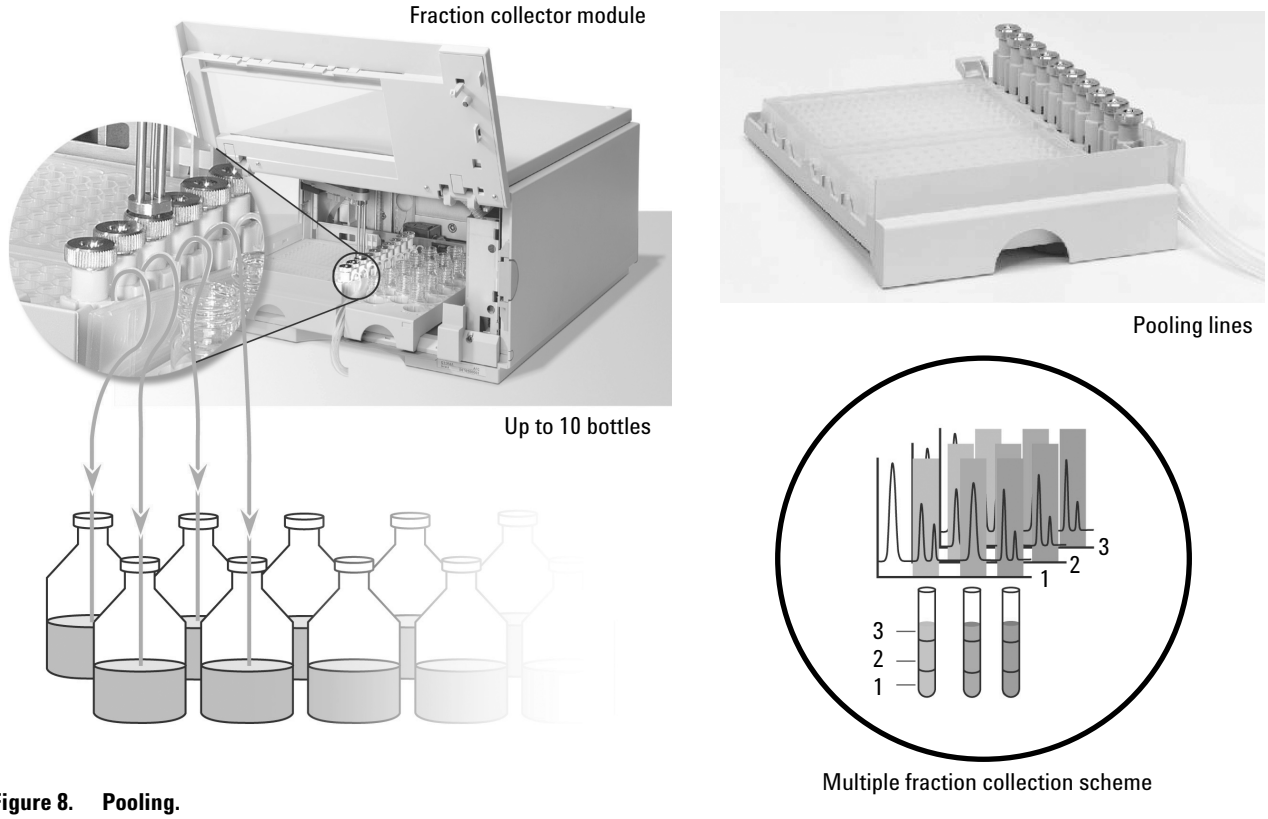


Figure 8. Pooling.

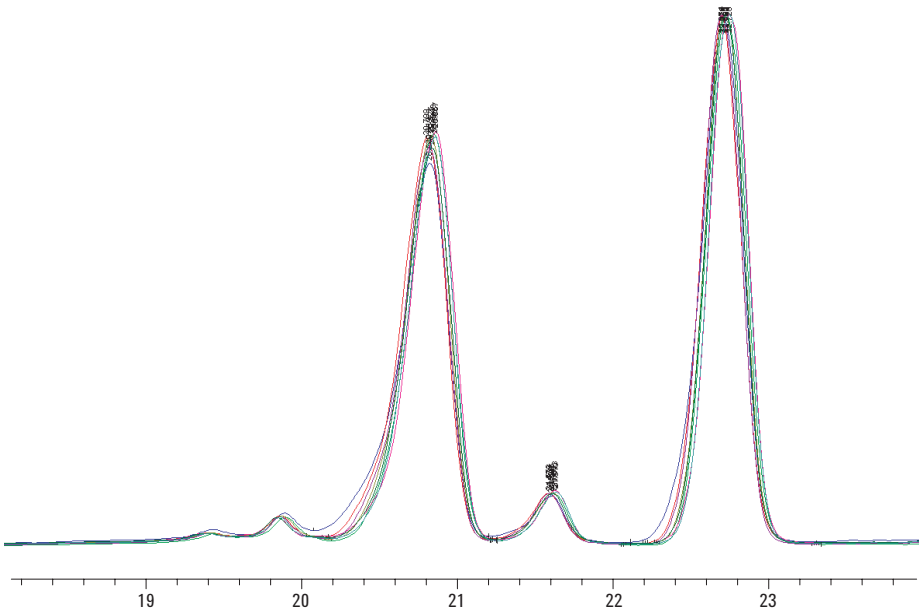


Figure 9. Robustness of the method - overlay of 10 mass-based fraction collection runs.

Conclusions

Configuration and operation of a combined LC/MS system to identify and isolate DSP toxins is described. In the analytical mode, OA and DTX-1 were monitored more selectively and sensitively than by using LC with fluorescence detection. With less sample preparation, the detection limits could be decreased by a factor of 3–5, depending on the matrix. In the semipreparative mode OA and DTX-1 could be isolated from crude extracts of *Prorocentrum lima* algae using mass-based fraction collection with a purity >98%. Due to this method reference standards of DSP toxins are now commercially available.

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Printed in the USA
May 24, 2005
5989-2912EN

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Analysis of Terbuthylazine in Olive Oil by LC/Ion Trap MS and LC/Time-of-Flight MS

Application

Food safety

Authors

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Abstract

This application note describes the use of liquid chromatography/ion trap mass spectrometry (LC/ITMS) and liquid chromatography/time-of-flight mass spectrometry (LC/TOFMS) for the identification and quantitation of terbuthylazine in olive oil samples. The method includes a sample treatment step based on a preliminary liquid-liquid extraction, followed by matrix solid-phase dispersion (MSPD) using an aminopropyl-bonded silica as a sorbent material. A final clean-up step is performed with Florisil using acetonitrile as an eluting solvent. The analysis by ion trap was achieved in MS/MS mode, monitoring the characteristic fragment ion at *m/z* 174. The identification by LC/TOFMS was accomplished with the accurate mass (and the subsequently generated empirical formula) of the protonated molecule ($[M+H]^+$ *m/z* 230), along with the accurate mass of the fragment ion and the characteristic chlorine isotope cluster present in terbuthylazine. The accuracy typically obtained was routinely better than 2 ppm. The method sensitivity, linearity, precision, accuracy, matrix effects, and limit of detection were also studied; they supported the potential of LC/TOFMS and LC/ITMS for the routine quantitative analyses of terbuthylazine in olive oil.

Introduction

Olive oil is one of the most-used food products in Mediterranean countries. The positive effects of olive oil on health have prompted a demand for this product world-wide. “Virgin” olive oil is obtained from the fruit of the olive tree (*Olea Europaea*) exclusively by mechanical and physical processes without any further treatment, which may alter the olive oil quality. The most extensively applied agrochemicals in olive plantations of Mediterranean countries (Greece, Spain, and Italy) are herbicides and insecticides. Although herbicides are mainly applied to soils, some residues can persist to the harvest stage, thus contaminating the olives picked up from soil. This can result in the presence of trace amounts of these pesticides in olive oil. Consequently, both the European Union and the Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) of the United Nations have established maximum pesticide residue levels in olives and olive oil. Currently, various olive oil pesticide residue regulatory programs are being carried out to update and to establish new and more stringent regulations concerning the maximum residue levels in these commodities [1]. This fact has fostered the development of more powerful analytical tools in order to provide enough sensitivity and selectivity to meet these requirements in food samples such as edible oils, which have a complex matrix due to the high fat content of the extracts obtained after the sample treatment step.

Many multiresidue procedures employing different clean-up techniques and a variety of detection methods are reported on the determination of pesticide residues in olive oil. The most commonly used methodology is based on GC after a comprehensive clean-up step, in most cases based on liquid-liquid partitioning or gel permeation chromatography to separate the low molecular mass pesticides from the higher molecular mass fat constituents of the oil, such as triglycerides [2, 3]. The preparation of oil samples for the determination of pesticides by GC requires the complete removal of the high-molecular-mass fat from the sample to maintain the chromatographic system in working order. Recently, a multiresidue method for the determination of triazines and organophosphorous pesticides using MSPD followed by GC/MS and ion trap MS techniques was reported [4]. In this work, we further explore the capabilities of LC/ITMS and LC/TOFMS techniques for the identification of terbuthylazine, one of the most common pesticides found in olive oil.

Experimental Methods

Olive Oil Extraction

MSPD was used for the extraction of terbuthylazine from olive oil after a preliminary liquid-liquid extraction with organic solvents.

1. The liquid-liquid extraction:
   - An aliquot of approximately 5 g (ca. 5.5 mL) of olive oil sample was mixed with 15 mL of petroleum ether, saturated with acetonitrile, in a 100-mL separatory funnel, in which a two-step liquid-liquid extraction was performed.
   - A solution of 25 mL of acetonitrile, saturated with petroleum ether, was added to the olive oil mix obtained previously. The funnel was shaken vigorously for 3 minutes, and the acetonitrile phase was separated from the petroleum ether phase.
   - Another 10 mL of acetonitrile saturated with petroleum ether was added to the petroleum ether extract. The mixture was shaken again for 3 minutes and the acetonitrile phase was collected and added to the previous one.
   - Finally, a 7-mL aliquot of the acetonitrile extract was transferred to a 10-mL glass test tube. The extract was then carefully evaporated down to a final volume of about 2 mL. This remaining extract was transferred to a glass mortar to be subjected to matrix solid-phase dispersion.
2. The MSPD:
   - The extract obtained in the liquid-liquid extraction step was homogenized with 2 g of aminopropyl-bonded sorbent (Bondesil-NH_2, 40-m particle size, Varian Inc.) until a fine powder was obtained.
   - The mixture was transferred to a commercially available minicolumn containing 2 g of Florisil (12-mL Bond-Elut Varian minicolumn, Varian Inc.). This minicolumn was connected to a vacuum system for solid phase extraction adjusting the flow to 3 mL/min.
   - An elution step was carried out with 2×5 mL of acetonitrile. The final extract was evaporated until near dryness, then dissolved with 1:1 acetonitrile:water.
   - Prior to LC/ITMS and LC/TOFMS analysis, the extract was filtered through a 0.45-m PTFE filter (Millex FG, Millipore, Milford, MA, USA).

Using the MSPD method, recoveries for terbuthylazine from olive oil samples were higher than 96% with a 6% relative standard deviation (RSD) (n = 5).

Agilent 1100 Series LC/MSD TOF Methods

LC conditions

LC Pumps were Agilent 1100 binary pumps

Injection volume:	50 µL with standard Agilent 1100 ALS
Column:	ZORBAX Eclipse XDB-C8, 4.6 mm × 150 mm, 5 µm, p/n 993967-906
Mobile phases:	A = acetonitrile and B = 0.1% formic acid in water
Gradient:	5 minutes isocratic at 10% A, followed by a linear gradient to 100% A in 25 minutes at a flow rate of 0.6 mL/min

MS conditions

LC/MSD TOF

Source:	Positive ESI (electrospray ionization)
Capillary:	4000 V
Nebulizer:	40 psig
Drying gas:	9 L/min
Gas temp:	300 °C
Fragmentor:	190 V
Skimmer:	60 V
Oct DC1:	37.5 V
Oct RF V:	250 V
Reference masses:	*m/z* 121.0509 and 922.0098
Resolution:	9500 ± 500 @ *m/z* 922.0098
Mass range:	50–1000 *m/z*
Reference A sprayer 2:	Constant flow during the run

Agilent 1100 Series LC/MSD Trap Methods

LC/MSD Trap

Methods identical to LC/MSD TOF for direct comparison of peaks.

LC Pumps:	HP 1100
Inject vol:	50 µL
Column:	ZORBAX Eclipse XDB-C-8, 4.6 mm × 150 mm, 5 µm, p/n 993967-906
Mobile phases:	A = acetonitrile (ACN) B = 0.1% formic acid in water
Gradient:	10% A, isocratic, for 5 minutes followed by linear gradient to 100% A in 25 min at a flow rate of 0.6 mL/min

LC/MSD Trap

Positive ESI:	Capillary 3200 V
Nebulizer:	40 psig
Drying gas:	9 L/min, gas temp 300 °C
Capillary exit:	70 V
Skimmer:	60 V
Trap accumulation:	50,000 counts with maximum accumulation time of 200 ms

Results and Discussion

Identification of Terbuthylazine by LC/ITMS and LC/TOFMS

Olive oil is one of the most difficult food matrices due to the presence of numerous interferences that show up in full-scan mode. For this reason, the ion trap method was optimized in MS/MS mode by isolating the precursor ion at *m/z* 230, which corresponds to $[M+H]^+$. An isolation mass window of *m/z* 2 and optimized fragmentation amplitude was used in order to enhance the signal-to-noise (S/N) ratio. An amplitude voltage of 0.8 V was used to obtain good fragmentation for terbuthylazine, which fragments by losing the terbutyl group (–56) forming the *m/z* 174 fragment ion.

Figure 1 shows the analysis of an olive oil sample (spiking level 0.025 mg/kg) by ion trap MS/MS. The extracted ion chromatogram (EIC) for *m/z* 174, the main fragment of terbuthylazine, as well as its mass spectrum is shown. The fragmentation occurs at the C–N bond via the cleavage of the terbutyl group. This represents a characteristic fragmentation for this analyte, allowing for the structural confirmation of terbuthylazine in a relative complex matrix such as olive oil.

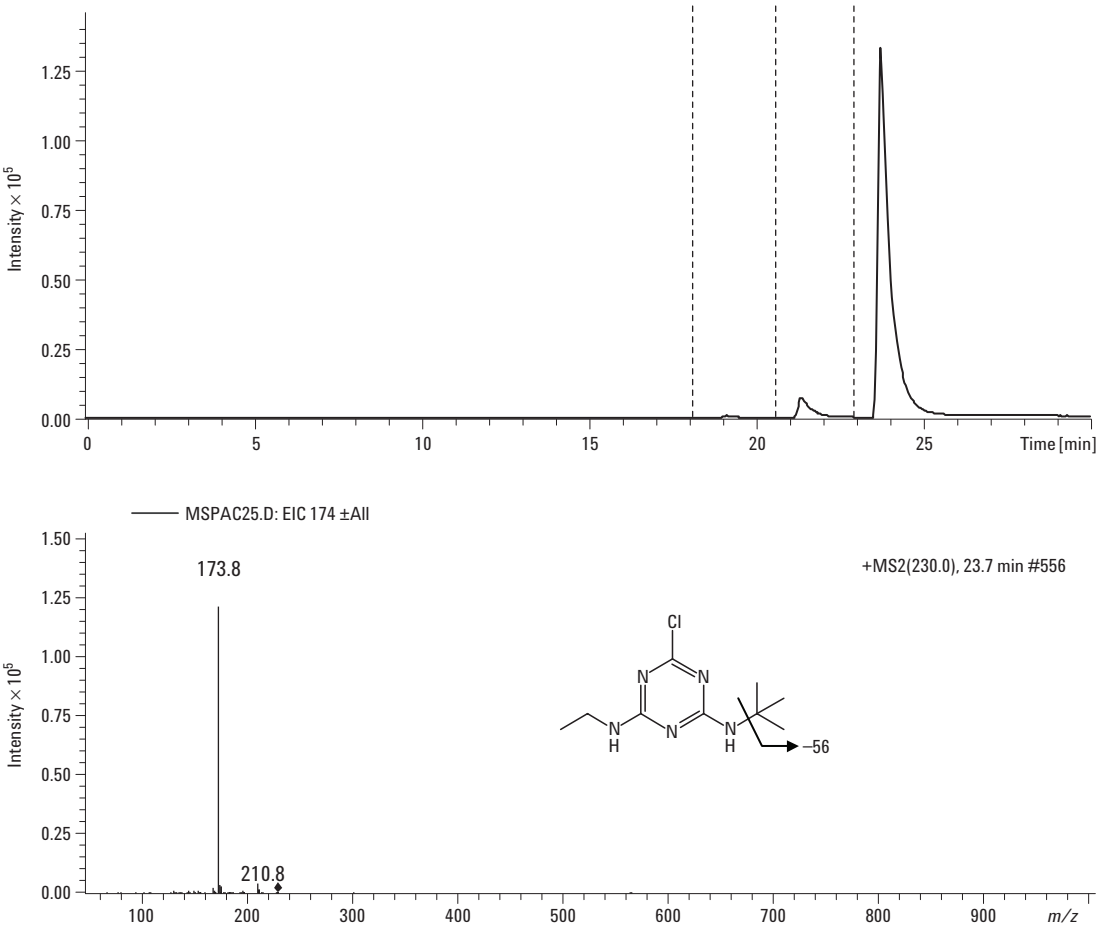

Figure 1. Ion Trap MS/MS chromatogram corresponding to the analysis of a spiked olive oil sample with terbuthylazine (0.025 mg/kg). The EIC for *m/z* 174 and its corresponding spectrum are shown.

LC/TOFMS analyses were optimized in terms of fragmentation. The in-source collisionally induced dissociation (CID) fragmentation is greatly enhanced at high fragmentor voltages. This provides highly valuable structural information since the accurate mass of the characteristic fragment ion can be used along with that of the molecular ion for confirmation purposes. The relative abundances for both the main fragment and the protonated molecule of terbuthylazine are summarized in Table 1 at three different voltages: 160 V (low), 190 V (medium), and 230 V (high). In order to obtain sufficient sensitivity for quantitative purposes (using the protonated molecule) and additional qualitative spectral information provided by the fragment ions generated by in-source fragmentation, a value of 190 V was chosen for further analyses. The accurate masses for both the main fragment and the protonated molecule of terbuthylazine in a spiked olive oil matrix are shown in Table 2.

Table 1. Effect of the Fragmentor Voltage on CID Fragmentation for LC/TOFMS

***m/z* ion**	**Relative abundance**		
	160 V	**190 V**	**230 V**
230 $[M+H]^+$	100	100	20
174 $[M+H–C_4H_8]^+$	5	30	100

Table 2. LC/TOFMS Accurate Mass Measurements for Terbuthylazine and its Main Fragment Ion in Olive Oil Matrix-Matched Standard (Fragmentor Voltage 190 V). Spiking Level: 0.025 mg/Kg

Elemental composition	Ion	Theoretical mass	Measured mass	Error mDa	Error ppm
$C_9H_{16}N_5{}^{35}Cl$	$[M+H]^+$	230.1167	230.1168	0.1	0.4
$C_9H_{16}N_5{}^{37}Cl$		232.1137	232.1134	–0.3	–1.5
$C_5H_9N_5{}^{35}Cl$	$[M+H–C_4H_8]^+$	174.0541	174.0542	0.1	0.6
$C_5H_9N_5{}^{37}Cl$		176.0511	176.0511	–0.05	–0.3

Along with the accurate mass of the protonated molecule and the information provided by the fragments obtained with an optimized in-source fragmentation, terbuthylazine presents another feature which enables its identification; the presence of a chlorine atom. The accurate mass pattern of the ^{37}Cl isotope signal confirms that the peak unequivocally contains only one chlorine atom (Figure 2). Therefore, the accurate mass of each ion as well as the presence of the chlorine signal, together with the characteristic retention time represent sufficient information to unequivocally identify and confirm this specie in such complex matrices.

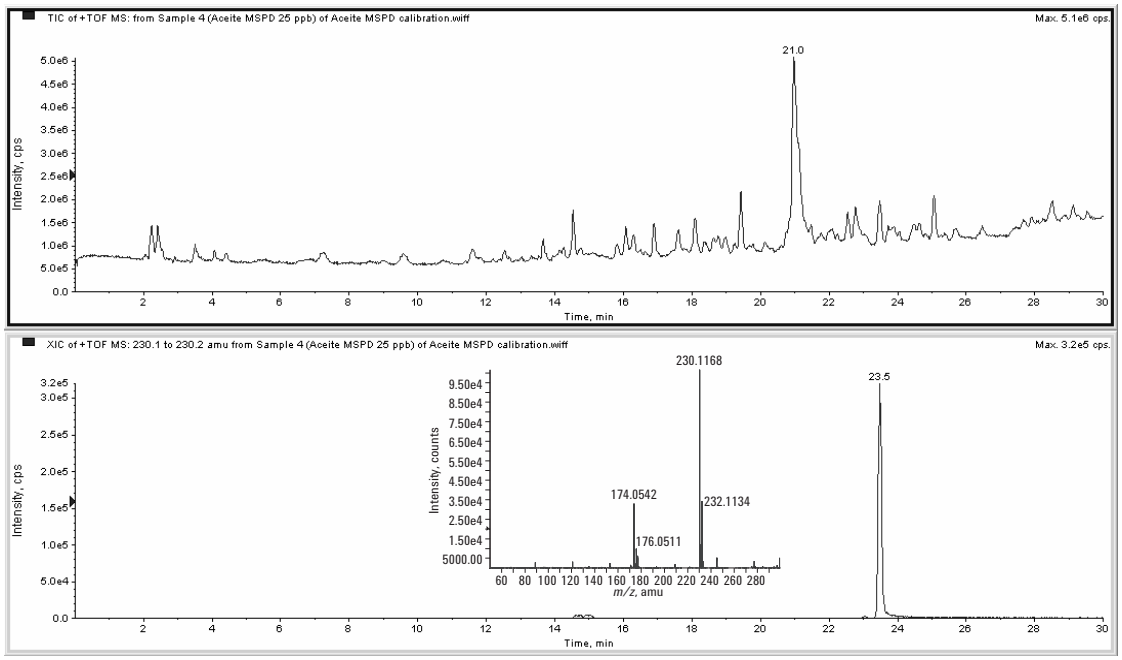

Figure 2. LC/TOFMS total ion chromatogram (TIC) corresponding to the analysis of a spiked olive oil sample with terbuthylazine (0.025 mg/kg). The EIC for *m/z* 230 and its corresponding spectrum are also shown.

Analytical Performance

The analytical performance of the proposed methods was studied in order to explore the ability to carry out quantitative analyses of terbuthylazine in these complex matrices with a high content of fat. The calibration was carried out using matrix-matched standards prepared by the extraction method based on MSPD described in the experimental section. Linearity was evaluated by analyzing solutions of matrix-matched standards at seven different concentration levels in the range 0.005–0.5 mg/kg. Using ion trap the quantitation was performed in MS/MS mode of the *m/z* 230 ion. Using LC/TOFMS the quantitation was carried out using the peak area from the EIC of the protonated molecule with a mass window of 0.1 Da. As an example, Figure 3 shows the linear calibration curve obtained by LC/TOFMS for terbuthylazine in an olive oil matrix compared to the curve obtained in pure solvent.

The limits of detection (LOD) were estimated from the injection of matrix-matched standard solutions with concentration levels giving a S/N ratio of about 3. The results obtained are shown in Table 3. The LOD obtained are remarkable since they are far below the maximum residue level regulations established for these pesticides. In this sense, LC/TOFMS analyses benefits of the use of narrow mass windows for quantitation purposes, which results in enhanced S/N ratio, thus providing lower detection limits. This fact illustrates the analytical potential of the proposed method based on MSPD and LC/TOFMS for the analyses of pesticides in complex matrices with high content of fat.

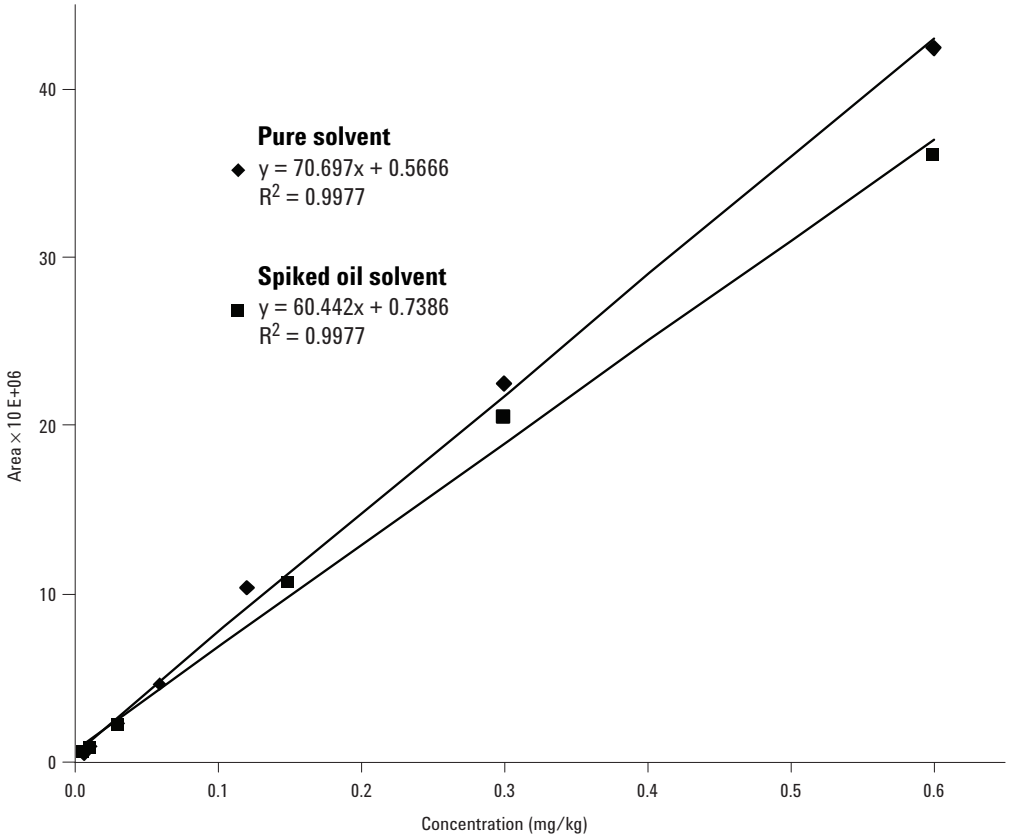

Figure 3. Calibration plot obtained from spiked olive oil samples versus solvent based samples by LC/TOFMS.

Table 3. Analytical Parameters for the Analysis of Terbuthylazine in Olive Oil Samples by Ion trap MS/MS and LC/TOFMS

Method	Concentration range (mg/kg)	Linearity (R^2)	LOD (µg/kg)	RSD (%) n = 5
LC/ITMS	0.005–0.5	0.991	0.2	5.5
LC/TOFMS	0.005–0.5	0.995	1	2

Analysis of Olive Oil Samples

To evaluate the effectiveness of the proposed method, it was applied to the analysis of olive oil samples. An example is shown in Figure 4 where traces of terbuthylazine were found in an olive oil extract; the EIC for *m/z* 230 is also shown. This is a real example illustrating the usefulness of the routine accurate mass measurement capabilities of LC/TOFMS, especially when analyzing traces of pesticides in complex samples such as olive oil. In this sense, the selectivity of LC/TOFMS relies on the resolving power of the instrument on the *m/z* axis, enabling discrimination between the target species and an "isobaric" interference within 0.05 Da of the mass difference (using 230 *m/z*, as example). In the case of terbuthylazine, it is easily overcome using the isotopic chlorine signal or a characteristic fragment not affected by other interfering species.

Conclusion

LC/ITMS and LC/TOFMS can be used for the identification and quantitation of terbuthylazine in olive oil samples after performing several preliminary sample treatments. The analysis by ion trap was achieved in MS/MS mode, monitoring the characteristic fragment ion at *m/z* 174. The identification by LC/TOFMS was accomplished with the accurate mass (and the subsequently generated empirical formula) of the protonated molecule ($[M+H]^+$ *m/z* 230), along with the accurate mass of the fragment ion and the characteristic chlorine isotope cluster present in terbuthylazine. The accuracy typically obtained was routinely better than 2 ppm. The method sensitivity, linearity, precision, accuracy, matrix effects, and LOD were also studied and they supported the potential of LC/TOFMS and LC/ITMS for the routine quantitative analyses of terbuthylazine in olive oil.

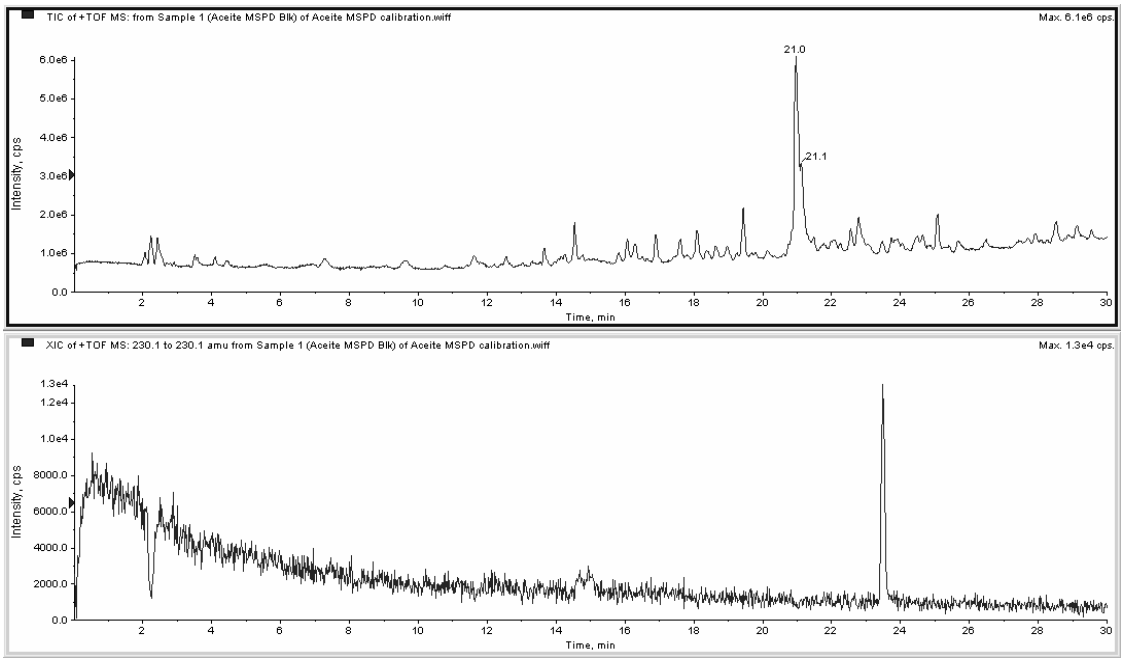

Figure 4. Upper: LC/TOFMS TIC of a "positive" for terbuthylazine in an olive oil sample. Lower: EIC of terbuthylazine using a 20 mDa mass window.

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Acknowledgments

We acknowledge Amadeo Fernandez-Alba, Juan-Francisco García-Reyes and Carmen Ferrer from the Department of Hydrogeology and Analytical Chemistry, University of Almería, for help, ideas, and analytical support.

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Printed in the USA
August 23, 2005
5989-3573EN

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Separation of Aflatoxins by HPLC

Application

Environmental, Food Safety

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Abstract

Four target aflatoxins (B_1, B_2, G_1, and G_2) were separated by HPLC using an isocratic ternary mixture of water, methanol and acetonitrile, and detected using UV 365 nm. A baseline separation was achieved in less than 5.5 min.

Introduction

Aflatoxins are mycotoxins that are produced by various *Aspergillus flavus* molds. Not only are these compounds extremely toxic, but they are also mutagenic, teratogenic (causing fetal abnormalities), and carcinogenic. Unfortunately, *A. flavus* is a common mold found in tropical and subtropical countries and has been found to cause aflatoxin contamination. This contamination is a result of inadequate storage conditions for certain agricultural commodities such as peanuts, cereal seeds, dried fruit, and a wide range of tree nuts such as pistachio, pecans, walnuts, almonds, and herbal seeds such as red and black pepper, cloves, and cinnamon. Because of their toxic and carcinogenic nature, there is a very low minimum detectable quantity (MDQ) for aflatoxin contamination in food.

Chemical Nature

Although 18 different aflatoxins have been identified, the four most prevalent aflatoxins are B_1, B_2, G_1, and G_2, whose chemical structures are shown in Figure 1. Aflatoxin B_1 is one of the most potent and abundant environmental mutagens and carcinogens known. Aflatoxins are quite stable in many foods and are fairly resistant to degradation. Collectively, the aflatoxins are chemical derivatives of difurancoumarin. Pure aflatoxin B_1 is a pale-white to yellow crystalline, odorless solid. Aflatoxins are freely soluble in moderately polar solvents such as chloroform, methanol, and dimethyl sulfoxide, and dissolve in water to the extent of 10–20 mg/L. In methanol, they have fairly strong extinction coefficients (around 10,000) at 265 nm and 360–362 nm. They fluoresce under UV radiation, and fluorescence detection is often used for trace analysis in HPLC. Since there are differences in fluorescence yields between B_1 and B_2, and between G_1 and G_2, it can be useful to run both UV and fluorescence detectors in series [1]. Aflatoxins have no polar functional groups, and can be separated by virtue of their hydrophobicity.

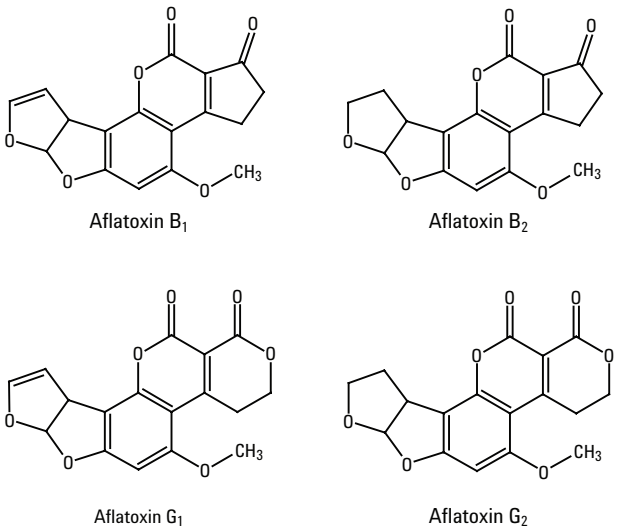

Figure 1. Chemical structures of target aflatoxins.

HPLC Methodology

While thin-layer chromatography was frequently used in the past, HPLC has been used in recent years because of its ease of operation and better quantitation. Most HPLC methods published to date have used reversed-phase HPLC on C18 bonded phases [1–4], where the aflatoxins are separated by their hydrophobicity. Most published separations have been performed on 5-µm columns of 25-cm in length. The use of smaller particle size packings in shorter columns with faster separation times are now in vogue. These columns show that the same separations can be achieved in less time than on the longer columns with similar resolution. In the present study, we desired to show that using such a column can provide improved results compared to the older methods. See Figure 2.

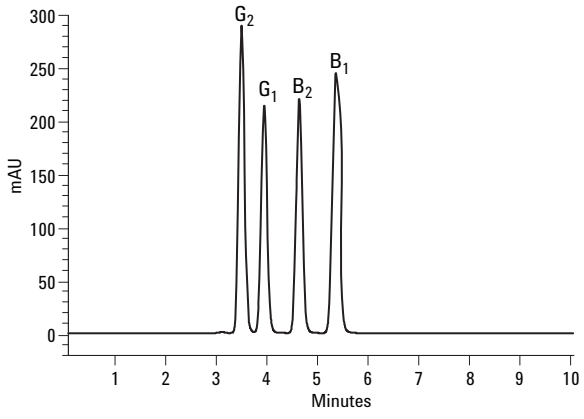

Figure 2. Reversed-phase separation of target aflatoxins using ZORBAX XDB-C18 Rapid Resolution column.

Experimental Conditions

Chemicals:	The aflatoxins were purchased from Sigma Aldrich (Madrid, Spain).

HPLC Conditions

Column:	ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 3.5 µm
Mobile phase:	Water/MeOH/ACN; 50/40/10 (V/V/V)
Flow rate:	0.8 mL/min
Temperature:	Ambient
Detector:	UV 365 nm
Injection volume:	10 µL (0.044 mg/mL)

Results and Discussion

All four aflatoxins were separated using an isocratic ternary mixture of water, methanol, and acetonitrile. A baseline separation was achieved in less than 5.5 min. Although UV detection was shown here, in some cases, lower levels of detection may be obtained for B_2 and G_2 using fluorescence (λ_{ex} = 365 nm, λ_{em} = 455 nm) detection. Mass spectroscopic detection has also been used [1].

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Printed in the USA
August 16, 2005
5989-3634EN

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.

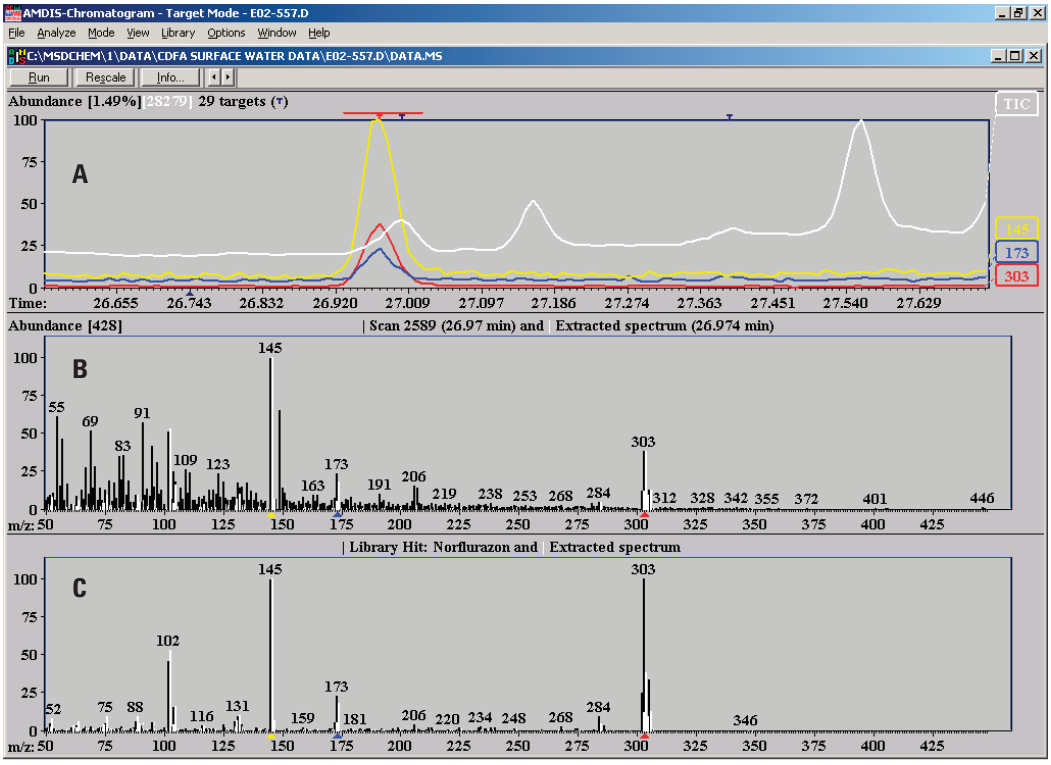

Figure 1. AMDIS screen showing the identification of norflurazon.
A) The total ion and extracted ion chromatograms where norflurazon elutes.
B) The deconvoluted component spectrum (white) juxtaposed with the spectrum at 26.972 min (black).
C) The deconvoluted component matched to the library spectrum 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).

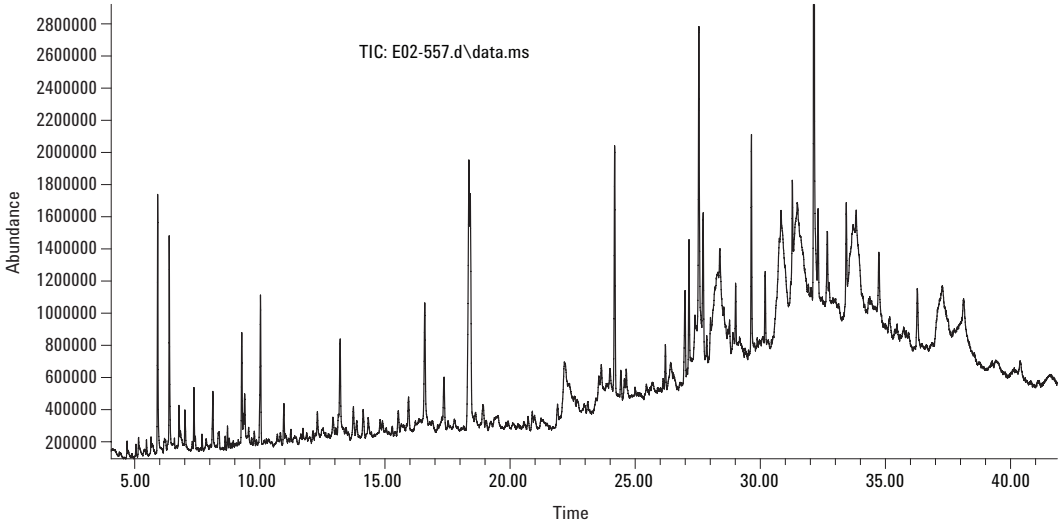

Figure 2. Chromatogram of a surface water extract that was analyzed by DRS using the new RTL Pesticide and Endocrine Disrupter Database. The results of this analysis are shown in Figure 3.

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.

			Agilent			NIST	
RT	**Cas #**	**Compound name**	**ChemStation amount (ng)**	**AMDIS match**	**RT Diff (sec.)**	**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).

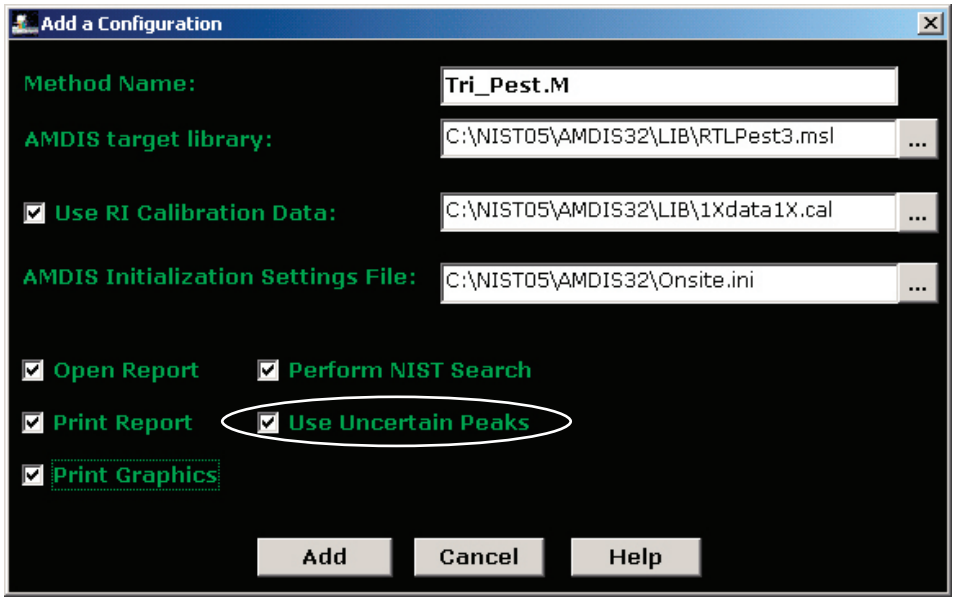

Figure 4. DRS configuration screen for the method called Tri_Pest. When the box labeled "Use Uncertain Peaks" is checked, AMDIS will use uncertain peaks for library searches. When unchecked, AMDIS ignores uncertain mass spectral peaks. Sometimes, this can affect the quality of a library match.

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.

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

Rapid Determination of Hydroxymethylfurfural in Foods Using Liquid Chromatography-Mass Spectrometry

Application

Food Industry

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Abstract

Hydroxymethylfurfural is a product of food deterioration and is still under investigation for possible toxic effects. It can also be used to monitor food quality. A sensitive and selective LC/MS method for monitoring this compound is presented. The method can quantitatively determine hydroxymethylfurfural in food with a detection limit of 0.005 µg/g. Sample preparation and analytical conditions are given.

Introduction

Hydroxymethylfurfural (HMF) is recognized as an indicator of quality deterioration in a wide range of foods. It is formed as an intermediate in the Maillard reaction and is also formed during acid-catalyzed dehydration of hexoses. Formation of HMF in foods is especially dependent on temperature and pH [1].

In recent years, the presence of HMF in foods has raised toxicological concerns: the compound and its similar derivatives were shown to have cytotoxic, genotoxic, and tumoral effects. However, further studies suggest that HMF does not pose a serious health risk, but the subject is still a matter of debate.

Several HPLC techniques were reported for the determination of HMF in various foods. These techniques use UV detection because of the strong absorption of furfurals at approximately 280 to 285 nm. However, many compounds naturally present or formed in foods during processing may also absorb at this wavelength. Poor chromatographic resolution of these compounds may adversely affect the quantification of HMF during UV detection.

A rapid and reliable liquid chromatography/mass spectrometry (LC/MS) method was developed for the determination of HMF in foods. The method entailed aqueous extraction of HMF, solid-phase extraction (SPE) cleanup and analysis by LC/MS. The separation was performed on a narrow-bore column to shorten the chromatographic run.

Experimental

LC/MS experiments were performed using an Agilent 1100 series HPLC system consisting of a binary pump, an autosampler, and a temperature-controlled column oven, coupled to an Agilent 1100 MS detector equipped with atmospheric pressure chemical ionization (APCI) interface.

Data acquisition was performed in selected ion monitoring (SIM) mode using the interface parameters: drying gas (N_2, 100 psig) flow of 4 L/min, nebulizer pressure of 60 psig, drying gas temperatures of 325 °C, vaporizer temperature of 425 °C, capillary voltage of 4 kV, corona current of 4 µA, fragmentor voltage of 55 eV, and dwell time of 439 ms. Ions monitored for HMF were *m/z* 109 and *m/z* 127. The quantification was performed based on the signal response of the ion having *m/z* of 109.

LC/MS

Flow rate:	0.2 mL/min
Gradient:	ZORBAX Bonus RP, 100 mm × 2.1 mm, 3,5 µm
Mobile phase:	0.01 mM acetic acid in 0.2% aqueous solution of formic acid
Injection:	20 µL out of 1000 µL

MS conditions

Ionization mode:	Positive APCI
Nebulizer pressure:	60 psi
Drying gas flow:	4 L/min
Drying gas temperature:	325 °C
Vaporizer temperature:	425 °C
Skimmer:	20 V
Capillary voltage:	4kV
Fragmentor voltage:	55 eV
Dwell time:	439 ms

The chromatographic separations were performed on a ZORBAX Bonus RP Narrow Bore column (2.1 mm × 100 mm, 3.5 µm) using the isocratic mixture of 0.01 mM acetic acid in 0.2% aqueous solution of formic acid at a flow rate of 0.2 mL/min at 40 °C.

Method

Sample Preparation

Finely ground sample (1 g) was weighed into a 10-mL glass centrifuge tube with cap. Carrez I and II solutions were prepared by dissolving 15 g of potassium hexacyanoferrate and 30 g of zinc sulfate in 100 mL of water, respectively. A total of 100 µL Carrez I and 100 µL Carrez II solutions were added to the sample and the volume completed to 10 mL with 0.2 mM acetic acid. HMF was extracted by mixing the tube for 3 min using a vortex mixer. It was then centrifuged for 10 min at 5,000 rpm at 0 °C. The clear supernatant was further cleaned up by using Oasis HLB SPE cartridge. Prior to use, the SPE cartridge was conditioned by passing 1 mL of methanol and equilibrated by passing 1 mL of water at a flow rate of approximately two drops per second using a plastic 2-mL syringe. The excess water was removed from the cartridge by passing 2 mL of air. One milliliter of aqueous extract was eluted through the preconditioned cartridge at a flow rate of approximately one drop per second using a plastic syringe and the eluate was discarded. The cartridge was washed by passing 0.5 mL of water. Then the cartridge was dried under a gentle stream of nitrogen. HMF was eluted from the cartridge by passing 0.5 mL of diethyl ether at a flow rate of approximately one drop per second using a plastic 2-mL syringe. The eluate was collected in a conical bottom glass test tube placed in a water bath at 40 °C (Zymark Turbo Vap® LV Evaporator) and evaporated to dryness under nitrogen at 3 psig. The remaining residue was immediately redissolved in 1 mL of water by mixing in a vortex mixer for 1 min. Twenty microliters of this test solution was injected onto the HPLC system.

Results and Discussion

Positive APCI-MS analysis of HMF showed both the precursor [M+1] ion and the compound-specific ion [$C_6H_5O_2$] due to loss of water from the protonated molecule. See Figure 1. These characteristic

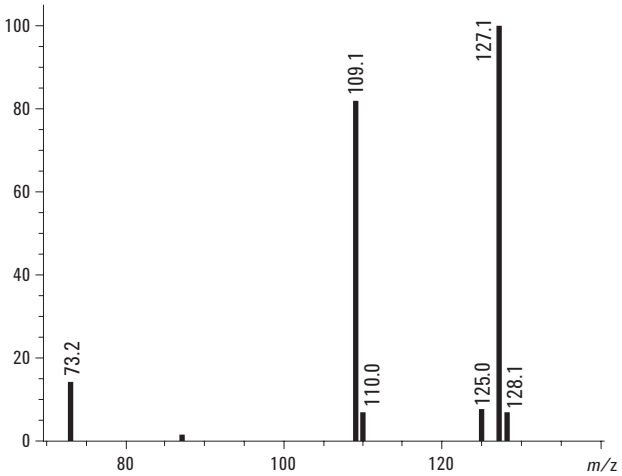

Figure 1. Mass spectrum for HMF obtained with positive APCI.

ions having *m/z* of 127 and 109 were used to monitor HMF in SIM mode. The ratio of these ions (response of ion 127/response of ion 109 = 1.12) was used to confirm the purity of HMF peak. The signal response was linear over a concentration range of 0.05 to 2.0 µg/mL for both ions with correlation coefficients of higher than 0.99. On the basis of a signal-to-noise ratio of 3, the limit of detection (LOD) was determined to be 0.005 µg/mL and 0.006 µg/mL for ions having *m/z* 127 and *m/z* 109, respectively. LC/MS with APCI was found to be a powerful tool that allowed us to determine HMF sensitively and precisely.

The chromatographic separation of HMF was performed on a ZORBAX Bonus RP narrow-bore column. The solution of 0.01 mM acetic acid in 0.2% aqueous solution of formic acid was used as the mobile phase at a flow rate of 0.2 mL/min to increase the ionization yield during MS detection with an adequate separation of HMF in the column from interfering matrix co-extractives. Under these conditions, HMF eluted at 5.087 min with good retention time reproducibility (5.09 ±0.04 min, n = 10). See Figure 2. The capacity factor (k') was determined to be 2.33 for HMF based on the hold-up time of 1.55 min.

Usual approach for the extraction of free furfurals from solid food matrices entails extraction with water followed by clarification using Carrez I and II reagents. Direct LC/MS analysis of aqueous extract showed the presence of interfering compounds. Oasis HLB cartridge packed with a macroporous copolymer of the lipophilic divinylbenzene and the hydrophilic N-vinylpyrrolidone was, there-

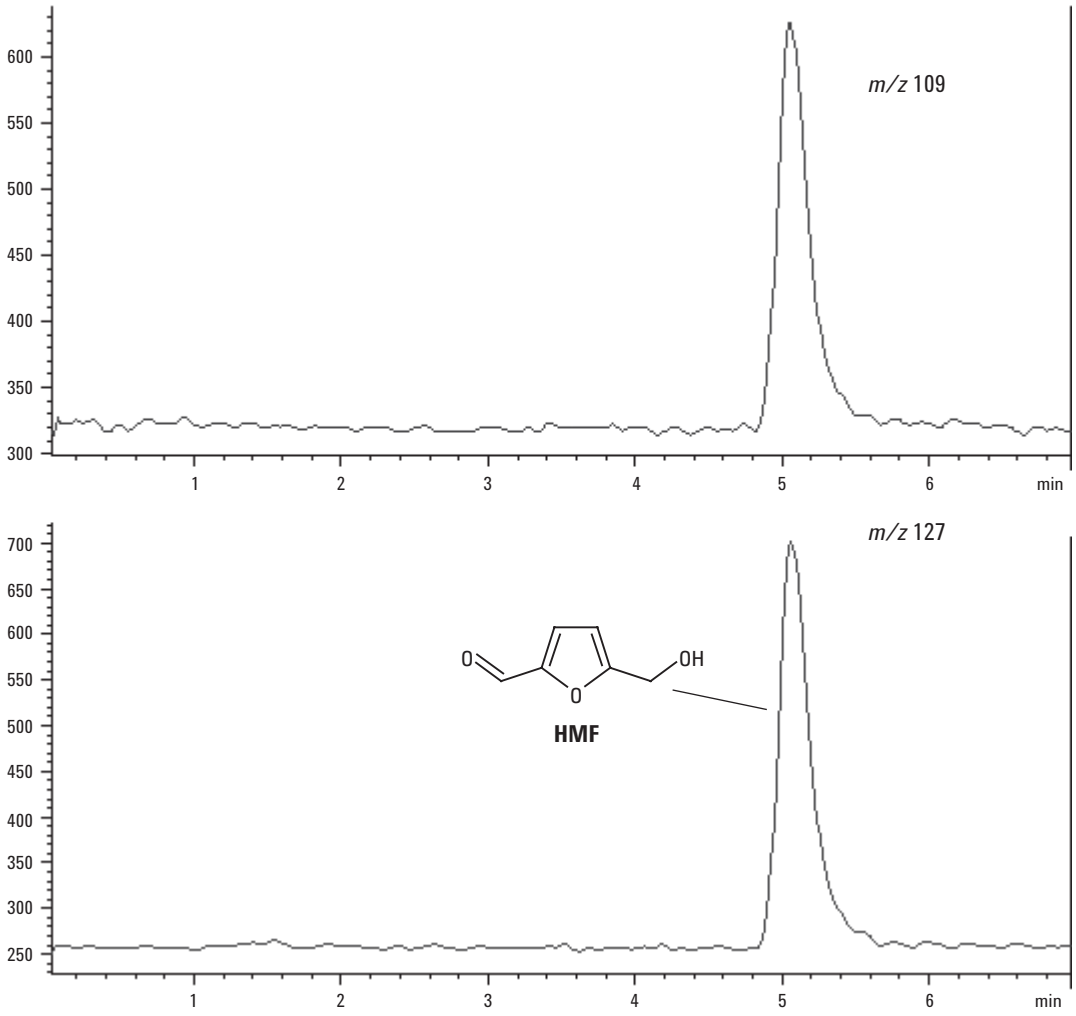

Figure 2. Extracted ion chromatogram (EIC) of an HMF standard (HMF concentration is 100 ng/mL).

fore, used to clean the extract prior to LC analysis. The clear aqueous extract was passed through a preconditioned cartridge. HMF present in the extract strongly interacted with the sorbent material while much of the co-extractives did not. HMF retained in the cartridge was then eluted with diethyl ether. It was determined that 0.5 mL of diethyl ether was sufficient to recover HMF from the cartridge completely.

SPE cleanup brought significant improvement for the detection of HMF using MS in SIM mode. Total ion chromatogram indicated the presence of three major peaks in the sample. HMF peak was identified by comparing both retention time and mass spectral data. The ratio of characteristic ions having *m/z* 127 and *m/z* 109 also confirmed the purity of HMF peak. The compound-specific ion [$C_6H_5O_2$] having *m/z* of 109 was found to be more selective than the parent compound ion. So, the quantification of HMF was performed using the signal response recorded for this ion.

The accuracy of the method was verified by analyzing spiked cereal-based baby foods. The recovery of HMF was determined by analyzing each of the

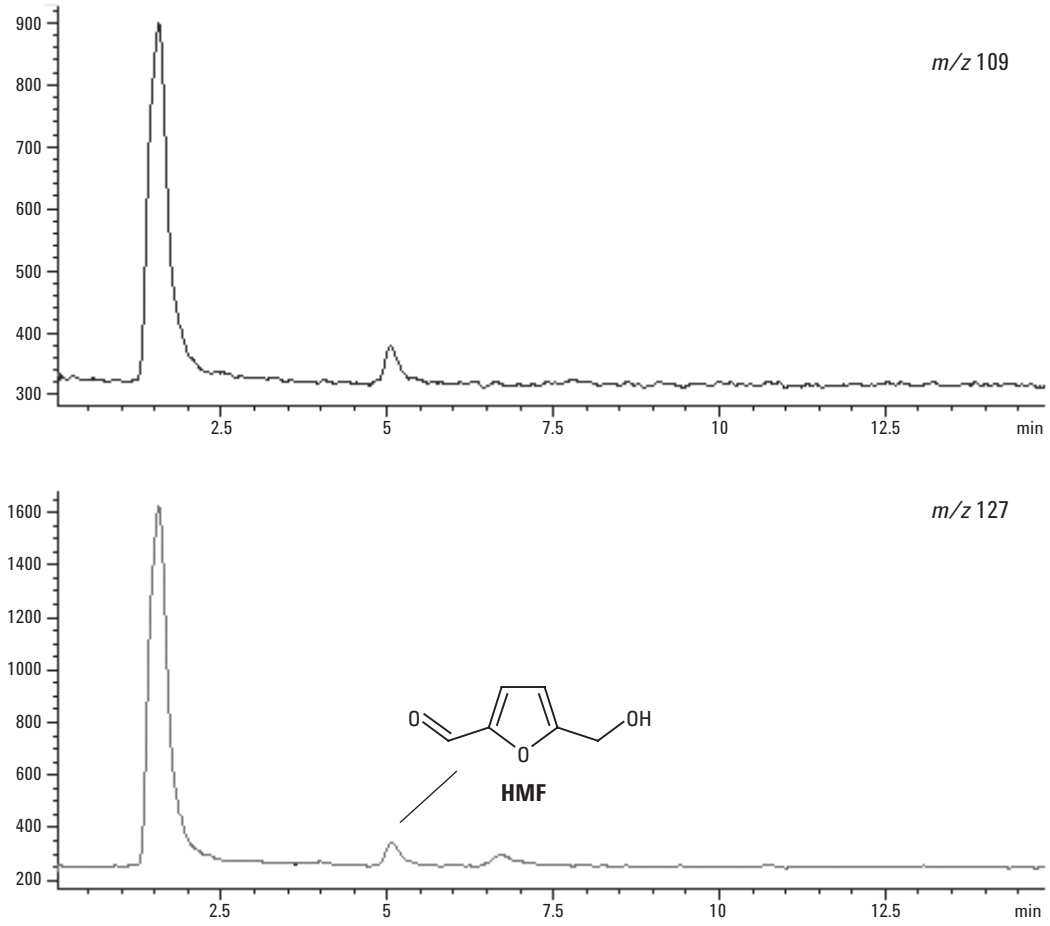

Figure 3. EIC of a fruited yogurt sample (HMF concentration is 0.2 µg/g).

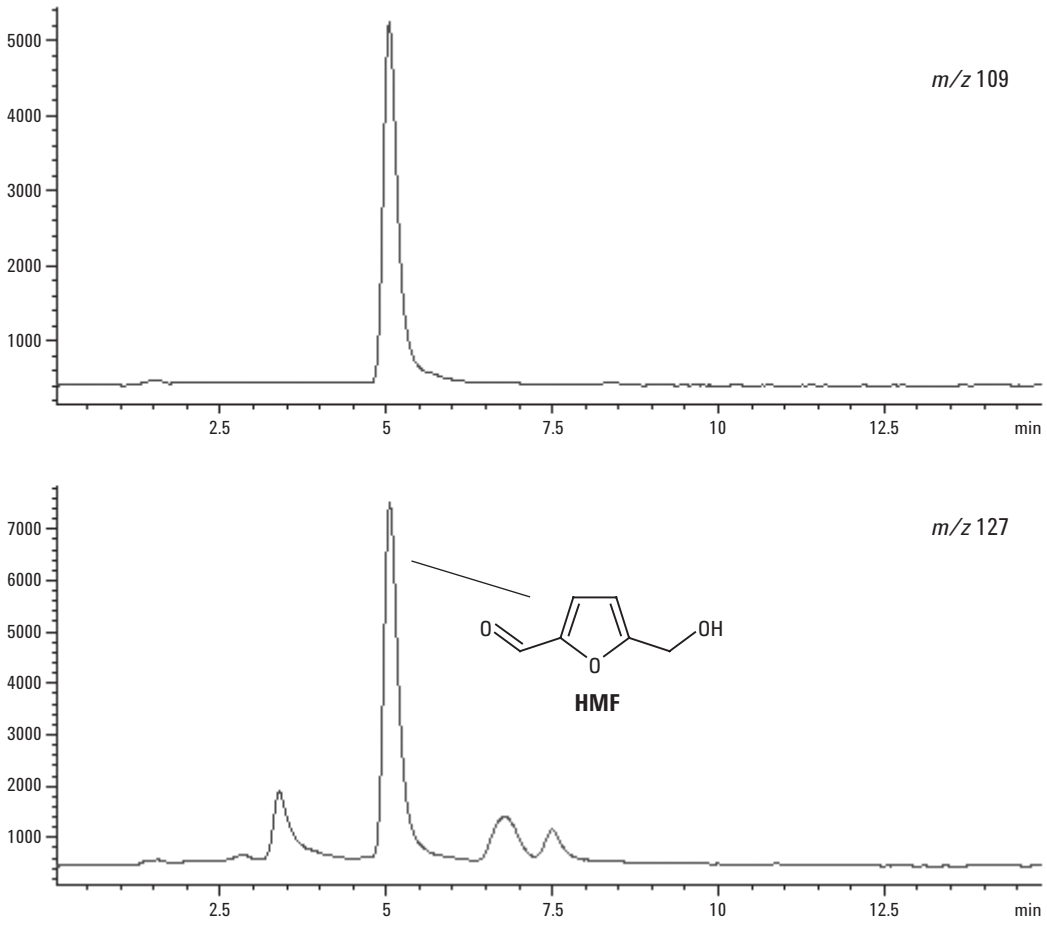

Figure 4. EIC of a crisp bread sample (HMF concentration is 17.5 µg/g).

spiked samples four times for spiking levels ranging from 0.25 to 5.0 µg/g. The mean percentage recoveries exceeded 90% for all levels.

The method is capable of low concentrations, but also high concentrations of HMF in foods precisely and accurately. Figure 3 illustrates the EICs of a fruited yogurt sample having 0.2 µg/g of HMF. It is difficult to measure such a low concentration of HMF using LC coupled to UV detection. Figure 4 illustrates the EICs of a crisp bread sample having 17.5 µg/g of HMF.

Conclusion

The growing attention of the scientific community with regard to the potentially toxic effects of HMF requires new efforts to be made to establish new rapid, reliable, and sensitive methods to determine HMF in real matrices. Previous methods usually dealt with the food items where HMF concentrations are comparatively higher and use extraction procedures that usually do not avoid potential interfering compounds prior to LC analysis. Presence of interferences may be problematic, particularly during the UV detection after LC separation when low concentrations of HMF are being measured in baby foods. The method described in this application combines 1) a rapid separation of HMF from the matrix co-extractives in a narrow-bore column, 2) an efficient cleanup of the extract using SPE, and 3) a selective detection of HMF using MS in a single analytical method.

Reference

1. V. Gökmen, H. Z. Senyuva, Improved method for the determination of hydroxymethylfurfural in baby foods using liquid chromatography-mass spectrometry, *Journal of Agricultural and Food Chemistry*, 2006, 54, 2845–2849.

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Printed in the USA
August 3, 2006
5989-5403EN

Agilent Technologies

Determination of 44 Pesticides in Foodstuffs by LC/MS/MS

Application

Food Safety

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Abstract

A sensitive and selective analytical method for the determination of 44 pesticide residues in several foodstuffs using the Agilent G6410AA Triple Quadrupole Mass Spectrometer (QQQ) was developed. This method use two different sample preparation methods followed by LC/MS/MS (liquid chromatography/tandem mass spectrometry). The limits of detection for all pesticides were less than 10 ng/mL in foodstuff. The sensitivity of QQQ easily met the maximum residue limits (MRLs) of all investigated pesticides in Japan Food Hygiene Law.

Introduction

Pesticides are widely used in agricultural practices. The main application can be classified in production and post-harvest treatment of agricultural commodities for transport purposes. In this sense, production agriculture comprises the main category of use of pesticides subject to control requirements and, therefore, maximum residue levels (MRLs) have been fixed to assess food safety. In recent years, the established regulations regarding MRLs in commodities have been more and more stringent. In Japan, the positive list system was introduced this year, and MRLs have been set for over 500 pesticides in all foodstuffs. This new system sets different MRLs for each pesticide within each food group. Typically, the MRLs range from 0.01 to 3 µg/g depending on the commodities and pesticides. The low MRLs fostered the development of more sensitive analytical methods to meet the requirements of complex samples. In this sense, liquid chromatography/tandem mass spectrometry (LC/MS/MS) with QQQ in multiple reaction monitoring (MRM) mode has become so far the most widely used techniques for the quantitation of polar pesticides in food. MRM mode provides for more specific detection in a complex matrix such as food. In this work, 44 pesticides (Tables 1 and 2) are analyzed in two separate runs with sample analytical conditions. The sensitivity requirements set by the positive lost system for these pesticides are easily met.

Experimental

Chemicals

The acetonitrile was of LC/MS grade from Wako Pure Chemical Ind (Japan). Toluene, acetone, n-hexane, formic acid, sodium chloride, and anhydrous sodium sulfate were of analytical grade from Wako Pure Chemical. All SPE cartridges were purchased from Spelco Japan (Japan). Pesticide standards were obtained from Hayashi Pure Chemical (Japan).

Sample Preparation

Extraction

Vegetable and fruit samples were obtained from the local markets. A sample of 10 to 500 g was chopped in a food processor to obtain thoroughly mixed homogenates. A 20-g portion of sample homogenate was weighed in a 200-mL PTFE centrifuge tube. Then 50 mL of acetonitrile was added and blended in a Polytoron. The extract was then filtered by applying vacuum. The filtrate was collected and the residue was re-extracted with 20 mL of acetonitrile. The filtrates were combined in a 100-mL volumetric flask and made up to volume with acetonitrile. A 20-mL portion of the extract was transferred into a PTFE centrifuge tube, and 10 g of NaCl and 20 mL of 0.5 M phosphate buffer (pH 7.0) were added to the extract followed by shaking for 5 min. Five grams of anhydrous Na_2SO_4 were added to the acetonitrile layer obtained after salting out. After removing anhydrous Na_2SO_4, the extract was evaporated to dryness by rotary evaporator (water bath temperature did not exceed 40 °C). The residue was dissolved in 2 mL of acetonitrile-toluene (3:1).

Cleanup

Group 1 - The extract was loaded into a GCB/amino propyl SPE cartridge (500 ng/500 mg) preconditioned with 10 mL of acetonitrile-toluene (3:1). The 20 mL of acetonitrile-toluene (3:1) was further added to the SPE cartridge. All eluate was collected and evaporated by rotary evaporator. The residue was dissolved in 4 mL of methanol.

Group 2 - The extract was loaded into a silica gel SPE cartridge (500 mg) preconditioned with 10 mL each of methanol, acetone, and n-hexane (10 mL of methanol, 10 mL of acetone, and 10 mL of n-hexane, total volume is 30 mL). The 10 mL of acetone-triethylamine-n-hexane (20:0.5:80) was further added to the SPE cartridge. All eluate was discarded. The 20 mL of acetone-methanol (1:1) was applied and the eluate was collected and evaporated by rotary evaporator. The residue was dissolved in 4 mL of methanol.

Standard Preparation

Stock solutions of individual pesticides were prepared in methanol at 1 µg/mL. Serial dilutions using methanol produced a range of standard mixture solutions at 0.001 µg/mL to 1 µg/mL.

The blank matrix residues were fortified with a mixture of pesticides studied at 10 ng/g.

LC/MS/MS Instrument

The LC/MS/MS system used in this work consists of an Agilent 1100-series vacuum degasser, binary pump, well-plate autosampler, thermostatted column compartment, and the Agilent G6410 Triple Quadrupole Mass Spectrometer with an electrospray ionization source (ESI). The objective of the method development was to obtain a fast and sensitive analysis for quantifying pesticides in fruits and vegetables. For chromatographic resolution and sensitivity, different solvents and columns were optimized. It was found that a simple solvent system using water, acetonitrile, formic acid, formic acetate, and a 1.8-µm particle size C18 column would work very well.

LC Conditions

Instrument:	Agilent 1100 HPLC
Column:	ZORBAX Extend C18, 100 mm × 2.1 mm, 1.8 µm (p/n 728700-902)
Column temp:	40 °C
Mobile phase:	A = 0.1% formic acid +5 mM ammonium formate in water B= Acetonitrile
Gradient:	10% B at 0 min, 80% B at 30 min
Flow rate:	0.2 mL/min
Injection vol:	5 µL

MS Conditions

Instrument:	Agilent 6410 QQQ
Source:	Positive ESI
Drying gas flow:	10 L/min
Nebulizer:	50 psig
Drying gas temp:	350 °C
V_{cap}:	4000 V
Scan:	*m/z* 100 to 550
Fragmentor:	Variable 100 V
MRM ions:	Shown in Tables 1 and 2
Collision energy:	Shown in Tables 1 and 2

LC/MS/MS Method

Quantitative analysis was carried out using MRM mode with time program. The parameters of MRM transition are shown in Tables 1 and 2.

Table 1. Data Acquisition Parameters of MRM Transitions of Each Pesticide in Group 1

No	Pesticides	RT (min)	Molecular weight	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy(V)
1	Thiabendazole	5.018	201	202	175	20
2	Thiamethoxam	6.16	291	292	211	5
3	Clothianidin	7.83	249	250	169	10
4	Chloridazon	8.19	221	222	104	10
5	Imidacloprid	8.39	255	256	209	20
6	Dimethirimol	8.8	209	210	171	20
7	Oxycarboxine	11.02	267	268	175	10
8	Thiacloprid	11.03	252	253	126	20
9	Azamethiophos	12.87	324	325	183	10
10	Ferimzone(E)	13.21	254	255	124	20
11	Ferimzone(Z)	13.7	254	255	132	20
12	Phenmedipham	17.77	317	318	136	20
13	Azinphos-methyl	17.9	318	132	77	15
14	Simeconazole	18.5	293	294	70	15
15	Isoxaflutol	18.7	359	360	251	15
16	Pyriftalid	18.7	318	319	139	20
17	Tridemorph	19.21	297	298	130	15
18	Methoxyfenozide	20.06	312	313	149	20
19	Chromafenozide	20.57	394	175	141	20
20	Fenoxycarb	20.63	301	302	88	15
21	Naproanilide	21.27	291	292	171	10
22	Butafenacil	21.55	491	492	331	20
23	Cyazofamide	21.7	324	325	108	10
24	Anilofos	22.5	367	368	199	10
25	Pyrazolate	23.5	438	439	173	15
26	Benzofenap	24	430	431	105	20
27	Cyflufenamid	24.3	412	413	241	20
28	Indoxacarb	24.37	527	528	150	15
29	Clomeprop	24.78	372	373	299	5
30	Cloquincet-mexyl	24.8	335	336	238	15
31	Furathiocarb	25.7	365	383	195	15
32	Lactofen	26.3	478	479	344	15
33	Tralkoxydim	26.7	329	330	284	10

Table 2. Data Acquisition Parameter of MRM Transitions of Each Pesticide in Group 2

No	Pesticides	RT (min)	Molecular weight	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)
1	Flumetsulam	9.96	325	326	129	20
2	Thidiazuron	11.95	220	221	102	10
3	Imazaquin	12.25	311	312	267	20
4	Thifensulfuron-methyl	12.89	387	388	167	10
5	Florasulam	13.75	359	360	129	20
6	Forchlorfenuron-methyl	14.63	247	248	129	10
7	Clorasulam-methyl	16.41	429	430	398	10
8	Diclosulam	16.83	405	406	161	20
9	Fomesafen	18.27	438	456	344	10
10	Triflusulfuron-methyl	19.29	492	493	264	15
11	Haloxyfop	19.67	361	362	316	15

Results and Discussion

Optimization of MRM Transitions

Determination of the optimal MRM transitions for each pesticide was carried out using full scan mode followed by product ion scan mode using two pesticide standard mixtures at 1 µg/mL. TICs of these standard mixtures in full scan mode and product ion scan mode are shown in Figures 1 and 2. The mass spectrum of each pesticide by full scan mode exhibited protonated molecular ions; $[M+H]^+$ as the base peak ion except azinphos-methyl, furathiocarb, and fomesafen, which exhibited fragment ion and ammonium adduct ion $[M+NH_4]^+$. These ions were selected as precursor ions for MRM mode. It was possible to generate individual product ion MS/MS spectrum of each pesticide by using multiple acquisition and time programming mode. As shown in Tables 1 and 2, 10 time segments for 33 pesticides in group 1 and 7 time segments for 11 pesticides in group 2 were used for MRM mode.

Total ion chromatograms of pesticide standard mixture corresponding to the minimum MRL value for pesticides (10 ng/mL) are shown in Figure 3. These show excellent signal-to-noise (S/N) ratios for all pesticides. The limit of detection (LOD) for each pesticide was determined using an S/N ratio of 3 with an MRM chromatogram of each pesticide at 1 ng/mL (see Table 3). To evaluate the linearity of the calibration curves, various concentrations of pesticide standard solutions ranging from 0.001 ng/mL to 1 ng/mL were analyzed. As shown in Table 3, the linearity was very good for all pesticides with correlation coefficients (r^2) greater than 0.998

The matrix effect of this method was investigated by using orange, apple, potato, and cabbage extracts spiked with pesticide standards at 10 ng/mL. Typical MRM chromatograms of orange extract are shown in Figures 4 and 5. The other chromatograms of apple, potato, and cabbage extract are shown in Figure 6. There was not additional peak from sample matrix in all food when compared with the pesticide standard mixture. These results indicate that MRM mode has very high selectivity.

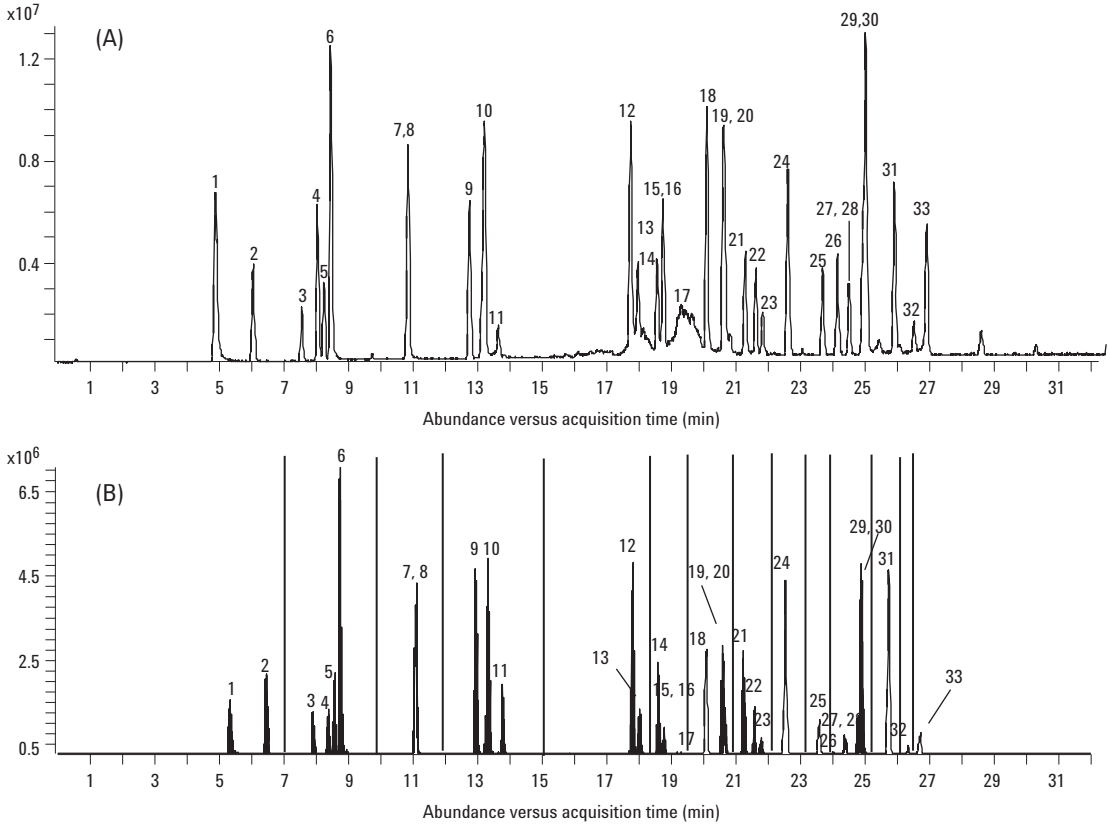

Figure 1. TIC of 33 pesticides standard in full scan mode (A) and product ion scan mode (B) at 1 µg/mL.

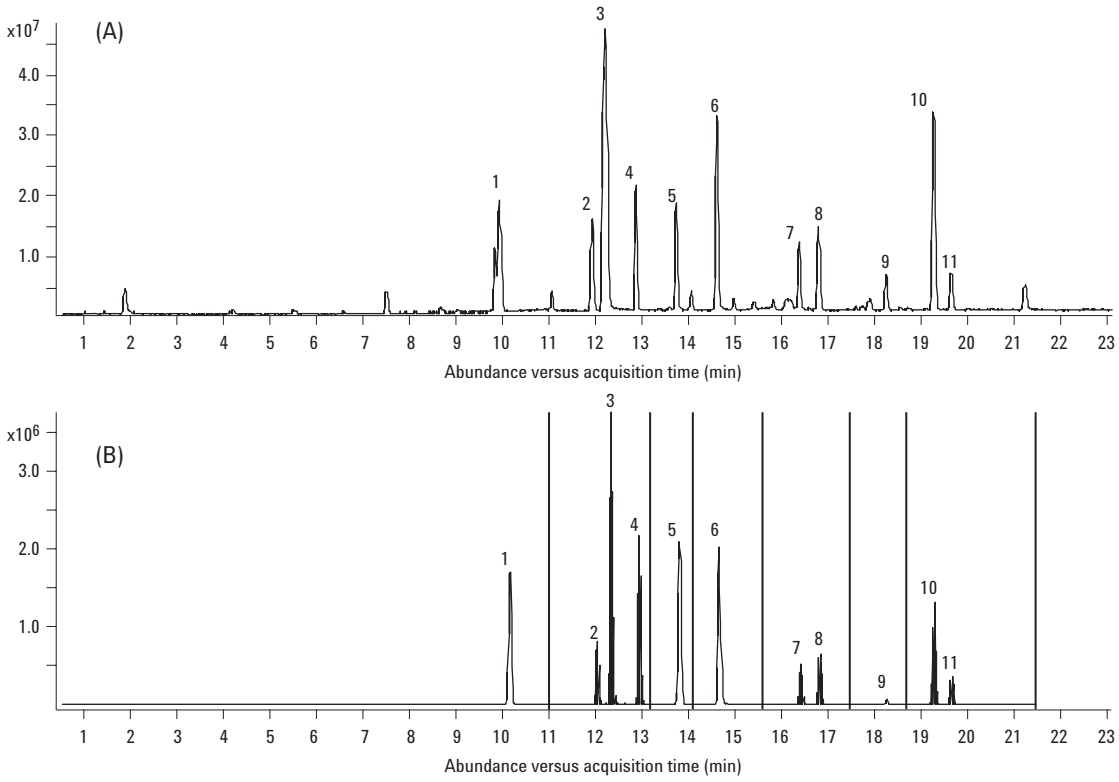

Figure 2. TIC of 11 pesticides standard in full scan mode (A) and product ion scan mode (B) at 1 µg/mL.

Furthermore, the change on the peak intensity of each pesticide by sample matrix was calculated by comparing with the peak intensity of pesticide standards. As these results show in Table 4, the relative intensity of each pesticide ranged from 91 to 116%. Thus, matrix effect such as ion suppression may be insignificant and it was possible to use external standards instead of matrix matched standards. The repeatability of each pesticide in orange extract is also shown in Table 4, and the RSD of each pesticide was in the range from 1.7 to 5.9%.

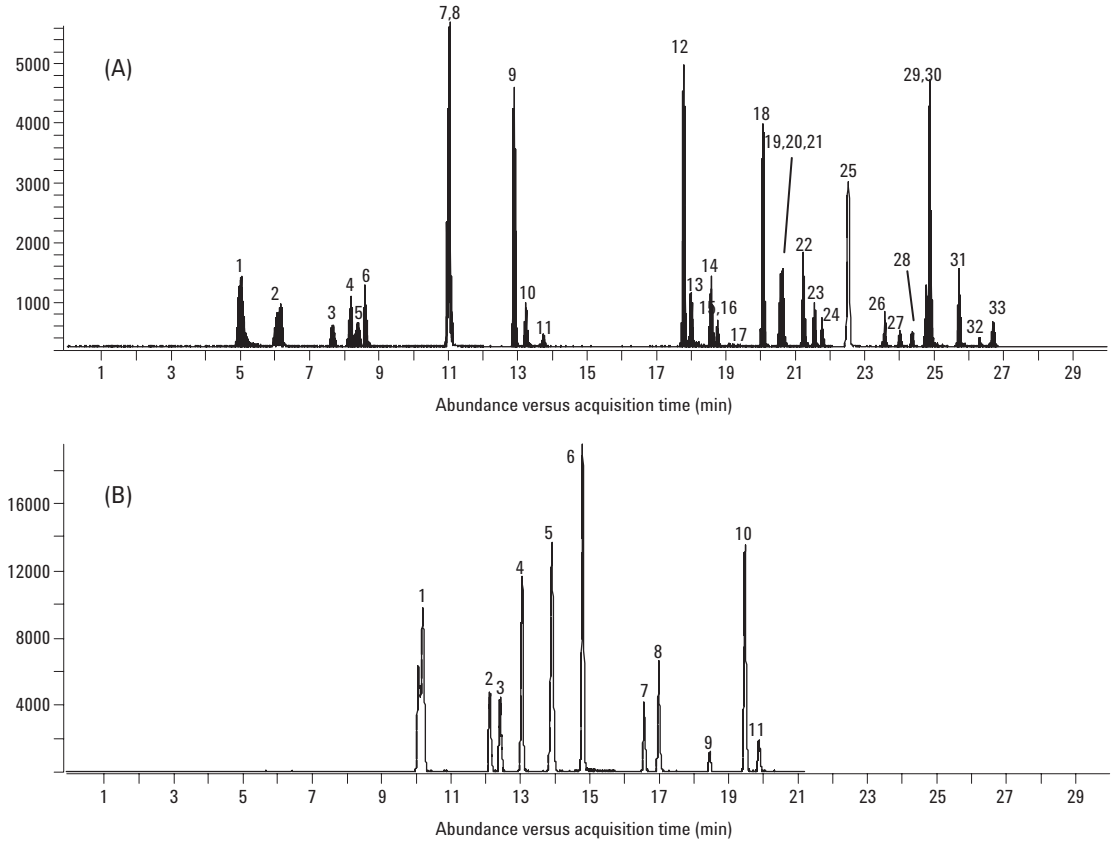

Figure 3. TIC of 33 pesticide standards (A) and 11 pesticides standard (B) at 10 ng/mL in MRM mode.

Table 3. Linearity and LOD of 44 Pesticide Standard Solutions

No	Pesticides	r^2	LOD (ng/mL)	No	Pesticides	r^2	LOD (ng/mL)
Group 1							
1	Thiabendazole	0.9999	<0.1	18	Methoxyfenozide	0.9993	0.55
2	Thiamethoxam	0.9992	<0.1	19	Chromafenozide	0.9992	0.49
3	Clothianidin	0.9999	<0.1	20	Fenoxycarb	0.9988	<0.1
4	Chloridazon	0.9993	<0.1	21	Naproanilide	0.9993	<0.1
5	Imidacloprid	0.9995	<0.1	22	Butafenacil	0.9994	<0.1
6	Dimethirimol	0.9989	<0.1	23	Cyazofamide	0.9987	0.43
7	Oxycarboxine	0.9993	<0.1	24	Anilofos	0.9991	<0.1
8	Thiacloprid	0.9991	<0.1	25	Pyrazolate	0.9990	0.51
9	Azamethiophos	0.9988	<0.1	26	Benzofenap	0.9982	0.49
10	Ferimzone(E)	0.9993	0.34	27	Cyflufenamid	0.9993	0.43
11	Ferimzone(Z)	0.9995	0.53	28	Clomeprop	0.9993	0.61
12	Phenmedipham	0.9993	<0.1	29	Indoxacarb	0.9991	1.04
13	Azinphos-methyl	0.9997	<0.1	30	Quinclorac-methyl	0.9988	0.63
14	Simeconazole	0.9992	<0.1	31	Furathiocarb	0.9987	<0.1
15	Isoxaflutol	0.9991	<0.1	32	Lactofen	0.9987	1.10
16	Pyriftalid	0.9988	<0.1	33	Tralkoxydim	0.9992	0.52
17	Tridemorph	0.9991	1.21				
Group 2							
1	Flumetsulam	0.9996	<0.1	7	Clorasulam-methyl	0.9987	<0.1
2	Thidiazuron	0.9994	<0.1	8	Diclosulam	0.9989	<0.1
3	Imazaquin	0.9992	<0.1	9	Fomesafen	0.9989	0.32
4	Thifensulfuron-methyl	0.9989	<0.1	10	Triflusulfuron-methyl	0.9992	<0.1
5	Florasulam	0.9969	<0.1	11	Haloxyfop	0.9995	0.19
6	Forchlorfenuron-methtyl	0.9977	<0.1				

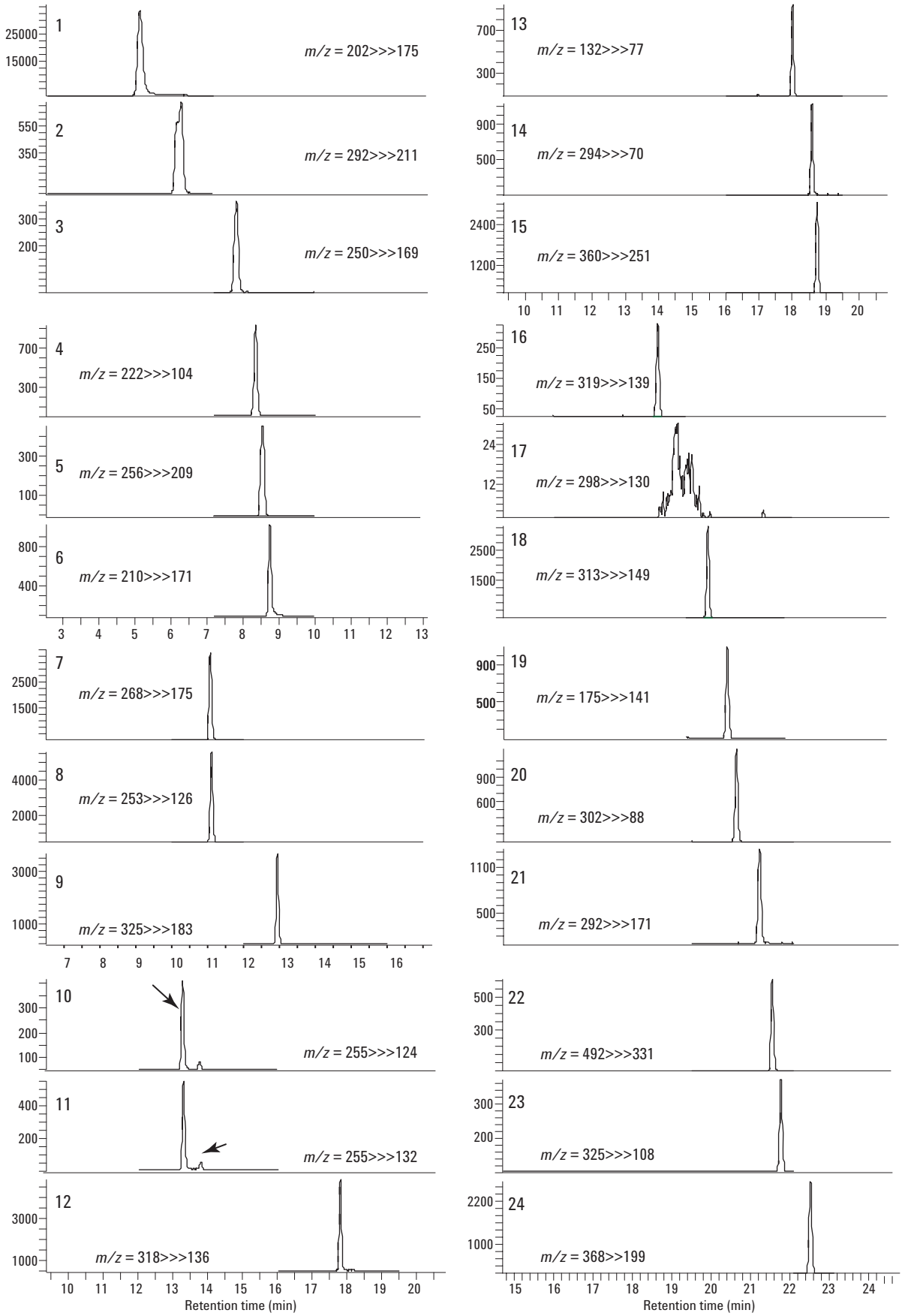

Figure 4. MRM of 33 pesticides in orange extract spiked at 10 ng / mL. (Continued)

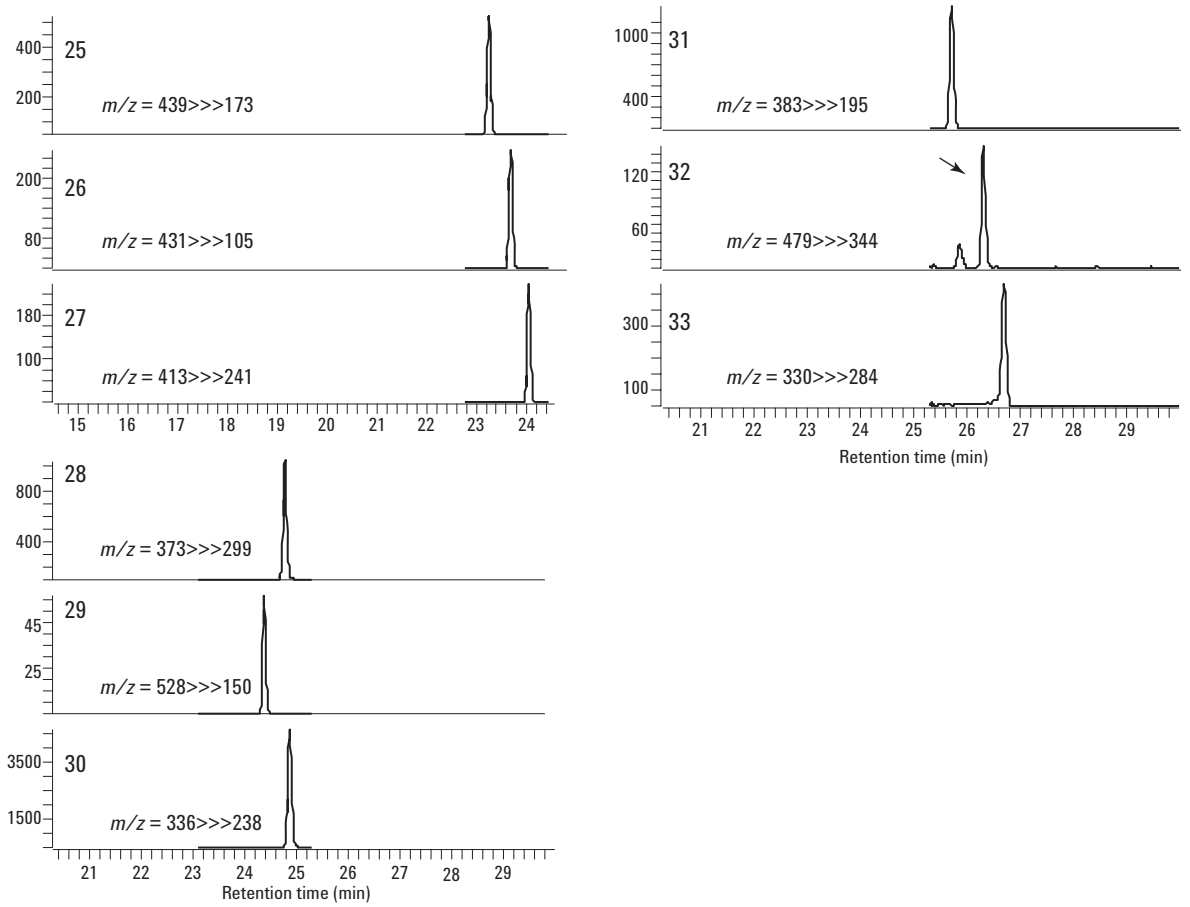

Figure 4. MRM of 33 pesticides in orange extract spiked at 10 ng / mL.

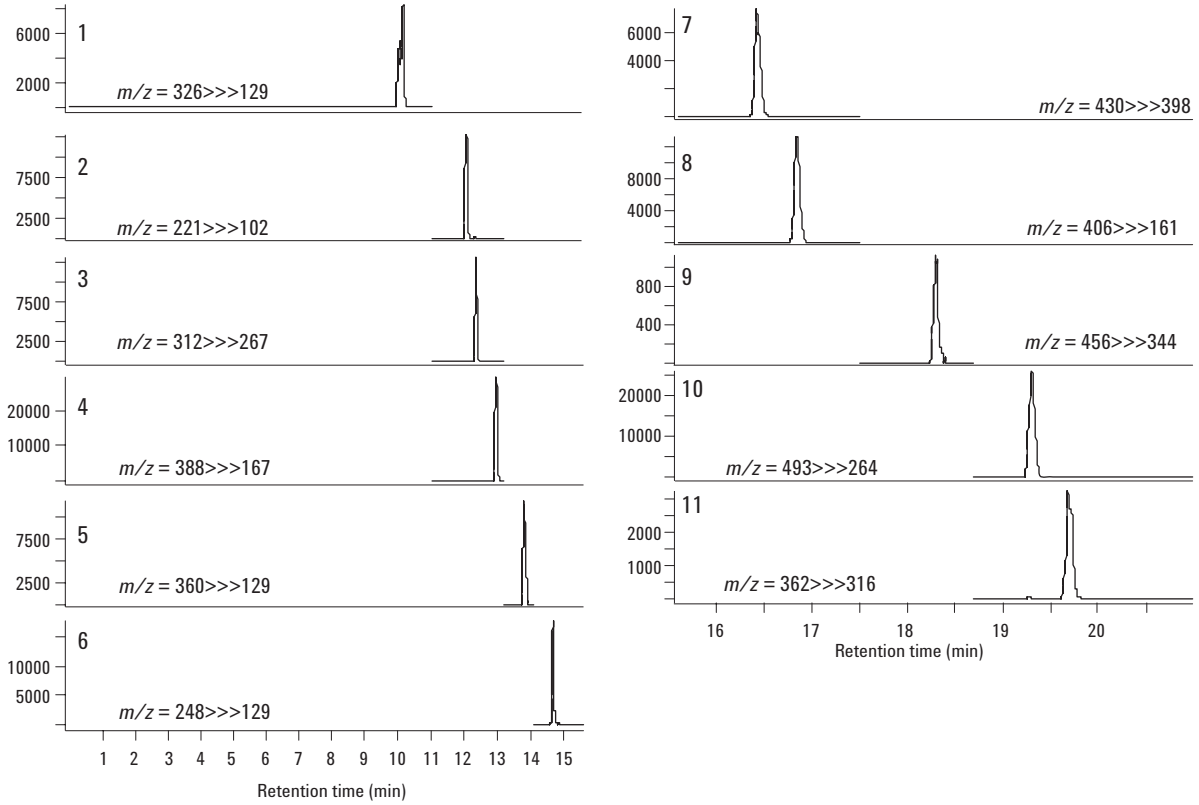

Figure 5. MRM of 11 pesticides in orange extract spiked at 10 ng / mL.

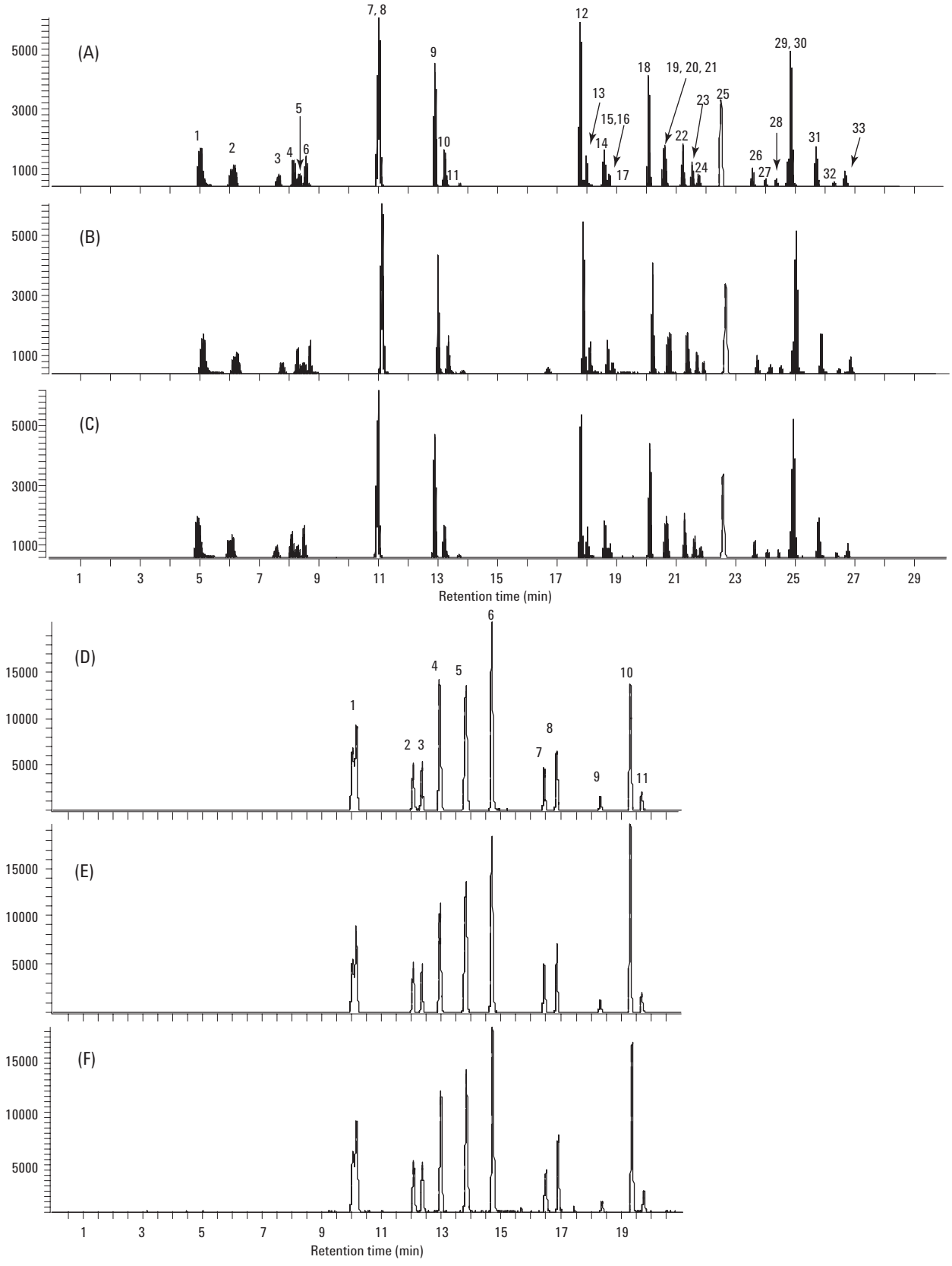

Figure 6. TIC of spiked at 10 ng/mL.

Table 4. Relative Intensity of Each Pesticide in Sample Extracts

No	Pesticides	Relative intensity(%)			
Group 1		**Orange***	**Cabbage**	**Apple**	**Potato**
1	Thiabendazole	105 (3.2)	101	116	107
2	Thiamethoxam	103 (2.1)	98	104	105
3	Clothianidin	106 (2.9)	101	109	112
4	Chloridazon	105 (3.3)	106	101	109
5	Imidacloprid	102 (1.7)	97	102	104
6	Dimethirimol	103 (4.6)	107	103	108
7	Oxycarboxine	106 (3.7)	102	104	106
8	Thiacloprid	104 (3.1)	104	106	108
9	Azamethiophos	93 (4.6)	90	94	84
10, 11	Ferimzone(E,Z)	116 (4.1)	109	102	112
12	Phenmedipham	96 (5.3)	99	100	104
13	Azinphos-methyl	90 (2.1)	103	104	110
14	Simeconazole	104 (4.4)	102	106	110
15	Isoxaflutol	102 (2.7)	104	108	103
16	Pyriftalid	97 (4.1)	103	104	93
17	Methoxyfenozide	92 (3.1)	99	104	97
18	Chromafenozide	96 (2.8)	102	103	101
19	Tridemorph	97 (3.4)	96	100	111
20	Fenoxycarb	99 (2.1)	105	102	101
21	Naproanilide	91 (4.3)	97	98	103
22	Butafenacil	102 (2.6)	114	104	114
23	Cyazofamide	93 (3.5)	92	87	95
24	Anilofos	102 (2.7)	105	103	107
25	Pyrazolate	103 (4.7)	101	103	97
26	Benzofenap	108 (5.2)	111	98	108
27	Cyflufenamid	108 (3.4)	110	105	101
29	Indoxacarb	109 (2.6)	105	100	111
28	Clomeprop	105 (4.2)	107	106	104
30	Cuinclorac-methyl	105 (4.1)	104	104	105
31	Furathiocarb	102 (1.8)	104	105	101
32	Lactofen	100 (3.7)	109	105	112
33	Tralkoxydim	101 (3.3)	111	102	117
Group 2					
1	Flumetsulam	97 (2.6)	110	156	104
2	Thidiazuron	104 (4.8)	101	102	113
3	Imazaquin	105 (3.1)	100	100	101
4	Thifensulfuron-methyl	106 (2.9)	112	116	113
5	Florasulam	99 (3.1)	106	103	109
6	Forchlorfenuron-methyl	101 (4.4)	103	100	108
7	Clorasulam-methyl	94 (3.9)	104	97	142
8	Diclosulam	95 (3.3)	102	96	107
9	Fomesafen	99 (5.9)	101	95	109
10	Triflusulfuron-methyl	97 (4.1)	111	104	108
11	Haloxyfop	108 (4.8)	114	110	124

*( ): RSD,% calculated based on five replicates within one day

Conclusions

The multiresidue method by LC/MS/MS described here was suitable for the determination of 44 pesticides in a variety of food samples due to its high sensitivity and high selectivity. Another advantage of this method is that ion suppression was not observed for all food samples studied. Thus, it may eliminate the need for matrix-matched standards, which make analysis more tedious for samples from different origins.

For more details concerning this application, please contact masahiko_takino@agilent.com

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Printed in the USA
August 8, 2006
5989-5459EN

Agilent Technologies

Multiresidue Analysis of 100 Pesticides in Food Samples by LC / Triple Quadrupole Mass Spectrometry

Application

Food Safety

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Abstract

An analytical methodology for confirming the presence of a group of 100 pesticides in vegetable and fruit samples was developed using the Agilent G6410AA Triple Quadrupole Mass Spectrometer (QQQ). One transition per parent compound was monitored in a single chromatographic run containing two time segments. The sensitivity obtained meets the maximum residue levels (MRLs) established by the European Union regulation for food monitoring programs. The analytical performance of the method was evaluated for different types of fruit and vegetables — orange, tomato, and green pepper — showing little or no matrix effects. Linearity of response over two orders of magnitude was demonstrated (r > 0.99). This study is a valuable indicator of the potential of the QQQ for routine quantitative multiresidue analysis of pesticides in vegetables and fruits.

Introduction

In recent years, the established regulations regarding the maximum residue limits (MRLs) in commodities have become more and more stringent. The European Union (EU) has set new directives for pesticides at low levels in vegetables in order to meet health concerns. For fruits and vegetables intended for production of baby food, an MRL of 10 µg/kg is applicable for all pesticides, and compounds without a stated regulation also have the lowest MRLs at 10 µg/kg. The low MRLs have encouraged the development of more sensitive analytical methods to meet the requirements in complex samples. In this sense, liquid-chromatography tandem-mass spectrometry (LC-MS-MS) with triple quadrupole in multiple reaction monitoring (MRM) mode has become, so far, the most widely used technique for the monitoring and quantitation of pesticides in food, as reported extensively in the literature. On the other hand, high-resolving power mass spectrometric techniques, such as time-of-flight mass spectrometry (TOF-MS), have been applied recently for screening purposes as well. Nevertheless, the simplicity of methodologies using triple quadrupole as a detection technique, together with the low limits of detection achieved and the MS/MS capability make this technique a valuable tool for routine

monitoring programs established in regulatory official laboratories. The easiness of use is sometimes an essential for these types of regulatory agencies, which lack the high-skilled personnel required for more sophisticated techniques such as TOF-MS. Triple quadrupole technology is not new in the sense that it needs to be validated for monitoring purposes and its basis is already well-established for routine analysis.

Our study in this report is one of the first of its kind to examine the new Agilent Triple Quad for the analysis of pesticides in fruit and vegetables. This topic was chosen because of the relevance of these compounds and their significant use on food commodities. The sensitivity of the QQQ easily meets the levels required by the regulations on pesticides in food.

Experimental

Sample preparation

Pesticide analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany). Individual pesticide stock solutions (around 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 mother solutions, working standard solutions were prepared by dilution with acetonitrile and water.

Vegetable samples were obtained from the local markets. "Blank" vegetable and fruit extracts were used to prepare the matrix-matched standards for validation purposes. In this way, two types of vegetables and one fruit (green peppers, tomatoes, and oranges) were extracted using the QuEChERS method already described in a previous application [1]. The vegetable extracts were spiked with the mix of standards at different concentrations (ranging from 2 to 100 µg/kg) and subsequently analyzed by LC/MS/MS.

LC / MS / MS Instrumentation

LC Conditions

Column:	Agilent ZORBAX Eclipse® XDB C-8, 4.6 mm × 150 mm, 5 µm, (p/n 993967-906).
Column temperature:	25 °C
Mobile phase:	A = 0.1% formic acid in water B= Acetonitrile
Flow-rate:	0.6 mL/min
Gradient:	10% B at 0 min 10% B at 5 min 100% B at 30 min
Injection volumes:	1-5 µL

MS Conditions

Mode:	Positive ESI using the Agilent G6410AA Triple Quadrupole Mass Spectrometer
Nebulizer:	40 psig
Drying gas flow:	9 L/min
V capillary:	4000 V
Drying gas temperature:	350 °C
Q1 resolution:	Unit
Q2 resolution:	Unit
Fragmentor voltage:	70 V
Collision energy:	5–25 V
MRM:	1 transition for every compound as shown in Table 1
Dwell time:	15 msec

Results and Discussion

Optimization of LC / MS / MS conditions

A preliminary study of the optimal MRM transitions for every compound was carried out by injecting groups of analytes (around 10 analytes in one chromatographic run) at a concentration level of 10 µg/mL. Various collision energies (5, 10, 15, 20, and 25 V) were applied to the compounds under study. The optimum energies were those that gave the best sensitivity for the main fragment ion and, as a general rule, left about 10% of parent compound in the spectra, and they were selected as optimum ones. Only one fragment ion was chosen as the most abundant product ion for every target compound. Results are shown in Table 1.

Table 1. Analytical Conditions and Limits of Detection (LOD) for Each of the Compounds Tested

Compound name	Retention time (min)	Protonated molecule $[M+H]^+$	Product ion (*m/z*)	Collision energy	LOD (pg)
Segment 1					
Cyromazine	2.7	167	125	20	10
Thiosultap	2.7	312	232	10	90
Cartap	3	150	105	15	10
Thiocyclam	4.5	182	137	10	8
Aldicarb sulfoxide	6.4	207	89	5	9
Carbendazim	6.6	192	160	15	5
Thiabendazole	7.9	202	175	25	10
Aldicarb sulfone	10.8	223	148	5	50
Nitenpyram	11	271	225	10	7
Hydroxyatrazine	11.2	198	156	15	3
Methomyl	11.5	163	88	5	4
Deisopropylatrazine	11.9	174	132	15	18
Imazapyr	12.5	262	234	15	8
Metamitron	13.9	203	175	15	8
Fenuron	14.5	165	72	15	2
Deethylatrazine	14.8	188	146	15	4
Imidacloprid	14.8	256	209	10	7
Dimethoate	15.4	230	199	5	7
Acetamiprid	15.5	223	126	15	6
Prometon	15.7	226	184	20	4
Irgarol metabolite	16	214	158	15	0.8
Methiocarb sulfone	16.4	258	122	5	6
Nicosulfuron	16.9	411	182	15	6
Thiacloprid	17	253	126	15	3
Imazalil	17.2	297	159	15	7
Mebendazole	17.2	296	264	20	2
Aldicarb	17.5	213	89	10	10
Imazaquin	17.8	312	284	20	15
Oxadixyl	17.9	279	219	10	10
Fluroxypyr	17.9	255	209	10	120
Simazine	18	202	132	15	5
Monuron	18	199	72	10	2
Lenacil	18.4	235	153	10	20
Cyanazine	18.5	241	214	10	70
Metolcarb	18.5	166	109	5	2
Spiroxamine	18.6	298	144	15	10
Dichlorvos	18.7	221	109	15	10
Metribuzin	18.9	215	187	15	5
Chlorotoluron	19.4	213	72	15	3
Prometryn	19.5	242	200	20	2
Terbutryn	19.5	242	186	15	1
Carbofuran	19.6	222	165	10	2
Bendiocarb	19.7	224	167	5	2
Segment 2					
Spinosad A	20	732	142	5	12
Carbaryl	20.1	202	145	5	2
Irgarol 1051	20.3	254	198	15	0.1
Atrazine	20.3	216	174	15	0.3
Metalaxyl	20.4	280	248	10	5
Difenoxuron	20.4	287	123	15	5
Isoproturon	20.4	207	72	15	1
Bensultap	20.5	432	290	15	6

Table 1. Analytical Conditions and Limits of Detection (LOD) for Each of the Compounds Tested (Continued)

Compound name	Retention time (min)	Protonated molecule $[M+H]^+$	Product ion (*m/z*)	Collision energy	LOD (pg)
Diuron	20.5	233	72	15	5
Spinosad D	20.7	746	558	5	100
Ethiofencarb	20.7	226	107	5	5
Dimethomorph isomer 1	21.3	388	301	20	11
Propachlor	21.6	212	170	10	1
Dimethomorph isomer 2	21.7	388	301	20	8
Prochloraz	21.9	376	308	10	6
Propanil	22.2	218	162	15	10
Cyproconazole	22.5	292	70	10	6
Methiocarb	22.6	226	169	5	15
Terbutylazine	22.7	230	174	15	0.3
Bromuconazole isomer 1	22.8	376	159	20	6
Fenamiphos	23	304	217	15	0.7
Methidathion	23	303	145	5	5
Azoxystrobin	23.2	404	372	10	0.4
Phosmet	23.2	318	160	5	2
Captan	23.2	300	264	10	50
Dimethenamide	23.3	276	244	10	1
Promecarb	23.3	208	151	10	5
Bromuconazole isomer 2	23.7	376	159	20	6
Molinate	23.7	188	126	10	5
Diflubenzuron	24.1	311	158	10	9
Iprodione	24.6	330	245	10	8
Propiconazole isomer 1	24.7	342	159	20	5
Malathion	24.8	331	127	5	5
Propiconazole isomer 2	24.9	342	159	20	5
Metolachlor	24.9	284	252	10	2
Triflumizole	24.9	346	278	10	7
Alachlor	25	270	238	10	8
Acetochlor	25.1	270	224	10	8
Flufenacet	25.2	364	194	5	5
Difenoconazole isomer 1	25.3	406	251	20	4
Difenoconazole isomer 2	25.4	406	251	20	4
Chlorfenvinphos	25.5	359	155	10	8
Benalaxyl	25.8	326	294	5	5
Parathion ethyl	26.2	292	236	10	9
Triclocarban	26.4	315	162	15	8
Hexaflumuron	26.5	461	158	10	7
Buprofezin	26.7	306	201	10	1
Diazinon	26.8	305	169	15	1
Teflubenzuron	26.9	381	158	15	22
Chlorpyrifos methyl	27.1	322	212	15	15
Profenofos	27.6	373	303	10	7
Lufenuron	27.9	511	158	10	10
Prosulfocarb	28	252	91	15	2
Flufenoxuron	28.5	489	158	10	6
Butylate	28.7	218	57	10	2
Pendimethalin	29.2	282	212	5	5
Trifluralin	29.7	336	236	15	30

The MRM transitions were included in the method with a dwell time of 15 msec, and two different time segments were recorded in the chromatographic run (each one of them containing about half of the pesticides studied). Figure 1 shows the chromatogram corresponding to 100 pg on column for all the compounds studied. Extracted ion chromatograms are overlaid for each one of the target analytes according to their respective protonated molecule and product ion MRM transition.

Linearity and Limits of Detection

Linearity was evaluated by analyzing the standards solutions at five different concentration levels in the range 2 to 100 pg on column. As an example, the calibration curve generated for atrazine is shown in Figure 2. As it can be observed in this figure, the linearity of the analytical response across the studied range is excellent, with a correlation coefficient of 0.998. Similar results were obtained for the rest of the compounds analyzed.

The limits of detection (LOD) were estimated from the injection of standard solutions at concentration levels corresponding to a signal-to-noise ratio of about 3. The results obtained are included in Table 1 as well. The best limits of detection were obtained for the triazines (from 100 fg to 2 pg on column) and the highest limits of detection were for fluoroxypyr and spinosad D (above 100 pg).

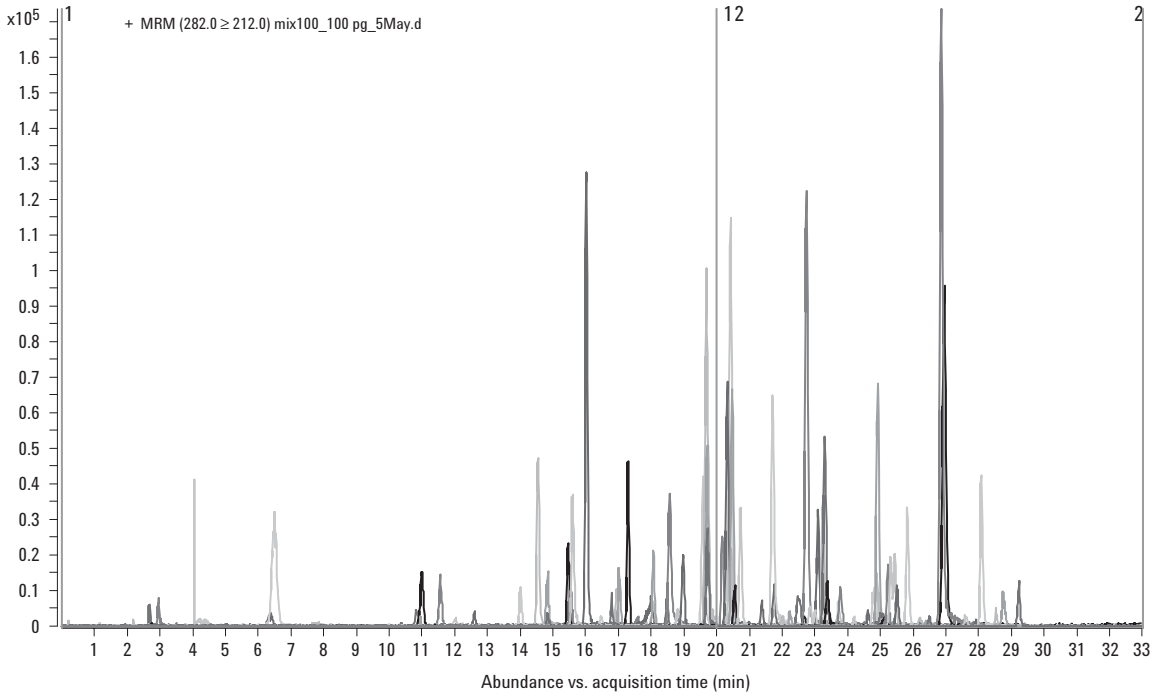

Figure 1. Product ion chromatograms of a mix of 100 pesticides (concentration: 100 pg on column).

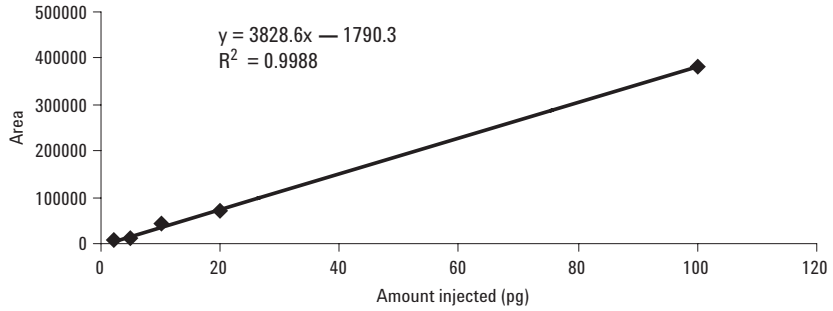

Figure 2. Calibration curve for atrazine using a linear fit with no weighting and no origin treatment.

Application to Vegetable Matrices

To confirm the suitability of the method for analysis of real samples, matrix-matched standards were analyzed in three different matrices — green pepper, tomato, and orange — at two different concentration levels (10 and 100 µg/kg). Figure 3 shows the analysis of a green pepper spiked with the pesticide mix at 10 µg/kg (10 pg on column). As it can be observed in two of the MS/MS extracted product ion chromatograms, for dimethoate and azoxystrobin, compounds can be easily identified in these complex matrices due to the selectivity of the MRM transitions, thus fulfilling the regulation limits imposed by the EU directives. In general, the LOD obtained meet the requirements regarding the MRLs imposed by the existing European regulations.

Reference

1. Imma Ferrer and E. Michael Thurman, "Determination of Fungicides in Fruits and Vegetables by Time-of-Flight and Ion Trap LC/MS" (2005) Agilent Technologies, publication 5989-2209EN www.agilent.com/chem.

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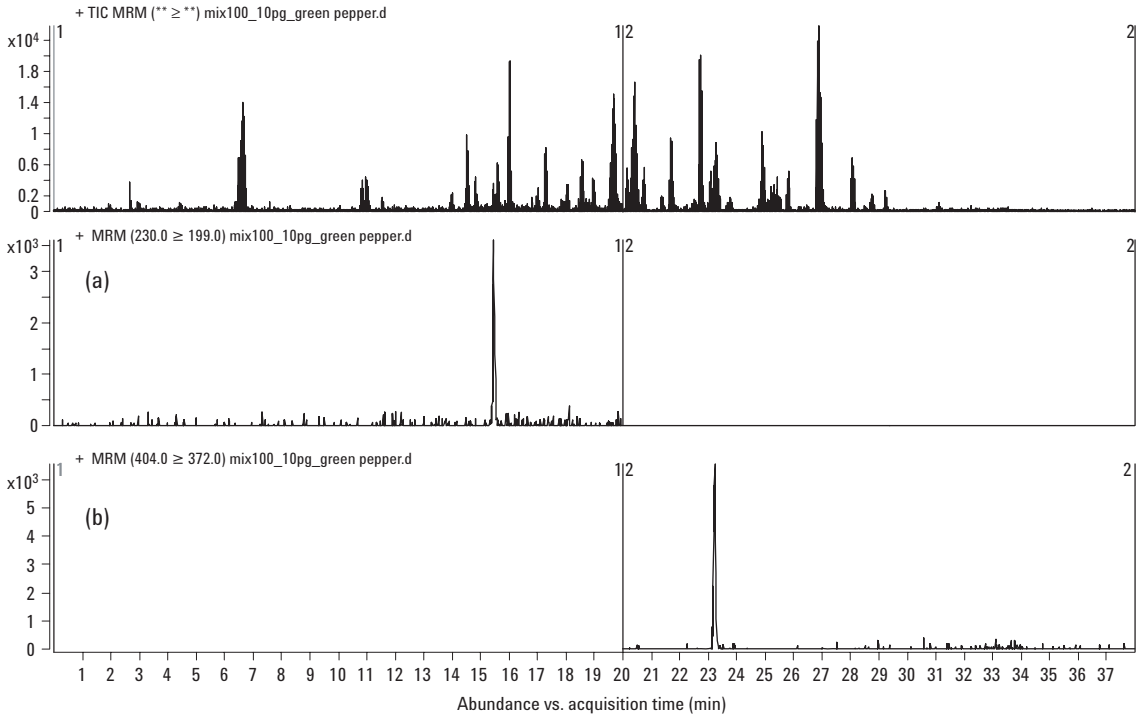

Figure 3. MRM chromatogram of a spiked green pepper sample at 10 µg/kg. Product ion chromatograms for (a) dimethoate and (b) azoxystrobin.

Printed in the USA
August 9, 2006
5989-5469EN

Agilent Technologies

Using RTL and 3-Way Splitter to Identify Unknown in Strawberry Extract

Application Brief

Chin-Kai Meng

Food Safety and Environmental

Fruit and vegetable extracts are usually very complex to analyze. It is common to use the very selective GC detectors, for example NPD, μECD, and FPD, to look for trace pesticide residues in the extracts. Mass spectrometry is most often used to confirm the hits from GC detectors. A previous application note [1] describes a GC/MS system with a three-way splitter added to the end of the column. The column effluent could be split three ways to two GC detectors and the MSD. The splitter system is therefore capable of providing up to four signals (two GC signals, SIM, and full-scan chromatograms) from a single injection. The combination of element selective detectors, SIM/scan, and Deconvolution Reporting Software (DRS) makes a very powerful pesticide analysis system [2]. The trade-off is the decrease of analyte concentration in any detector due to the flow splitting at the end of the column.

The system used for this study consists of an Agilent 7890A GC with split/splitless inlet, a three-way splitter, μECD, dual flame photometric detector (DFPD), and 5975C MSD. Figure 1 shows chromatograms from 2 separate injections (each injection provides two GC signals) of the same strawberry extract without any hardware or filter changes. All of the target compounds were found and confirmed by DRS, GC, and MS signals except the unknown peak at about 41 minutes. The peak shows responses from μECD, DFPD(S) and DFPD(P). However, no peak was observed in the MS full-scan signal. This makes it difficult to confirm the unknown peak using the full-scan TIC.

Since the analysis was retention time locked, it is therefore possible to find potential matches by examining the RTL pesticide database (part number G1672AA). The unknown compound, containing electron-capturing atoms (for example, Cl or O), P, and S atoms, would have a target retention time inside the

Highlights

Splitter—an inert, easy-to-use capillary flow technology that splits column effluent to multiple detectors (for example, MSD, DFPD, and μECD). The splitter configuration provides a comprehensive screening and quantitative system.

By combing RTL, element-selective detector chromatograms, and the RTL pesticide database, a trace level pesticide residue was identified without the full-scan mass spectrum.

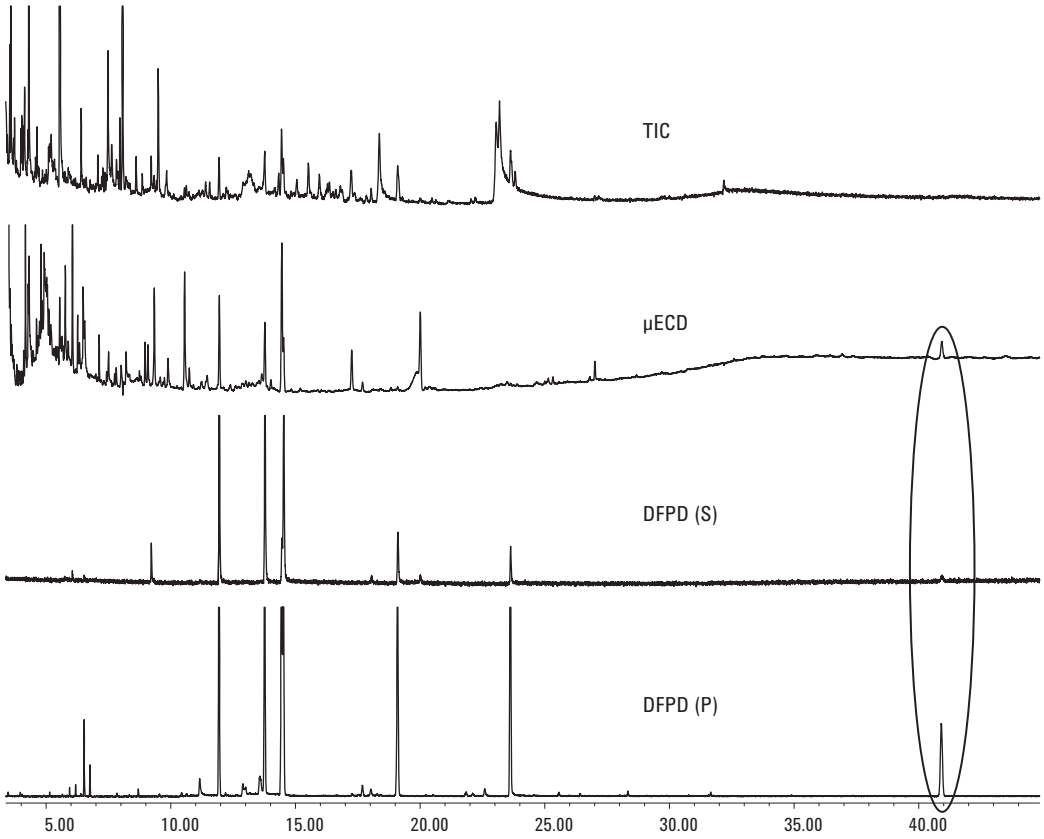

Figure 1. Unknown compound detected by GC signals not found in strawberry extract TIC.

Table 1. Compound List Extracted from the RTLPest3.tab File

Name	CAS	Mol form	Mol wt	R.T.	Target Ion	Q1	Q2	Q3
Fluthiacet-methyl	117337196	$C_{15}H_{15}ClFN_3O_3S_2$	403.9	39.10	403	56	405	232
Benzo[g,h,i]perylene	191242	$C_{22}H_{12}$	276.3	39.13	276	277	138	275
Temephos	3383968	$C_{16}H_{20}O_6P_2S_3$	466.5	40.74	466	125	93	109
PBB 169 hexabrombiphenyl	60044260	$C_{12}H_4Br_6$	627.6	40.93	308	468	148	154
Rotenone	83794	$C_{23}H_{22}O_6$	394.4	41.70	192	191	394	177

41 ± 0.5-minute window (that is, 40.5 to 41.5 min) in the database, if it is included in the database. Table 1 is a portion of the RTLPest3.tab file[1] opened in Microsoft Excel. The compound temephos at locked retention time 40.74 min meets all the criteria for the unknown peak. To further confirm peak identity, extracted ion chromatograms (EICs) of the four major ions of temephos were plotted. Figure 2 shows EICs of target ion and three qualifiers (ions 466, 125, 93, and 109 from Table 1) of temephos. Although the ion intensities were weak, the noticeable presence of all four ions at 40.9 min helped to confirm that the unknown peak was temephos.

1. The RTLPest3.tab file is created in the C:\Database directory while executing the Tools\List Screen Database... command (in MSD Enhanced Data Analysis software) and selecting the RTLPest3.scd from the C:\Database directory.

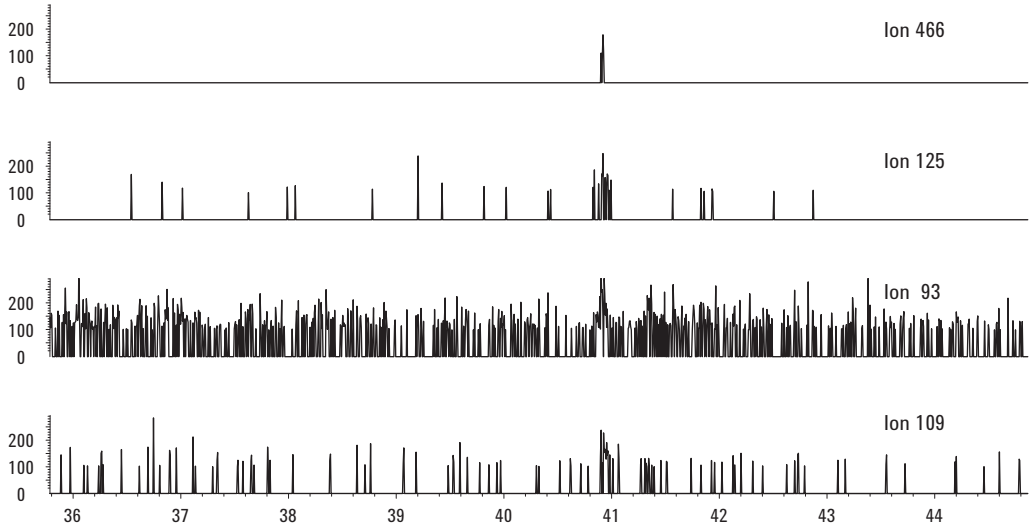

Figure 2. EICs of target ion 466 (temephos) and three qualifier ions.

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1. Chin-Kai Meng and Bruce Quimby, "Identifying Pesticides with Full Scan, SIM, μECD, and FPD from a Single Injection," Application Note, 5989-3299, July 2005.

2. Mike Szelewski and Bruce Quimby, "New Tools for Rapid Pesticide Analysis in High Matrix Samples," Application Note, 5989-1716, October 2004.

Acknowledgement

Strawberry extract is courtesy of Dr. Steven Lehotay from USDA Agricultural Research Service in Wyndmoor, Pennsylvania, USA.

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Printed in the USA
December 13, 2006
5989-6007EN

Agilent Technologies

Direct Injection of Fish Oil for the GC-ECD Analysis of PCBs: Results Using a Deans Switch With Backflushing

Application

Environmental and Pharmaceutical

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Abstract

A Deans switch, employing Agilent's Capillary Flow Technology, was configured on an Agilent 7890A gas chromatograph (GC) equipped with dual electron capture detectors (ECDs). A method was developed for the analysis of fish oil for polychlorinated biphenyl (PCB) contamination. The Deans switch was used to heart cut 7 indicator PCBs (IUPAC congeners 28, 52, 101, 118, 138, 153, and 180) from the primary DB-XLB column onto a DB-200 column for further separation. Fish oil from a supplement capsule was simply diluted 1:10 in isooctane and injected directly. In a separate experiment, the fish oil was analyzed by GC with a flame ionization detector (GC/FID) without backflushing. From these analyses, it was estimated that about two-thirds of the fish oil components would remain on the column after the 17.4-minute GC/ECD run. To prevent carryover, contamination, and retention time shifts, the Deans switch was used to backflush the primary column at the end of each run. Evidence shows that backflushing removed the fish oil residue, which otherwise would quickly degrade the chromatography.

Introduction

Fish oils contain high levels of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), omega-3 fatty acids that are thought to have beneficial health affects. In addition to eating fish, many people take fish oil as a supplement to their daily diet. However, fish, especially those high on the aquatic food chain, can bioaccumulate fat-soluble pollutants. Among these are polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). Therefore, fish oil used in supplements undergoes a variety of analyses, including tests for halogenated pollutants.

One of the quality assurance tests is to analyze fish oil for PCB contamination. This is complicated by the fact that fish oil is a very complex mixture containing high-boiling fatty acids and triglycerides of fatty acids; chain lengths are mostly between 14 and 22 carbons. They also contain varying amounts of phospholipids, glycerol ethers, wax esters, and fatty alcohols. PCB analysis is complex by itself, with 209 possible congeners. Of these, 140 to 150 have been observed in commercial PCB mixtures called Aroclors. PCB analysis usually focuses on the 12 planar, dioxin-like PCBs and/or on seven indicator PCBs (IUPAC Numbers 28, 52, 101, 118, 138, 153, and 180).

To obtain sufficient sensitivity and selectivity for these compounds, analysts have traditionally employed very expensive techniques such as high-resolution mass spectrometry (HR/MS) or HR/MS/MS. Analysis of the fish oil generally follows a series of extraction and cleanup steps. This paper focuses on the analysis of the seven indicator PCBs in fish oil using an Agilent 7890A GC configured with a Deans switch, two columns of differing selectivity, and dual electron capture

detectors (ECDs). Fish oil from a commercially available supplement was simply diluted 10:1 in isooctane and injected into the GC. No cleanup steps were employed.

Experimental

The fish oil supplement was obtained from a local grocery store. According to the bottle's label, each gelatin capsule contains 1.0 g of fish oil of which 180 mg is EPA and 120 mg is DHA. Oil was removed from a capsule and diluted with isooctane (pesticide grade from Sigma-Aldrich, Milwaukee, WI, USA) to make a 10% solution. This solution was spiked with various Aroclors (Supelco, Bellefonte, PA, USA) or with individual PCB congeners (AccuStandard, New Haven, CT, USA).

Table 1 lists the instrumentation and experimental conditions for the analysis.

Table 1. Instrumentation and Experimental Conditions

Instrumentation and Software	
Gas chromatograph	Agilent 7890A
Automatic sampler	Agilent 7683B Series injector and tray
Primary column	J&W 30-m × 0.18-mm × 0.18-µm DB-XLB (P/N 121-1232)
Primary column connections	Split/splitless inlet to Deans switch
Secondary column	J&W 30-m × 0.25-mm × 0.50-µm DB-200 (P/N 122-2033)
Secondary column connections	Deans switch to back ECD
Restrictor	76.8-cm × 0.100-mm deactivated fused silica tubing
Restrictor connections	Deans switch to front ECD
Inlet liner	Agilent deactivated single taper with glass wool (P/N 5062-3587)
Auxiliary pressure control device	Agilent 7890A Pneumatic Control Module (PCM) Option # 309
Deans switch calculator software	Agilent Technologies Deans Switch Calculator (Rev. A.01.01)
Software for data acquisition and analysis	Agilent GC ChemStation (Rev. B.03.01)
Instrumental Conditions for Analysis	
Inlet	Split/splitless at 330 °C
Oven temperature program	80 °C (1 min), 50 °C/min to 200 °C (0 min), 10 °C/min to 290 °C (5 min)
Detectors	Dual ECD at 340 °C
ECD make-up gas	N_2 at 60 mL/min
Inlet pressure	H_2 at 41.040 psig (constant pressure mode)
PCM pressure to Deans switch	H_2 at 20.610 psig (constant pressure mode)
Post-Run Backflush Conditions	
Post-run duration	2.4 min
Inlet pressure	H_2 at 0 psig
PCM pressure	H_2 at 80 psig
Oven temperature during backflush	290 °C for 2.4 min

Results and Discussion

Without backflushing, the high-boiling components of fish oil can be retained by the GC column, causing severe carryover problems from one run to the next. After a few injections, so much of the fish oil residue builds up on the column that it causes PCB retention times to shift by a minute or more. Such dramatic retention time shifts would prevent the use of the Deans switch, where heart cuts are just a few seconds wide.

Deans Switch–Heart Cutting

The Deans switch is one of Agilent's new devices that employ Capillary Flow Technology. These devices have extremely low dead volumes, are inert, and do not leak, even with large cycles in oven temperature. Columns are easy to install into the Deans switch, which is mounted on the side of the oven wall (Figure 1).

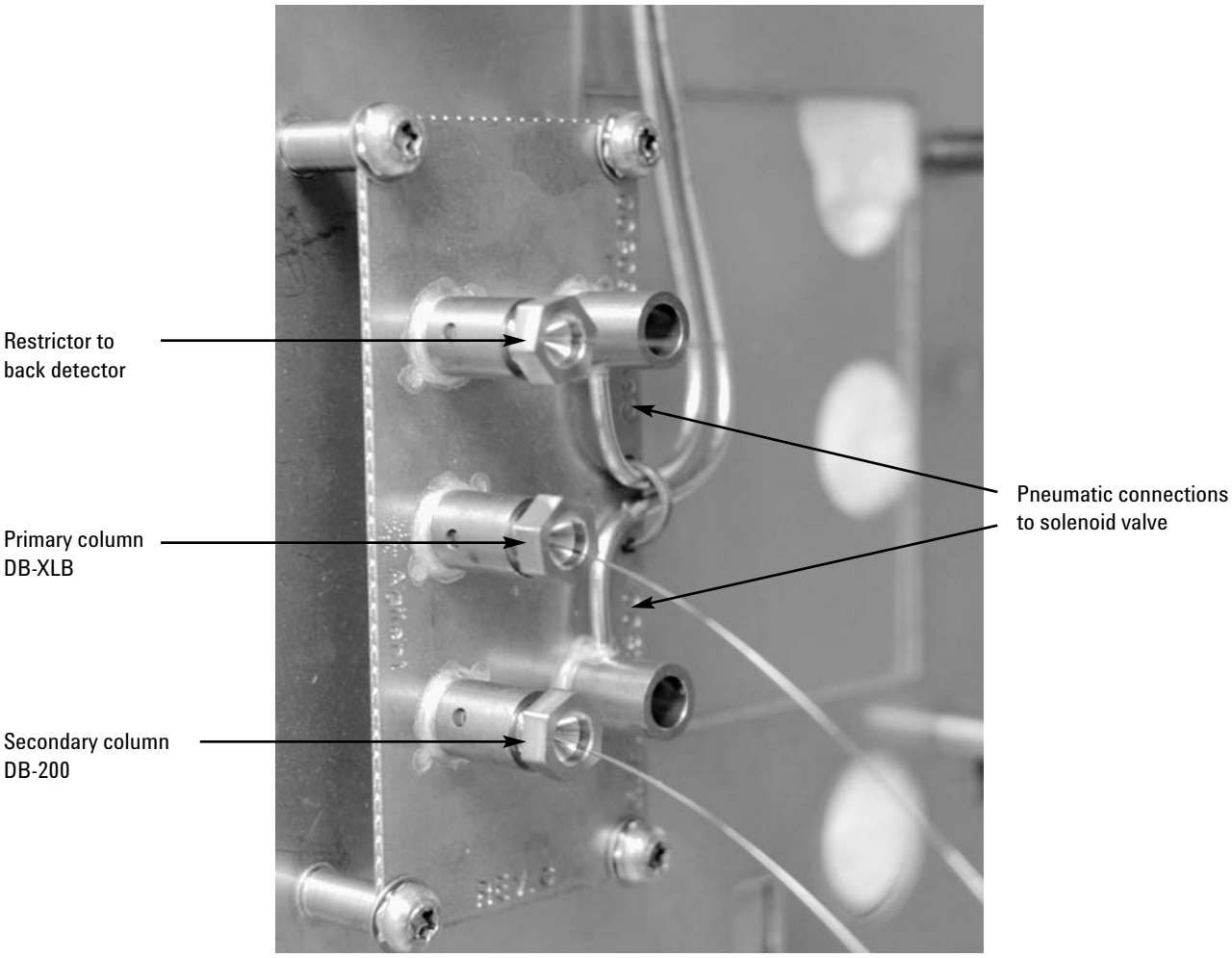

Figure 1. Photograph of the Deans switch installed on the side of the 7890A GC oven. The column and restrictor connections are indicated by an * in Figure 2a.

As shown in Figure 2a, the 30-m × 0.18-mm × 0.18-µm DB-XLB column is connected between the split/splitless inlet and the Deans switch. A short length of deactivated fused silica tubing (76.8 cm × 0.100 mm) connects the Deans switch to the front ECD. The secondary column (30-m × 0.25-mm × 0.5-µm DB-200) was chosen because it is more polar than the DB-XLB column and has a different selectivity for PCBs. It has an upper temperature limit of 300 °C, which is high enough to elute the PCBs of interest.

Figure 2a shows the Deans switch in the "normal" mode with the solenoid valve in the off position. In this mode, the effluent from the primary DB-XLB column is directed through the restrictor to the front ECD. When the solenoid valve is switched, the effluent is directed through the secondary DB-200 column to the back ECD (Figure 2b). The retention times for the seven indicator PCBs were initially determined with the valve in the *off* position. Using the timed events table in the ChemStation, the valve was switched to *on* just before each PCB peak and *off* immediately after. This produced seven heart cuts that were directed through the DB-200 column to the back ECD.

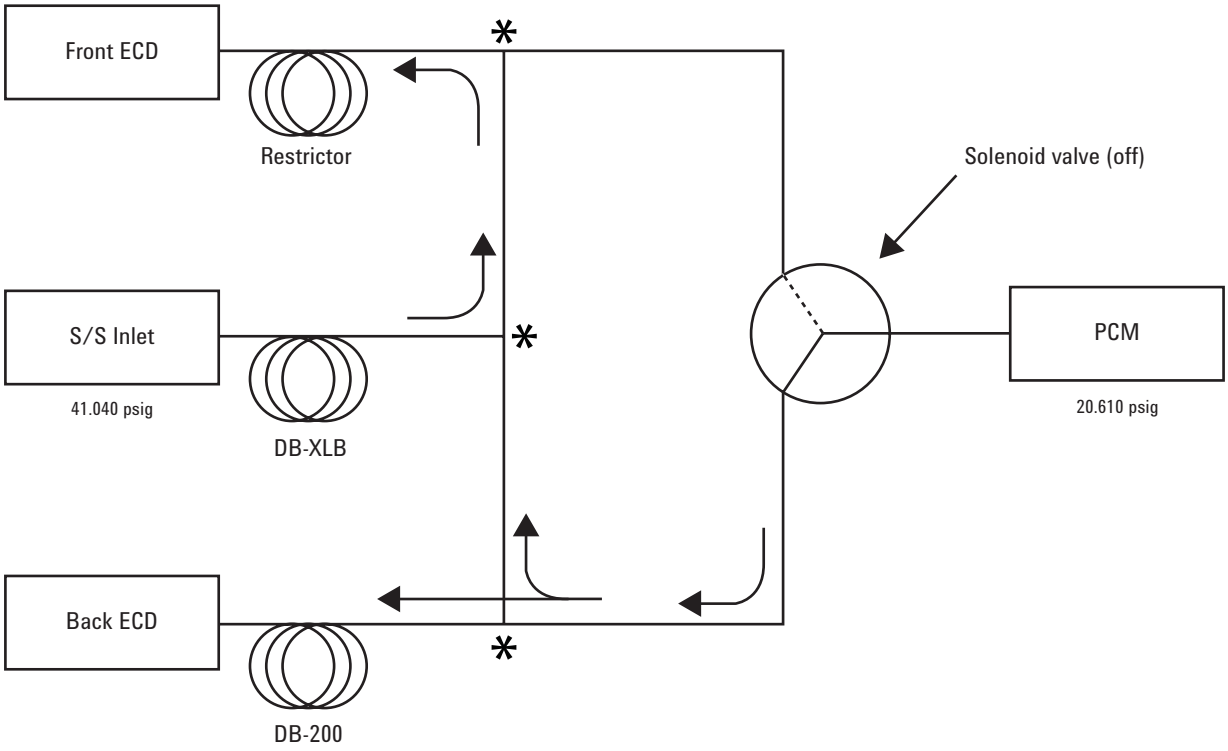

Figure 2a. Deans switch in the "no cut" position. The effluent from the DB-XLB column goes directly to the front ECD through the short restrictor. The intersections marked with an * are column and restrictor connections to the Deans switch plate (Figure 1).

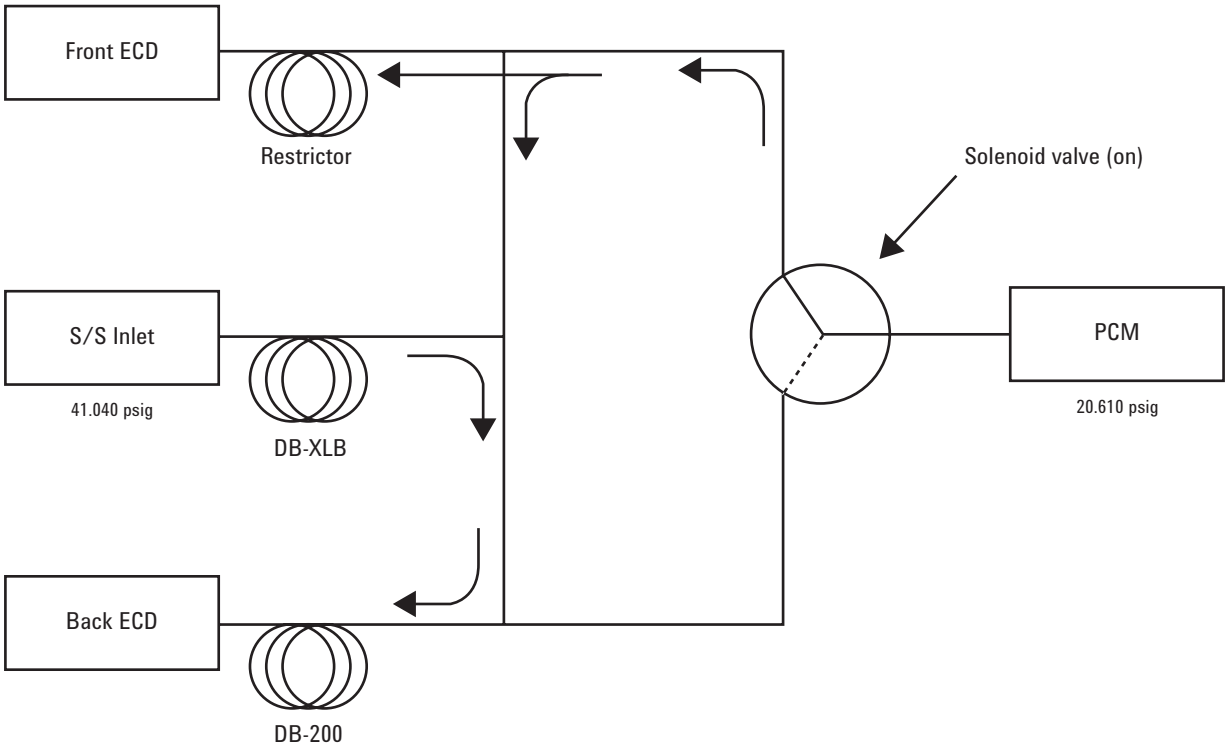

Figure 2b. Deans switch in the "cut" position. The effluent from the DB-XLB column goes to the DB-200 column and then to the back ECD.

In some Deans switch systems, the second column is placed in a separate GC oven or cryogenic cooling is used to trap the heart cut components at the head of the second column. In this case, both columns were mounted inside of the 7890A oven and cooling was not used to focus compounds at the head of the DB-200 column.

Figure 3a shows the chromatogram for a fish oil sample spiked with Aroclor 1260. PCBs 28, 52, 101, 118, 138, 153, and 180 were cut out of the primary chromatogram (Figure 3b) and sent to the second column (Figure 3c). The purpose of the DB-200 column is to resolve the target PCBs from other PCBs and matrix components that co-elute with them on the DB-XLB column. Six of the 7 PCBs appear to be well resolved on the DB-200 column. PCB 118 is only partially resolved by this method.

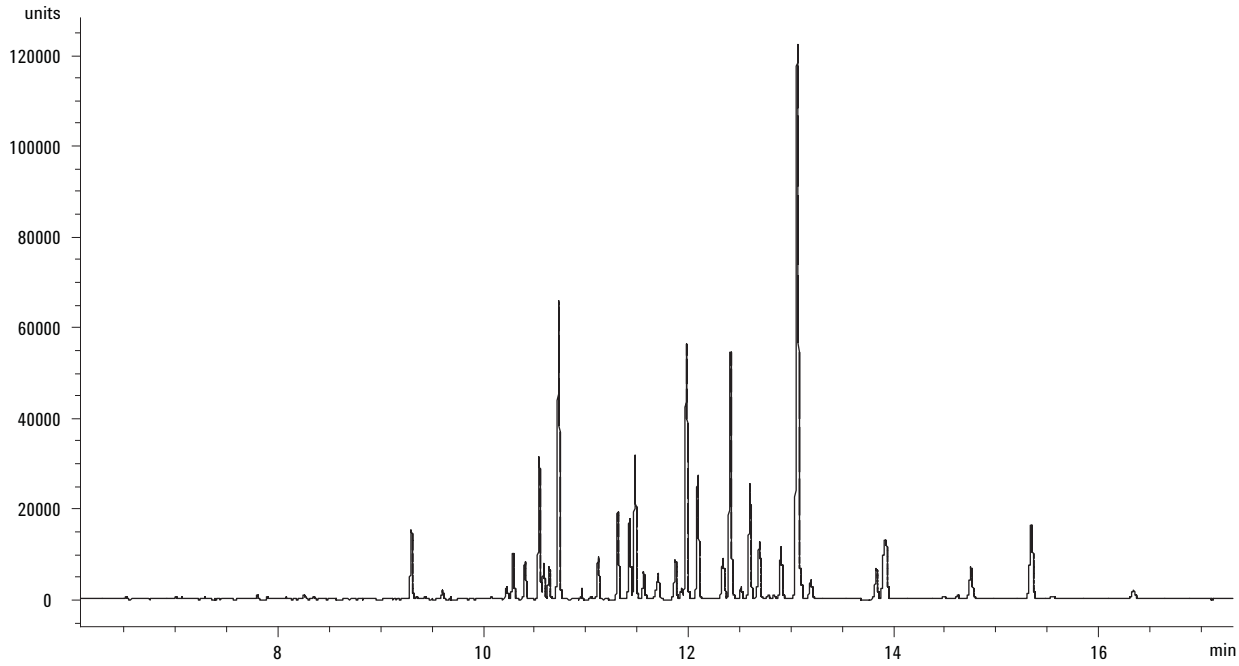

Figure 3a. GC/ECD chromatogram of Aroclor 1260 spiked into fish oil. This is the effluent from the primary DB-XLB column with seven heart cuts.

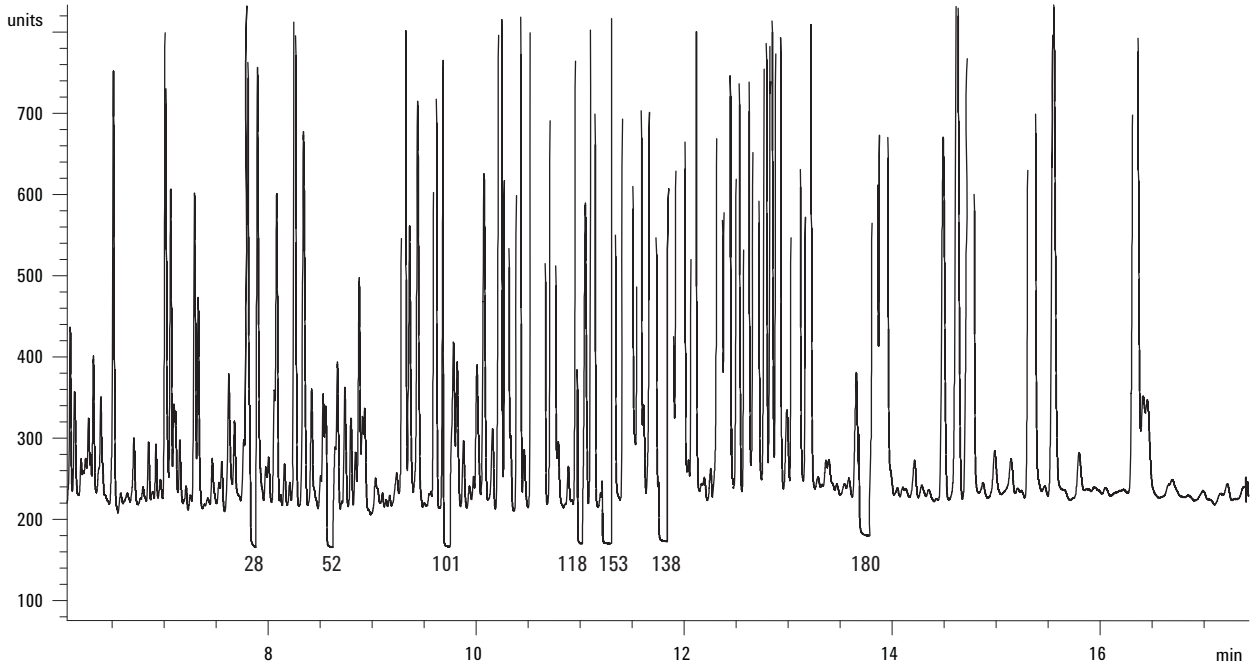

Figure 3b. Enlargement of the chromatogram in Figure 3a showing where heart cuts were made for the seven target PCBs.

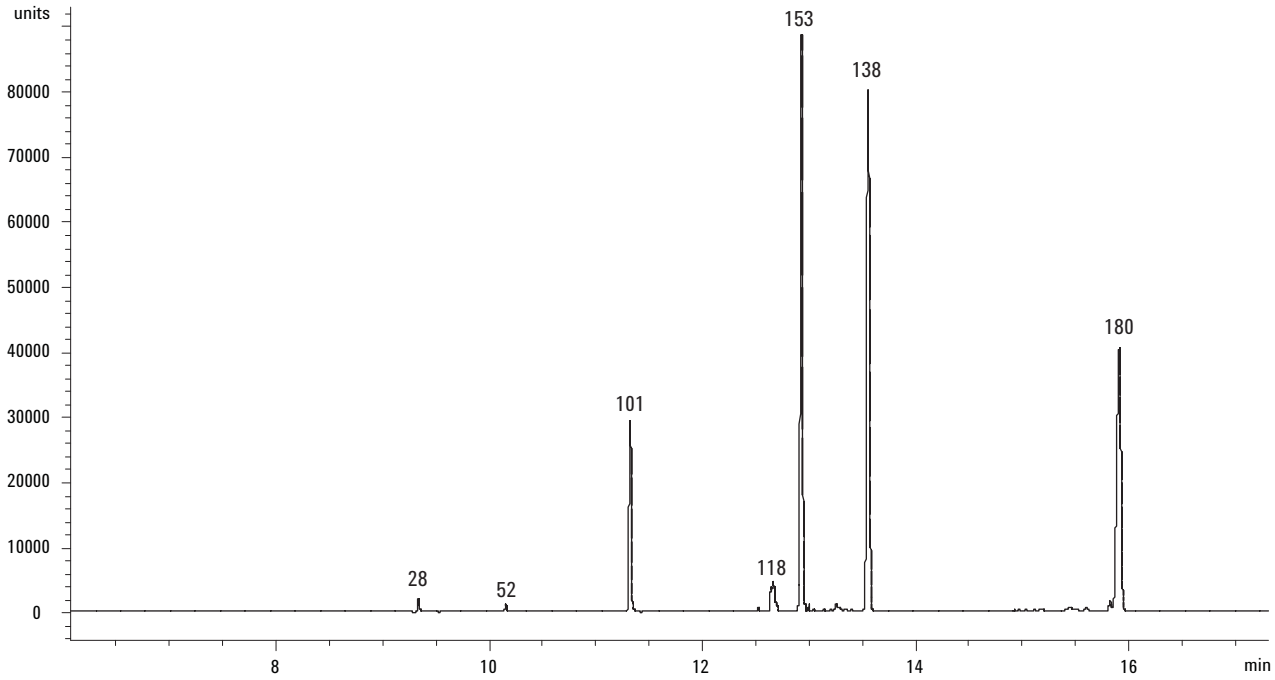

Figure 3c. GC/ECD chromatogram from the DB-200 column. The peaks in this chromatogram were heart cut from the DB-XLB column. Except for congener 118, the target PCBs were separated from co-eluting interferences by the DB-200 column.

Deans Switch–Backflushing

Data collection with the Deans switch system ended at 17.4 min with the oven at 290 °C. While it was assumed that a lot of the fish oil components remained on the column at this point, it was impossible to tell because the ECD responds poorly to these compounds. The fish oil does contribute some small peaks (both positive and negative) to the chromatogram, but it is impossible to see the full contribution of the fish oil. So a sample of the fish oil was analyzed on an identical DB-XLB column using a flame ionization detector (FID) with no Deans switch installed. The temperature was held at 290 °C for an extra 25 minutes to determine if high boiling compounds were still eluting.

Figure 4 shows that a great deal of the fish oil continued to elute after 17.4 minutes (arrow in figure). When a blank run was made with a final oven temperature of 310 °C, much more of the fish oil eluted from the column (Figure 4, middle chromatogram). A second blank run (Figure 4, top chromatogram) showed that fish oil components were still eluting from the column. In actuality, only about a third of the fish oil comes off the column under the Deans switch conditions. This is why other fish oil methods begin with a solvent extraction followed by solid phase extraction for sample cleanup.

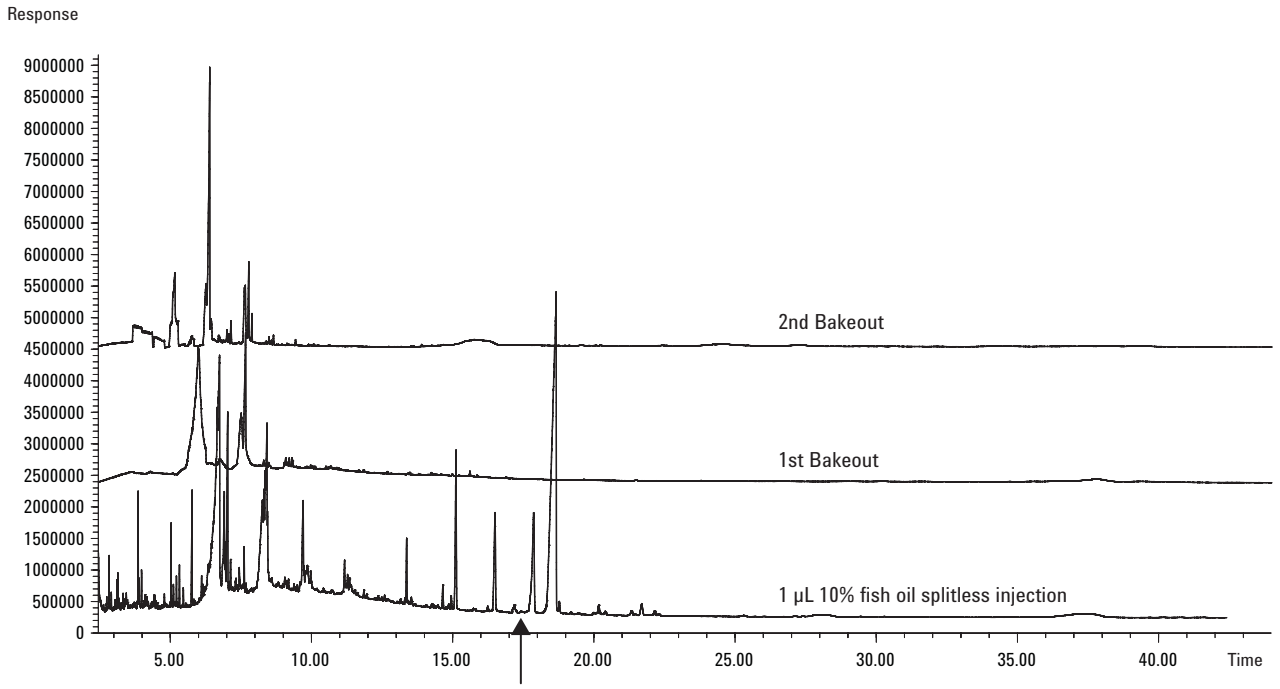

Figure 4. GC/FID chromatogram from a 1 µL splitless injection of 10% fish oil using a 30-m × 0.18-mm × 0.18 µm DB-XLB column. The arrow indicates where the GC/ECD method ends and the post-run backflush begins. In this case, there was no backflushing so the oven was held at 290 °C for an extra 25 min. The run was repeated two more times without injection but with the oven held at 310 °C for 30 minutes at the end of the run. Residue from the fish oil injection continued to elute, even during a second bakeout step.

The 7890A has been designed to make column backflushing a routine process. It has been shown empirically that backflushing should continue for about five times the holdup time. In this case the column was held at 290 °C during the post run backflush. At the same time, the inlet pressure was dropped to 0 psig while the PCM pressure was increased to 80 psig. Using Agilent's GC Pressure/Flow Calculator software, the H_2 flow rate backwards through the column was 3.81 mL/min and the holdup time was 0.466 min. Backflushing was, therefore, continued for 2.4 minutes, which is slightly more than five times the calculated holdup value. Figure 5 shows the Deans switch in the backflush mode.

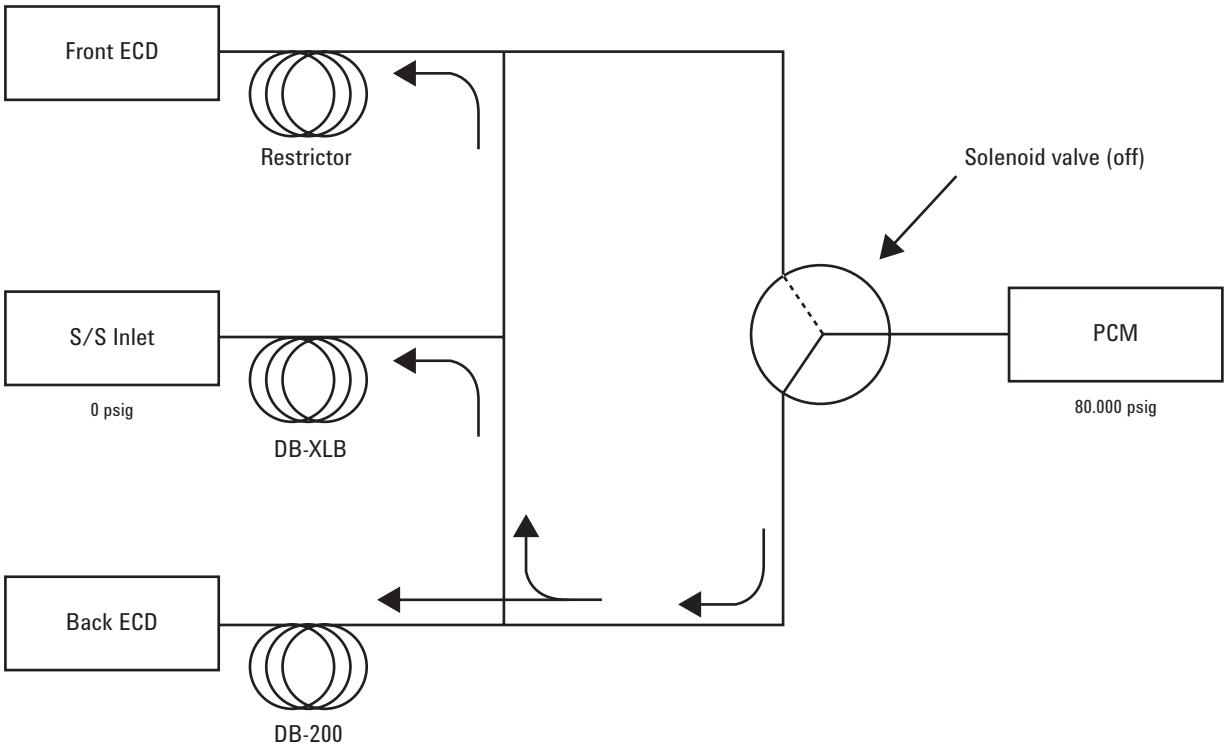

Figure 5. Deans switch in the "backflush" mode. The inlet pressure is dropped to 0 (or 1) psig while the PCM pressure is raised to 80 psig. This causes the carrier gas to flow backwards through the DB-XLB column. The reverse flow sweeps high-boiling fish oil components off the head of the column and out the split vent.

As mentioned earlier, just a few injections of fish oil can cause dramatic shifts in PCB retention times. Backflushing forces the remaining fish oil components backwards through the primary column and out through the split vent. This prevents fish oil buildup on the column, thus eliminating carryover and retention time shifts. Figure 6a compares the first and last chromatograms in a six-run sequence. One-µL splitless injections were made of 10% fish oil spiked with Aroclor 1260. This sequence was run after many previous injections of fish oil using this method, and it is clear that the retention times did not shift.

Figure 6b shows the seven PCBs that were heart cut from the two analyses shown in Figure 6a. Figure 6b shows no differences in the first and last heart cut chromatograms, providing further proof that there were not even subtle shifts in the PCB retention times. Each heart cut was just 4 to 5 seconds wide, so very small RT shifts in the first column would dramatically alter the results in the second.

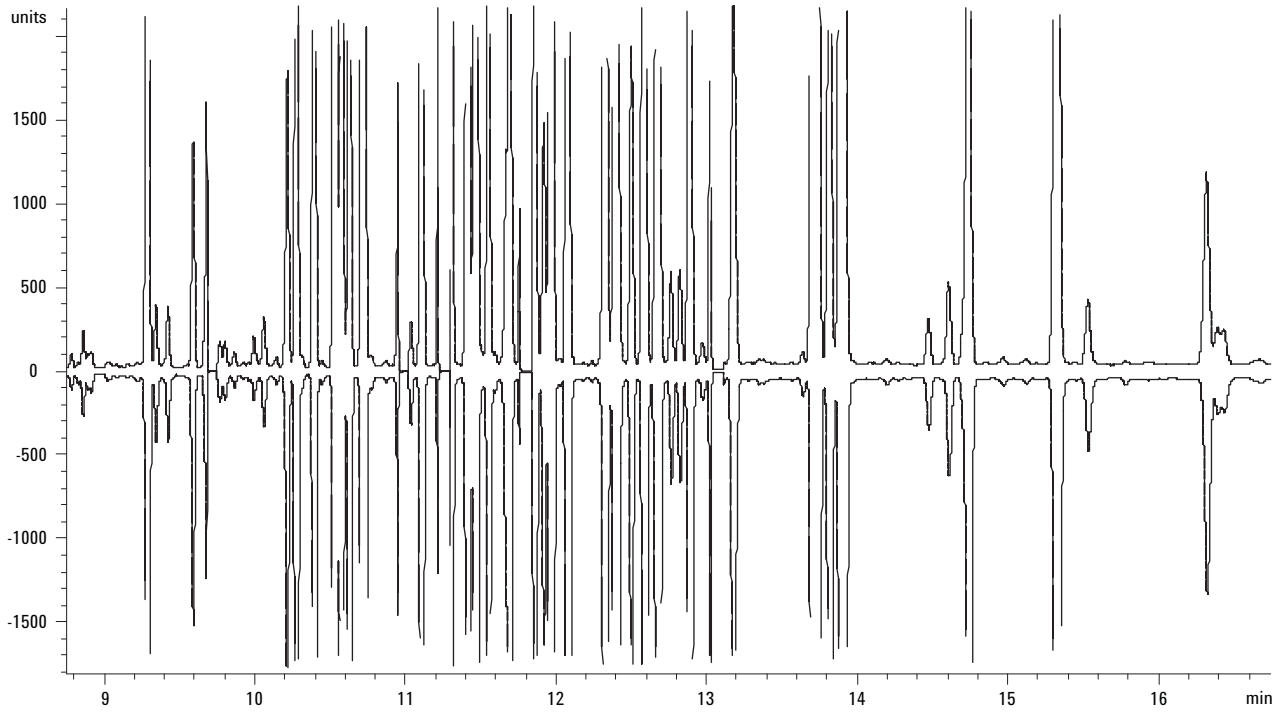

Figure 6a. First (top) and sixth (inverted) injections of 10% fish oil spiked with Aroclor 1260. Seven Deans switch cuts were made from this DB-XLB column in order to isolate PCBs 28, 52, 101, 118, 138, 153, and 180. The DB-XLB column was back-flushed after each run, preventing build-up of fish oil residue. The comparison shows that there was no shift in retention times caused by fish oil accumulation.

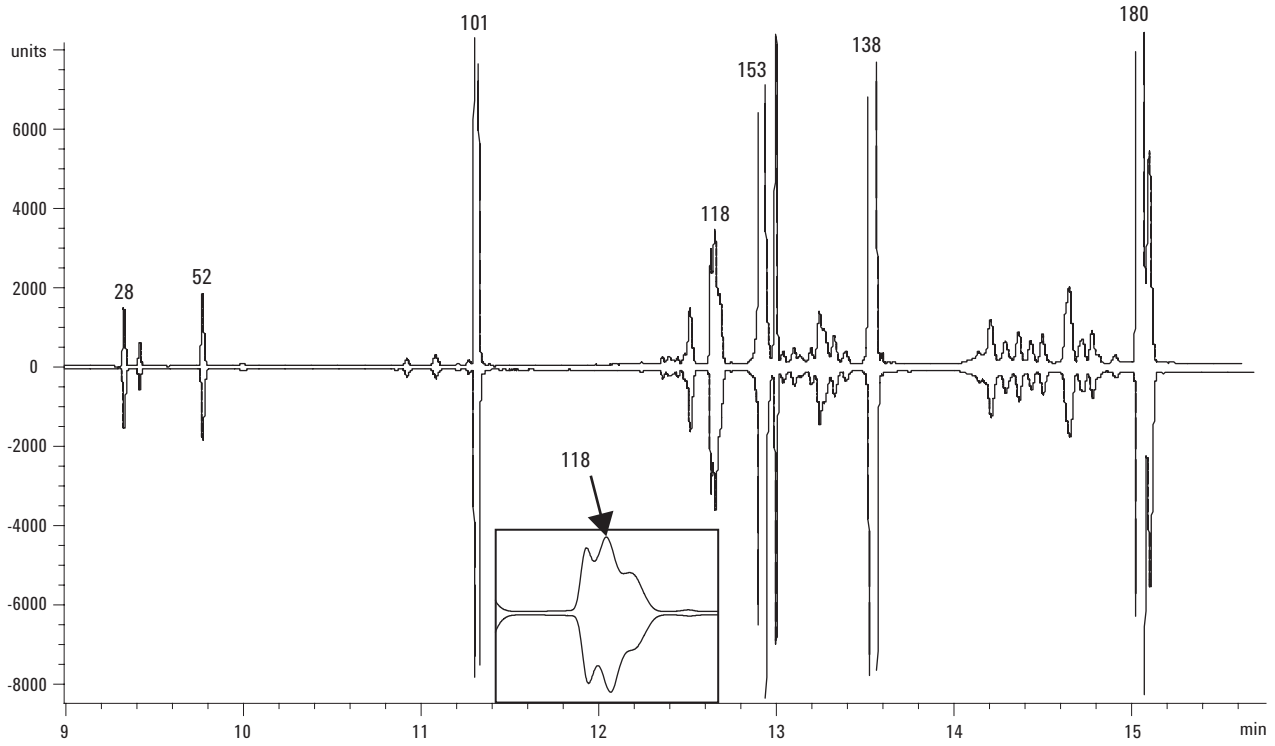

Figure 6b. Chromatogram of the seven PCB congeners and interferences that were cut from the DB-XLB column to the DB-200. The first chromatogram (top) and sixth (inverted) are identical, providing further proof of retention time stability. Any retention time shift on the primary column would severely alter the appearance of the secondary chromatogram.

Conclusions

This paper demonstrates that it is possible to analyze PCBs in fish oil without performing laborious sample cleanup prior to GC injection. A Deans switch was used to cut seven target PCBs (28, 52, 101, 118, 138, 153, and 180) from a DB-XLB column for further separation on a DB-200 column. This produced nearly baseline separation of the target PCBs. Only congener 118 was not well separated from co-eluting PCBs. Further refinement of the oven temperature program would be needed to isolate this congener.

It has been estimated that about two-thirds of the fish oil remained on the primary GC column at the end of the run. By setting the Deans switch to the backflush mode for just 2.4 minutes at the end of each run, this material was swept backwards through the column and out the split vent. There was no evidence for retention time shifts or carryover from run to run.

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Printed in the USA
January 5, 2007
5989-6095EN

Agilent Technologies

Rapid Analysis of Crude Fungal Extracts for Secondary Metabolites by LC/TOF-MS – A New Approach to Fungal Characterization

Application

Food

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Abstract

A novel approach to studying the production of secondary metabolites by fungi using LC/TOF-MS has been developed. Fungi grown on culture media are solvent-extracted and directly analyzed by LC/TOF-MS. Searching against a database of 465 secondary metabolites, mycotoxins and other compounds of interest can be readily identified. The methodology was validated by spiking culture media with 20 mycotoxin standards and identifying these toxins in the crude solvent extracts. Subsequently, using seven different fungi from culture collections, after culturing for 7 to 14 days in three different media, anticipated metabolites were readily identified.

Introduction

From a food safety perspective there is a need to characterize molds (fungi) isolated from agricultural products, as these may represent a potential source of mycotoxin contamination in food.

Traditionally, this fungal characterization has been based on classical mycology, involving culturing the fungi on different media and then classifying depending on morphological and growth behavior characteristics. However, such classification can be time-consuming and is somewhat subjective, being dependant on the skill and experience of the mycologist. Additionally, such typing of fungi only provides anecdotal evidence about actual profiles of secondary metabolites, as it is based on previously observed secondary metabolism of particular fungal species. This empirical approach is further confounded by the fact that fungi of the same species can be both toxigenic and nontoxigenic; that is, some readily produce mycotoxins, but some otherwise indistinguishable fungi of the same species are genetically incapable of toxin production. Classification of fungal species alone therefore provides no real insight into mycotoxin production.

In the past, direct analysis of fungal culture media for the presence of mycotoxins has of necessity involved “target” analysis with the inevitable assumption as to which toxins should be sought. However, LC/TOF-MS offers new possibilities for studying the behavior of fungi with regard to toxin production. Providing that efficient extraction from the medium of toxins with widely differing polarity can be demonstrated, the specificity of TOF-MS means that any further sample clean-up is not necessary. Furthermore, targeted analysis is also unnecessary as the instrument can provide accurate mass measurement of molecular ions of any components detected in an LC run, and these can be identified by searching a database of exact masses of relevant secondary metabolites.

In this note we describe suitable conditions for extraction of secondary metabolites from cultured fungi and LC/TOF-MS conditions for subsequent analysis. The methodology has been validated by spiking aflatoxins, ochratoxin A, trichothecenes, zearalenone, and fumonisins into various growth media, and demonstrating good recovery from the media at low levels and subsequent identification by searching against a database of 465 secondary metabolites. The methodology has been applied to one *Penicillium* species and six *Aspergillus* species, which were obtained from a culture collection, and their secondary metabolites have been compared with the anticipated toxin profiles.

Experimental

All analytical work was performed using an Agilent 6210 TOF-MS coupled to an Agilent 1200 Series HPLC. The separation of mycotoxins and other fungal metabolites was also carried out using an HPLC system (consisting of vacuum degasser, autosampler with thermostat, binary pump, and DAD system) equipped with a reversed-phase C18 column (ZORBAX Eclipse XDB 100 × 2.1 mm, 1.8 µm). The TOF-MS was equipped with a dual-nebulizer electrospray source, allowing continuous introduction of reference mass compounds. The instrument was scanned from *m/z* 100 to 1,000 for all samples at a scan rate of 1 cycle/sec in 9,429 transient/scan. This mass range enabled the inclusion of two reference mass compounds, which produced ions at *m/z* 121.0508 and 922.0097. The injected sample volume was 5 µL.

The HPLC analysis used a mobile phase of acetonitrile and 2 mM ammonium acetate in an aqueous solution of 1% formic acid at a flow rate of 0.3 mL/min. The gradient elution started with 15% acetonitrile and reached 100% acetonitrile in 20 min. The column was washed with 100% acetonitrile for 5 min. and equilibrated for 5 min between chromatographic runs. UV spectra were obtained using diode array detection scanning every 0.4 sec from 200 to 700 nm with a resolution of 4 nm. The optimum TOF-MS conditions are given in Table 1. The data recorded were processed with Analyst-QS software with accurate mass application. The database of 465 mycotoxins and other fungal metabolites was created in Excel from reference sources [1,2], which were easily adapted to use in a search capacity using Agilent software.

Table 1. LC/MS-TOF Operational Conditions in ESI+ Ion Mode

Parameter	
Capillary voltage	3000 V
Nebulizer pressure	40 psig
Drying gas	10 L/min
Gas temperature	300 °C
Fragmentor voltage	150 V
Skimmer voltage	60 V
OCT* RF	250 V
OCT* DC	37.5 V
Mass range (*m/z*)	100–1000
Reference masses	121.050873; 922.009798

*Octapole

Fungal Extraction

Well-characterized isolates of *A. paraciticus (NRRL 2999)*, were obtained from the USDA culture collection and isolates of *A. flavus, (200198), A. ochraceus (200700), A. oryzae (200828), A. niger (200807), A. fumigatus (200418),* and *P. citrinum (501862)* were obtained from the TÜBITAK Mamara Research Center culture collection. Fungi were inoculated onto malt extract agar (MEA), potato dextrose agar (PDA), and yeast extract sucrose agar (YES) in petri dishes. After allowing the fungi to grow for 7 to 14 days at 25 °C, typical prolific growth of fungal colonies was observed on the surface of the media. Samples of fungal hyphae, together with underlying culture media, were taken by vertically cutting two 6-mm diameter plugs using a cork borer. The plugs were transferred to 5-mL disposable screw-cap bottles. Extraction conditions were modified from previous published methods [3,4]. One of the plugs was extracted twice with 2 mL ethyl acetate with 1% formic acid and then 2 mL isopropanol. The second plug was extracted twice with 2 mL ethyl acetate with 1% formic acid and then 2 mL acetonitrile, followed by 1 min vortexing and 30 min total ultrasonication. The extracts were filtered and evaporated gently under a nitrogen stream. The residues in both cases were dissolved in 1 mL methanol, ultrasonicated for 10 min and passed through a 0.2-µL disposable filter prior to HPLC analysis.

Results and Discussion

Optimization of LC/TOF-MS Conditions

The most important instrumental parameters, which were capillary voltage, nebulizer pressure, drying gas, gas temperature, and skimmer voltage, were initially optimized by autotune to achieve

maximum sensitivity. However, the fragmentor voltage also needed to be optimized to provide maximum structural information, which sometimes required a compromise. Optimization was carried out by varying the fragmentor voltage in the range of 55 to 250 V without changing any other conditions. The fragmentor voltage that provided minimum fragmentation was found at 150 V.

To validate the whole procedure, 20 commercially available standards (aflatoxins B_1, B_2, G_1, and G_2; aflatoxin M_1; ochratoxin A; zearalenone; 4-deoxynivalenol; 3-acetyldeoxynivalenol; 15-acetyldeoxynivalenol; diacetoxyscirpenol; fusarenone X; neosolaniol; fumonisins B_1, B_2, and B_3; nivalenol; HT-2 toxin; T2 toxin; and kojic acid) and internal standard (benzophenone) were mixed together. Using positive electrospray, the accurate masses of protonated molecule ions, retention times, and UV spectra were obtained in each case.

Construction of Database of Accurate Masses of Fungal Metabolites

An Excel spreadsheet was constructed containing the exact mass data for each of the 465 mycotoxins and fungal metabolites, together with their empirical formulas [1,2]. Theoretical monoisotopic exact masses of the compounds were calculated based on their molecular formula using an Excel spreadsheet (called "Formula DB Generator" and provided with the Agilent TOF) and put into csv (comma-separated values) format for use by the Agilent TOF automated data analysis software. The csv file is searched automatically by the LC/TOF-MS instrument at the completion of the sample run and a report is generated on compounds that were found in the database. The creation of the data analysis method is done using a data analysis editor. The editor allows selection of adducts (for example, in positive ion H^+, NH_4^+, Na^+, etc.) and neutral losses to be searched automatically, as well as mass accuracy and retention time tolerances, report options, and other search and detection criteria. Retention times are not required but if they are known add a degree of confidence to the identification.

We use samples of various growth media that had been spiked with the standard mixture of 20 mycotoxin standards to determine retention times. The standards were injected 10 times to establish the repeatability of those retention times. The criteria used for identification were a fit for the accurate mass of the M+1 ion to a mass tolerance of ± 5 ppm, a retention time match to ± 0.2 min (if standards available), a minimum peak height count, which is called the compound threshold of 1,000 counts (or a signal-to-noise ratio of ~10:1 or 0.06% relative volume), and, if present, good correspondence (to ± 5 ppm) with predicted adducts and neutral fragment losses.

Method Validation by Spiking and Analysis

Based on the above detection criteria, all 20 standards were correctly identified when spiked at 25 to 100 µg/kg into growth media, and analyzed as described above.

Utilization of the Method to Determine Metabolite Production from Well-Characterized Fungi

Rather than simply looking at theoretical situations with spiked growth media, the above technique was applied to the real situation of well-characterized fungi being cultured on various media. One *Penicillim* species and six *Aspergillus* species were grown on three different media. Using the simple solvent extraction described above, the crude extracts were directly analyzed by LC/TOF-MS. By way of illustration, Figure 1 shows the total ion chromatogram for an *A. flavus* extract indicating about 20 components detected. The peak eluting at 8.9 min on database searching was found to have an accurate mass of *m/z* 313.0712. Based on the $M+H^+$ ion this corresponded to aflatoxin B_1 with a 0.2 ppm mass match as compared to the database exact mass for aflatoxin B_1.

The software uses a molecular feature (MFE) algorithm that removes all ions that are not real peaks and groups the real ions into "molecular features." Those molecular features can be characterized by their relationship with each other and adducts, dimers, trimers, etc., and their isotopes (depicted as +1, +2, etc.) are deduced. The molecular features and accurate mass measurement of these species for the peak at a retention time of 8.9 min identified as aflatoxin B_1 are summarized in Table 2. Selecting a molecular feature, the software will calculate possible empirical formulas and score the isotopes for the "fit" to the proposed formula; this is also shown in Table 2. The formula with the score of 100 is that of aflatoxin B_1. This formula then can be automatically translated to a Web connection search with NIST, ChemIndex, and Medline. The search results in NIST indicated the formula and structure of aflatoxin B_1 as illustrated in Figure 2.

In addition to the identification of aflatoxin B_1 as a secondary metabolite from *A. flavus*, this fungi was also found to produce aflatoxin B_2, aflatoxin B_3, and aflatoxin G_1, which are consistent with

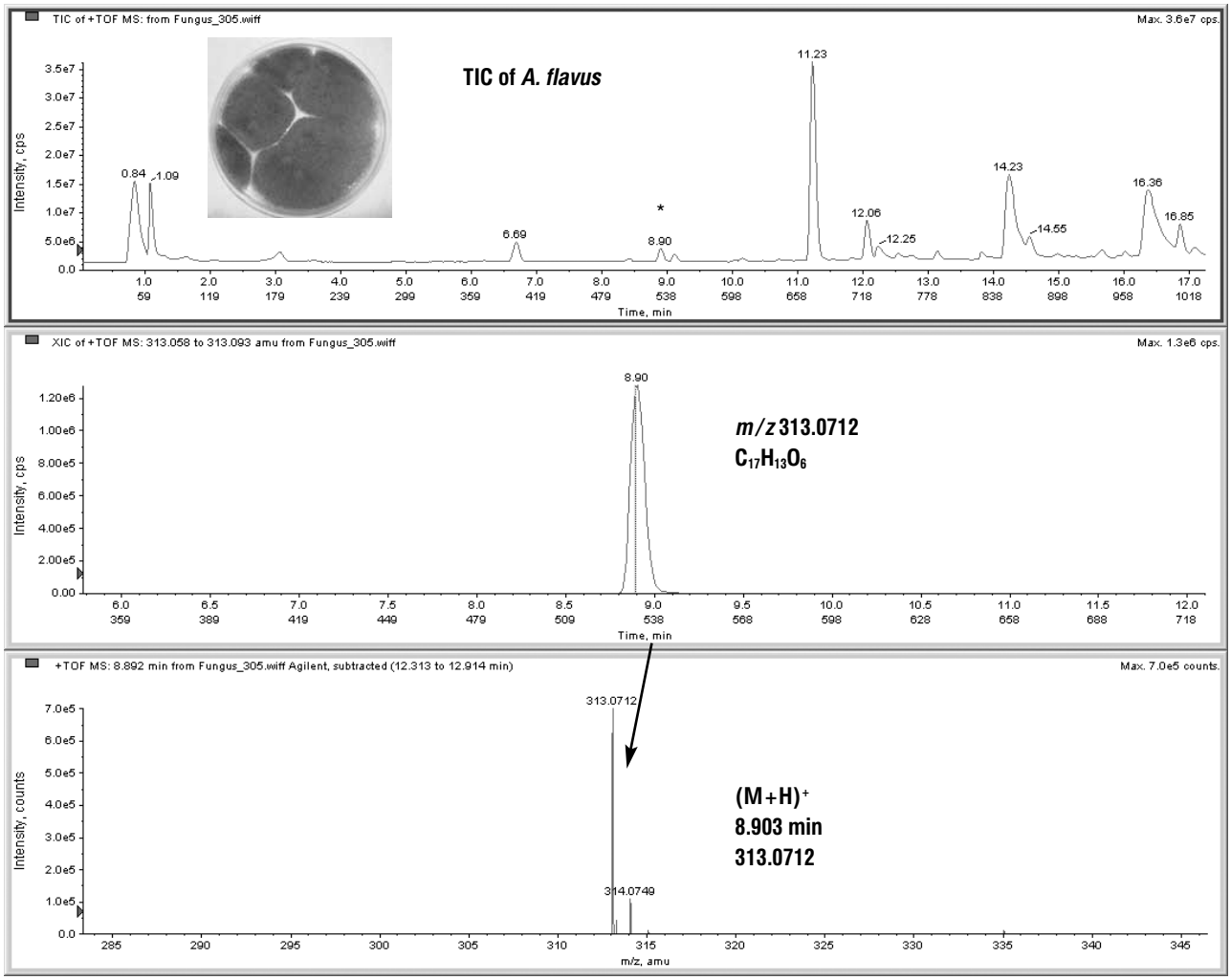

Figure 1. Analysis of an extract from *A. flavus* by LC/TOF-MS illustrating:
(a) Total ion chromatogram (TIC) with * peak corresponding to aflatoxin B_1,
(b) Extracted ion chromatogram from *m/z* 313.058 to 313.093 for aflatoxin B_1,
(c) Full-scan spectrum showing accurate mass with 0.2 ppm error for M+1 ion for aflatoxin B_1.

Table 2. Typical Clusters Seen in ESI+ LC/MS-TOF on the Peak Retention Time of 8.90 min, *m/z* 313.0706

MFE

Feature #27 (RT = 8.903)

Species	RT	*m/z*	Mass	Abundance	Width
M	8.903		312.0633	5541933	0.088
M+H	8.903	313.0706	312.0633	4035186	0.09
M+H+1	8.903	314.0744		622147	0.088
M+H+2	8.902	315.0766		74349	0.09
M+H+3	8.906	316.0795		7943	0.085
M+Na	8.904	335.0529	312.0637	86580	0.094
M+Na+1	8.904	336.0563		15898	0.097
M+Na+2	8.916	337.0593		1848	0.091
2M+H	8.906	625.1357	312.0642	741	0.049
2M+Na	8.902	647.1164	312.0636	226965	0.061
2M+Na+1	8.902	648.1202		65639	0.063
2M+Na+2	8.900	649.1228		14941	0.065
2M+Na+3	8.899	650.1256		2677	0.058
2M+Na+4	8.897	651.1290		257	0.048

MFE

Composition's chemical formula	dm(Da)	dm(ppm)	dm(ppm)	DBE	Score
$C_{17}H_{12}O_6$	0.0001	0.2	0.2	12	100
$C_{18}H_8N_4O_2$	0.0014	4.5	4.5	17	77
$C_{14}H_4N_{10}$	–0.0013	–4.2	4.2	18	68
$C_9H_{16}N_2O_8S$	–0.0006	–1.9	1.9	3	58
$C_{13}H_8N_6O_4$	–0.0026	–8.4	8.4	13	55

NIST
National Institute of Standards and Technology

Standard Reference Data Program | Data Gateway | Online Databasis | Chemistry WebBook

Aflatoxin B_1

- **Formula:** $C_{17}H_{12}O_6$
- **Molecular weight:** 312.27
- **IUPAC International Chemical Identifer:**
  - o InChI=1/C17H12O6/c1-20-10-6-11-14(8-4-5-21-17(8)22-11)15-13(10)7-2-3-9(18)12(7)16(19)23-15/h4-6,8,17H,2-3H2,1H3
- **CAS Registry Number:** 1162-65-8
- **Chemical structure:**

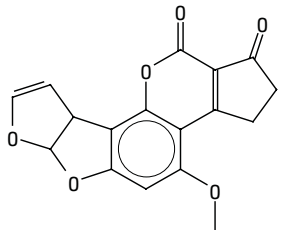

Figure 2. Database search result for emprical formula using NIST (Medline and ChemIndex results were the same but are not given here). Note molecular weights should not be searched in these databases as they are often the "average" molecular weight and not the monoisotopic weight.

known metabolic behavior. In Table 3 the screening results from the database search with a 5 ppm tolerance are shown with the accurate masses of some other peaks, which corresponded to known compounds. Kojic acid and methoxysterigmatocystin, which are a good match, are both well-known fungal metabolites that might be expected to be found from *A. flavus*. A good match was also found for cinnamic acid, which is not known as a metabolite.

When this new approach was applied in a preliminary study of a total of seven different fungi obtained from culture collections and grown on three different media, the results shown in Table 4 were obtained. In most cases the predicted metabolites were found, which gives good confidence in the methodology. Some of these initial results showed that predicted mycotoxins were not detected and unexpected metabolites were found. The possibility of a misidentified culture exists or that metabolites not previously reported were detected. While this demonstrates the power of the approach, these results do need to be followed up with more in-depth study.

Future Prospects

The use of accurate mass LC/TOF-MS combined

Table 3. Results of Automated Mycotoxin Database Search for *A. flavus* Extract (Extraction compound list is sorted in ascending order of retention time within 5 ppm error. Benzophenone was used as an internal standard.)

Mass Value = 142.03					
Formula	**Compound**	**Mass**	**Error (mDa)**	***Error (ppm)**	**Ret. Time Error**
$C_6H_6O_4$	Kojic acid	142.03	–0.10	–0.7	–
Mass Value = 148.05					
Formula	**Compound**	**Mass**	**Error (mDa)**	**Error (ppm)**	**Ret. Time Error**
$C_9H_8O_2$	Cinnamic acid	148.05	–0.08	–0.5	–
Mass Value = 328.06					
Formula	**Compound**	**Mass**	**Error (mDa)**	**Error (ppm)**	**Ret. Time Error**
$C_{17}H_{12}O_7$	Aflatoxin G_1	328.06	1.01	1.4	–0.05
Mass Value = 354.07					
Formula	**Compound**	**Mass**	**Error (mDa)**	***Error (ppm)**	**Ret. Time Error**
$C_{19}H_{14}O_7$	5-Methoxysterigmatocystin	354.07	0.99	2.8	–
Mass Value =312.06					
Formula	**Compound**	**Mass**	**Error (mDa)**	***Error (ppm)**	**Ret. Time Error**
$C_{17}H_{12}O_6$	Aflatoxin B_1	312.06	–0.05	–0.2	–0.11
Mass Value =312.06					
Formula	**Compound**	**Mass**	**Error (mDa)**	***Error (ppm)**	**Ret. Time Error**
$C_{17}H_{14}O_6$	Aflatoxin B_2	314.08	0.06	0.2	0.06
Mass Value = 338.08					
Formula	**Compound**	**Mass**	**Error (mDa)**	***Error (ppm)**	**Ret. Time Error**
$C_{19}H_{14}O_6$	Methylsterigmatocystin	338.08	–0.16	–0.5	–
Mass Value = 182.07					
Formula	**Compound**	**Mass**	**Error (mDa)**	***Error (ppm)**	**Ret. Time Error**
$C_{13}H_{10}O$	Benzophenone	182.07	0.73	4.0	–0.15

Table 4. A Comparison of Detected and Predicted Metabolites from Culture Collection Fungi Grown in MEA, YES, and PDA Medium

	Fungi						
Metabolites	*P. citrinum*	*A. flavus*	*A. paraciticus*	*A. niger*	*A. fumigatus*	*A. oryzae*	*A. ochraseus*
AFB1		✱ √	✱ √				
AFB2		✱ √	✱ √				
AFB3		✱ √	✱				
AFG1		✱ √	✱ √				
AFG2		✱	✱ √				
KA	✱ √	✱ √	✱ √		✱	✱	
MST		✱ √	√				
5-MST		√					√
OTA				✱ √			✱
RO-A					√		
FU-B					✱ √		
MA				✱ √			
AA		✱					√
Nig				✱ √			
Ter							√
Cit	✱						

√ - metabolites detected by LC/TOF-MS; ✱ - metabolites predicted to be present

Key:

AFB1	Aflatoxin B_1 etc.	FU-B	Fumigaclavine B
KA	Kojic acid	MA	Malformin (peptides)
MST	Methylsterigmatocystin	AA	Aspergillic acid
5-MST	5-methoxysterigmatocystin	Nig	Nigragillin
OTA	Ochratoxin A	Ter	Terrein
RO-A	Roquefortine A (isofumigaclavine A)	Cit	Citrinin

with database searching is a powerful example of a new, versatile identification technique that can be used in targeted analysis. In the area of fungal metabolites, the potential to screen fungi for a range of metabolites for which dedicated methods are not available has been demonstrated. This approach offers new possibilities for fungal typing based on metabolite production and rapid screening of agricultural products for mycotoxins of food safety interest. Where previously unknown metabolites are detected, although LC/TOF-MS can provide some insight, further work with a hybrid quadrupole time-of-flight LC/MS system (LC/QTOF-MS) will be required for structural elucidation.

Conclusions

A simple and rapid method has been developed using LC/TOF-MS to determine the profile of secondary metabolites produced by fungi under various culture conditions. The approach has been validated by spiking representative metabolites into solid cultures and demonstrating good recovery and identification by searching accurate masses against a metabolite database. Results for a range of well-characterized fungi from a culture collection showed that the anticipated toxins could be readily detected.

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Printed in the USA
June 12, 2007
5989-6820EN

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Determination of Aflatoxins in Food by LC/MS/MS

Application

Food Safety

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Abstract

A sensitive and selective analytical method for the determination of aflatoxin G1, G2, B1, and B2 residues in cereals using the Agilent G6410AA LC/MS Triple Quadrupole Mass Spectrometer was developed. This method uses simple sample preparation methods followed by LC/MS/MS. The limits of detection for all aflatoxins were less than 1 ng/mL in cereals.

Introduction

Aflatoxins (AFs) belong to a closely related group of secondary fungal metabolites. These mycotoxins are severely toxic metabolites produced mainly by *Aspergillus flavus* and *A. parasiticus*, and exposure to them can cause cancer in humans and livestock [1]. Based on epidemiological evidence, AFs have been classified as human liver carcinogens by the World Health Organization and by the U.S. Environmental Protection Agency. Thus, accurate determination of AFs is required to avoid human disease from AF exposure and to advance worldwide surveillance of food. Analysis of AFs in food products is routinely performed by thin-layer chromatography (TLC) and liquid chromatography (LC) with fluorescence detection (FD) in combination with both precolumn derivatization and postcolumn derivatization. The LC/FD technology is often used due to the high selectivity and sensitivity of these methods. Furthermore, hyphenated techniques such as LC coupled to mass spectrometric (MS) detection have been developed and applied in residual analysis of foods. The high selectivity and sensitivity of MS detection methods associated with the resolution of LC provide decisive advantages to perform qualitative as well as quantitative analysis of a wide range of molecules at trace levels. Several papers describing different kinds of MS methods for the analysis of AFs have been published [2-4.]

Experimental

Sample Preparation

The samples analyzed (peanuts, corn, nutmeg, and red pepper) were obtained from a local market and did not include any AFs. The extraction and cleanup steps for AFs were carried out according to validated methods reported by Tanaka [5]. Briefly, 20 g fine ground sample was poured into a 200-mL Erlenmeyer flask, followed by adding 40 mL acetonitrile-water (9:1, v/v) for corn and cereals. After shaking for 30 min, the mixed solution was centrifuged for 5 min at 1,650 g. The supernatant obtained was filtered through a glass microfiber GF/B grade filter (Whatman Interna-

tional Ltd, Maidstone, UK). A 5-mL portion of the filtrate was applied to a MultiSep number 228 cartridge column for the cleanup. After passing through at a flow rate of 1 mL/min, 2 mL of the first eluate was collected. The eluate was evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 1 mL methanol-water (4:6 v/v) containing 10 mM ammonium acetate.

Standard Preparation

Each of the standard reagents, aflatoxin G_2 (AFG_2), aflatoxin G_1 (AFG_1), aflatoxin B_2 (AFB_2) and aflatoxin B_1 (AFB_1), was dissolved in acetonitrile at 1 mg/mL and was stored at 4 °C in the dark until use. To prepare the working standard for LC/MS analysis, each AF stock solution was equally pipetted and transferred to a vial, and it was then diluted with the mobile phase. The final concentration of each AF was 1 ng/mL.

Chemicals

The standards AFG_2, AFG_1, AFB_2, and AFB_1 were obtained from Sigma Aldrich Japan (Tokyo, Japan). The purity of these compounds was greater than 99%. Ammonium acetate, toluene, HPLC-grade acetonitrile, and HPLC-grade methanol were obtained from Wako Chemical (Osaka, Japan). Water was purified in-house with a Milli-Q system (Millipore, Tokyo, Japan). The cartridge column of MultiSep number 228 was purchased from Showa Denko (Kanagawa, Japan).

LC/MS Instrument

The LC/MS/MS system used in this work consists of an Agilent 1200 Series vacuum degasser, binary pump, well-plate autosampler, thermostatted column compartment, the Agilent G6410 Triple Quadrupole Mass Spectrometer with an electrospray ionization (ESI) source. The objective of the method development was to obtain a fast and sensitive analysis for quantifying AFs in foods. For chromatographic resolution and sensitivity, different solvents and columns were optimized. It was found that a simple solvent system using water, methanol, ammonium acetate, and a 1.8-µm particle size C18 column worked very well.

LC Conditions

Instrument:	Agilent 1200 HPLC
Column:	ZORBAX Extend C18, 100 mm × 2.1 mm, 1.8 µm (p/n 728700-902)
Column temp:	40 °C
Mobile phase:	A = 10 mM ammonium acetate in water B= Methanol 40% A/60% B
Flow rate:	0.2 mL/min
Injection volume:	5 µL

MS Conditions

Instrument:	Agilent 6410 LC /MS Triple Quadrupole
Source:	Positive ESI
Drying gas flow:	10 L/min
Nebulizer:	50 psig
Drying gas temp:	350 °C
V_{cap}:	4000 V
Scan:	*m/z* 100 – 550
Fragmentor:	Variable 100 V
MRM ions:	Shown in Table 1
Collision energy:	Shown in Table 1

LC/MS/MS Method

Quantitative analysis was carried out using MRM mode. The parameters for MRM transitions are shown in Table 1.

Table 1. Data Acquisition Parameters of MRM Transitions for Each Aflatoxin

No	Mycotoxins	RT (min)	Molecular weight	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)
1	Aflatoxin G_2	5.21	330	331	245	30
2	Aflatoxin G_1	6.61	328	329	243	30
3	Aflatoxin B_2	8.44	314	315	259	30
4	Aflatoxin B_1	10.89	312	313	241	30

Results and Discussion

Optimization of MRM Transitions

Determination of the optimal MRM transitions for each aflatoxin was carried out using single-MS full-scan mode followed by product ion scan mode using taflatoxin standard mixtures at 1 ug/mL. Mass spectra of these standard mixtures in full scan mode and product ion scan mode are shown in Figures 1 and 2. The mass spectrum of each aflatoxin by full-scan mode exhibited the protonated molecule $[M+H]^+$ as the base peak ion. These ions were selected as precursor ions for MRM mode. The optimum collision voltage is compound dependent. To establish the optimum collision voltage, this parameter was varied from 5 to 40 V using a step size of 5V. As shown in Figure 2, a distinct optimum in the intensity of the product ion of each AF was observed at 30 V. The product ions that indicated the highest intensity were *m/z* 245 (AFG_2), 243 (AFG_1), 259 (AFB_2), and 241 (AFB_1), respectively. On the basis of the above results, the collision voltage was set to 30 V.

Table 1 shows the parameters of MRM mode of each aflatoxin.

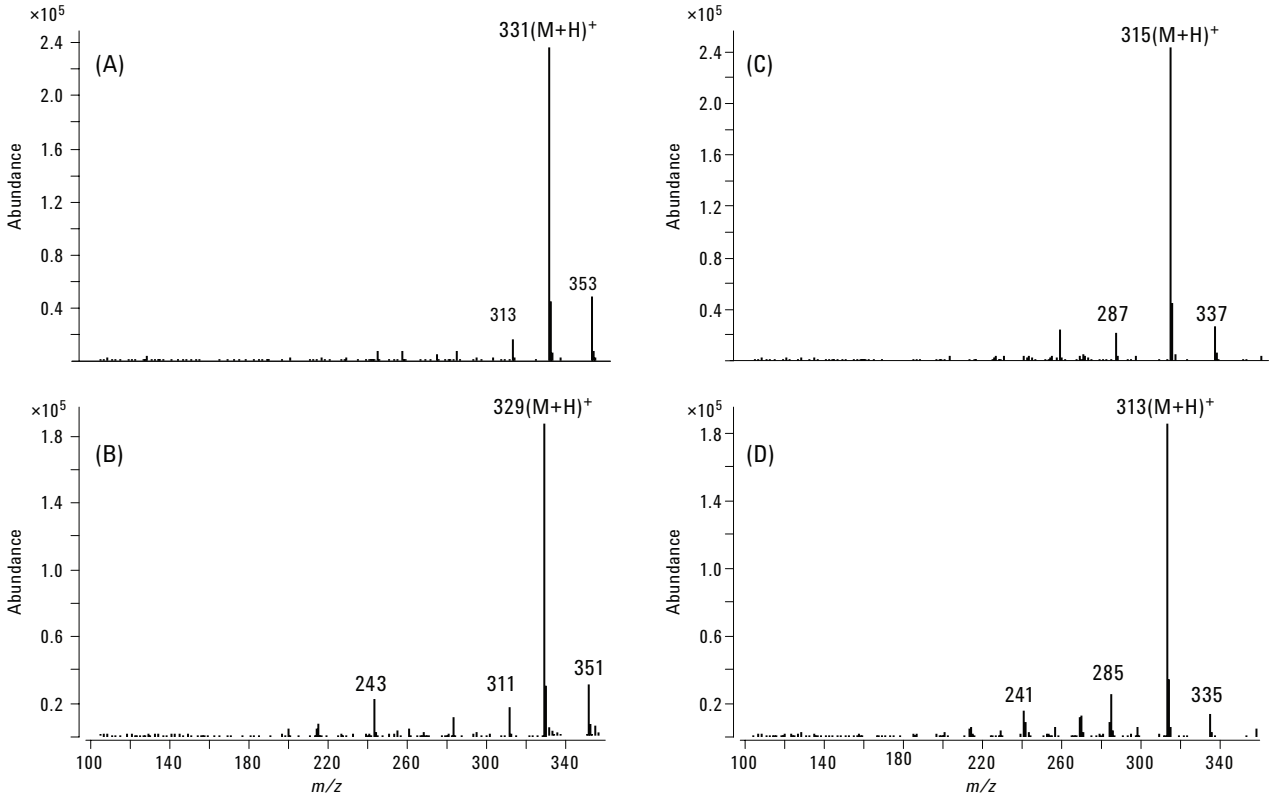

Figure 1. Mass spectra of four aflatoxins standard in single-MS full-scan mode at 1 µg/mL (A): aflatoxin G_2, (B): aflatoxin G_1, (C): aflatoxin B_2, and (D): aflatoxin B_1.

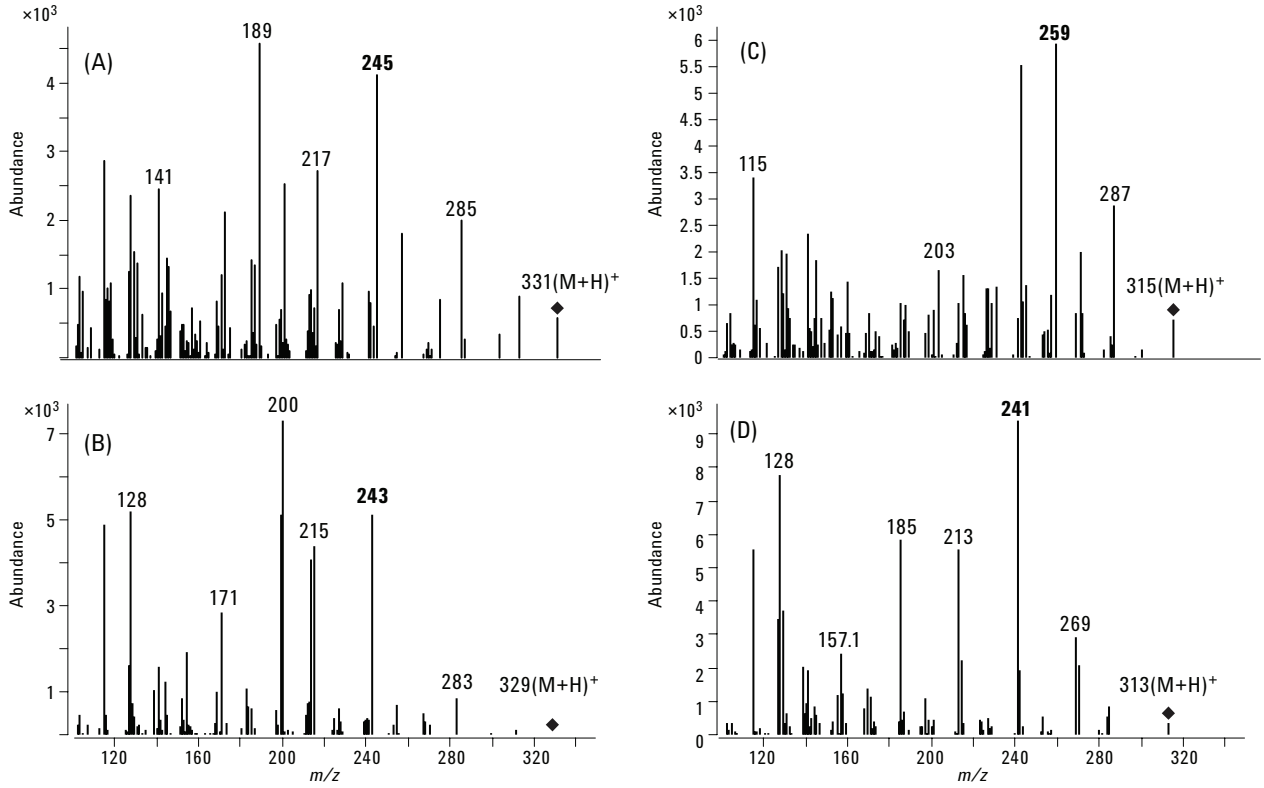

Figure 2. Mass spectra of four aflatoxins standard in product ion scan mode at 1µg/mL (A): aflatoxin G_2, (B): aflatoxin G_1, (C): aflatoxin B_2, and (D): aflatoxin B_1.

The MRM chromatogram of each aflatoxin at 0.1 ng/mL is shown in Figure 3. These show excellent signal-to-noise (S/N) ratios for all aflatoxins. The limit of detection (LOD) for each aflatoxin was determined using an S/N ratio of 3 with this MRM chromatogram and is shown in Table 2. To evaluate the linearity of the calibration curves, various concentrations of aflatoxin standard solutions ranging from 0.1 ng/mL to 100 ng/mL were analyzed. As shown in Figure 4 and Table 2, the linearity was very good for all aflatoxins with correlation coefficients (r^2) greater than 0.999.

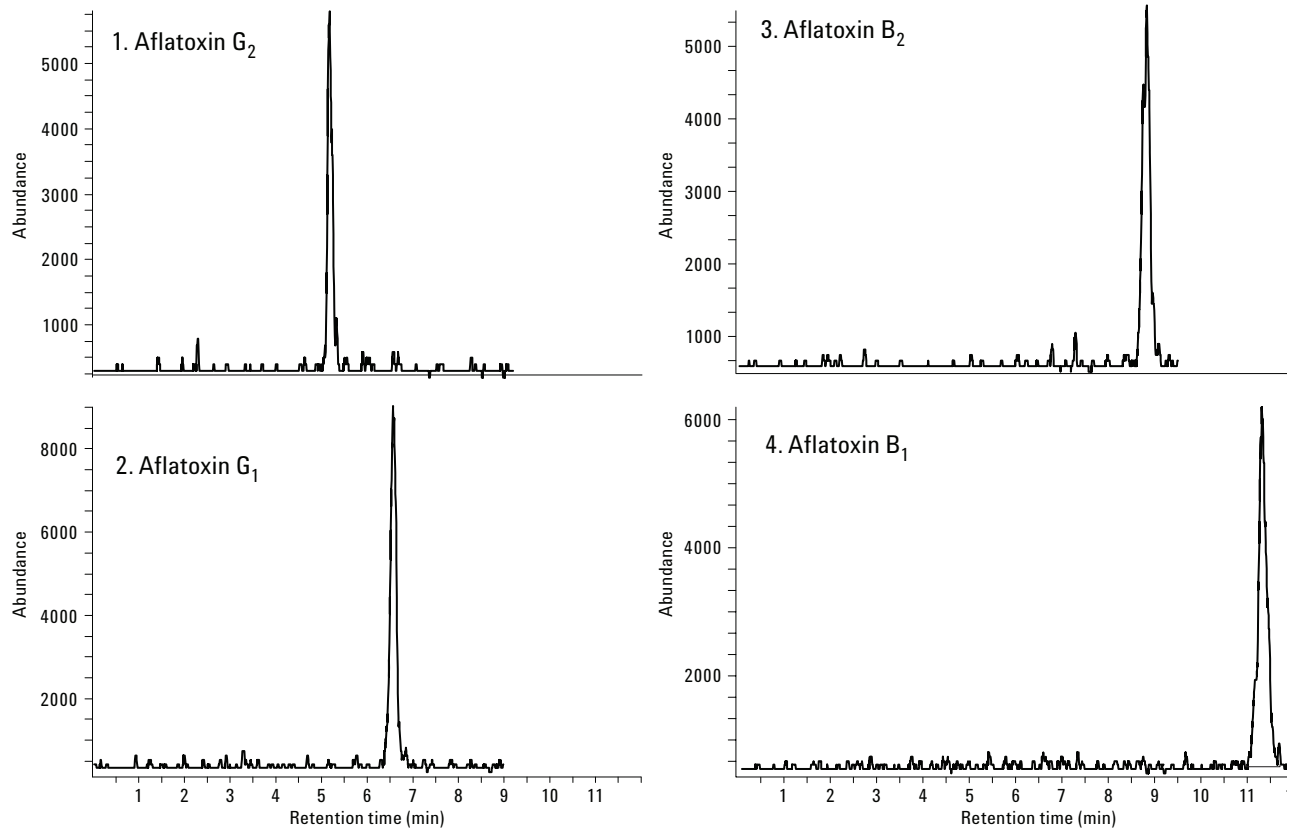

Figure 3. MRM chromatograms of four aflatoxin standards at 0.1 ng/mL in MRM mode.

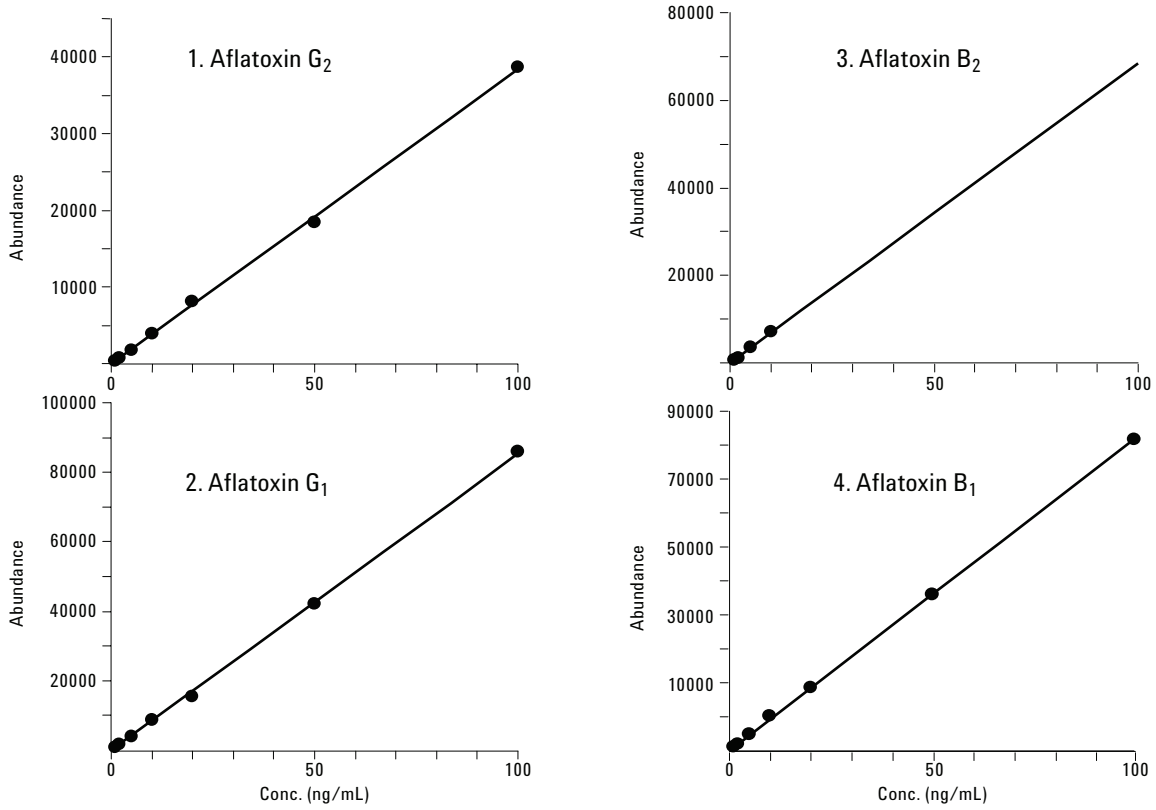

Figure 4. Calibration curves of four aflatoxins ranged from 0.1 ng/mL to 100 ng/mL.

Table 2. Linearity and LODs of Four Aflatoxins

No	Mycotoxins	r^2	LOD (ng/mL)
1	Aflatoxin G_2	0.9999	0.025
2	Aflatoxin G_1	0.9992	0.020
3	Aflatoxin B_2	0.9999	0.025
4	Aflatoxin B_1	0.9993	0.020

The matrix effect of this method was investigated by using cereal and corn extracts spiked with mycotoxin standards at 0.2 ng/mL. Typical MRM chromatograms of cereal and corn extract are shown in Figures 5 and 6, respectively. There were no additional peaks from sample matrix in either food when compared with the mycotoxin standard mixture. These results indicate that MRM mode has very high selectivity.

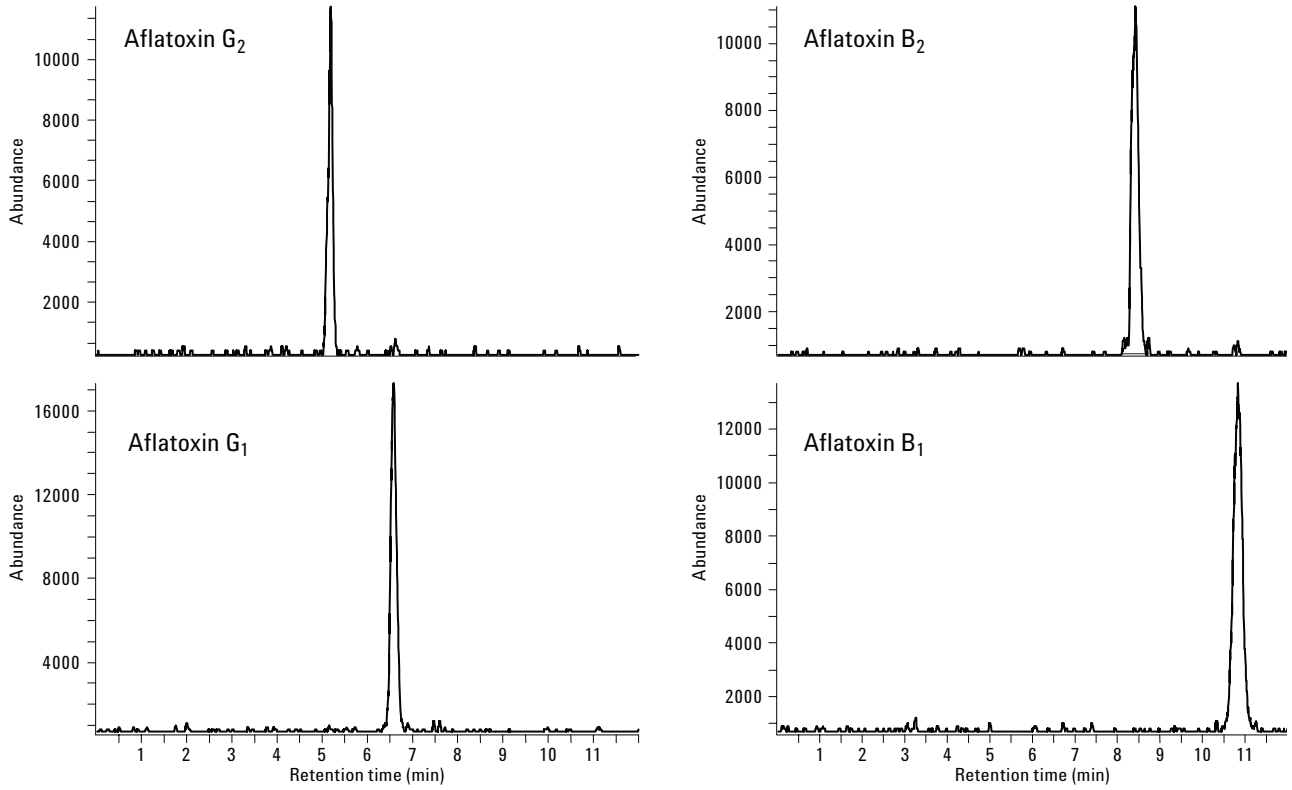

Figure 5. MRM of four aflatoxins in cereal extract spiked at 0.2 ng/g.

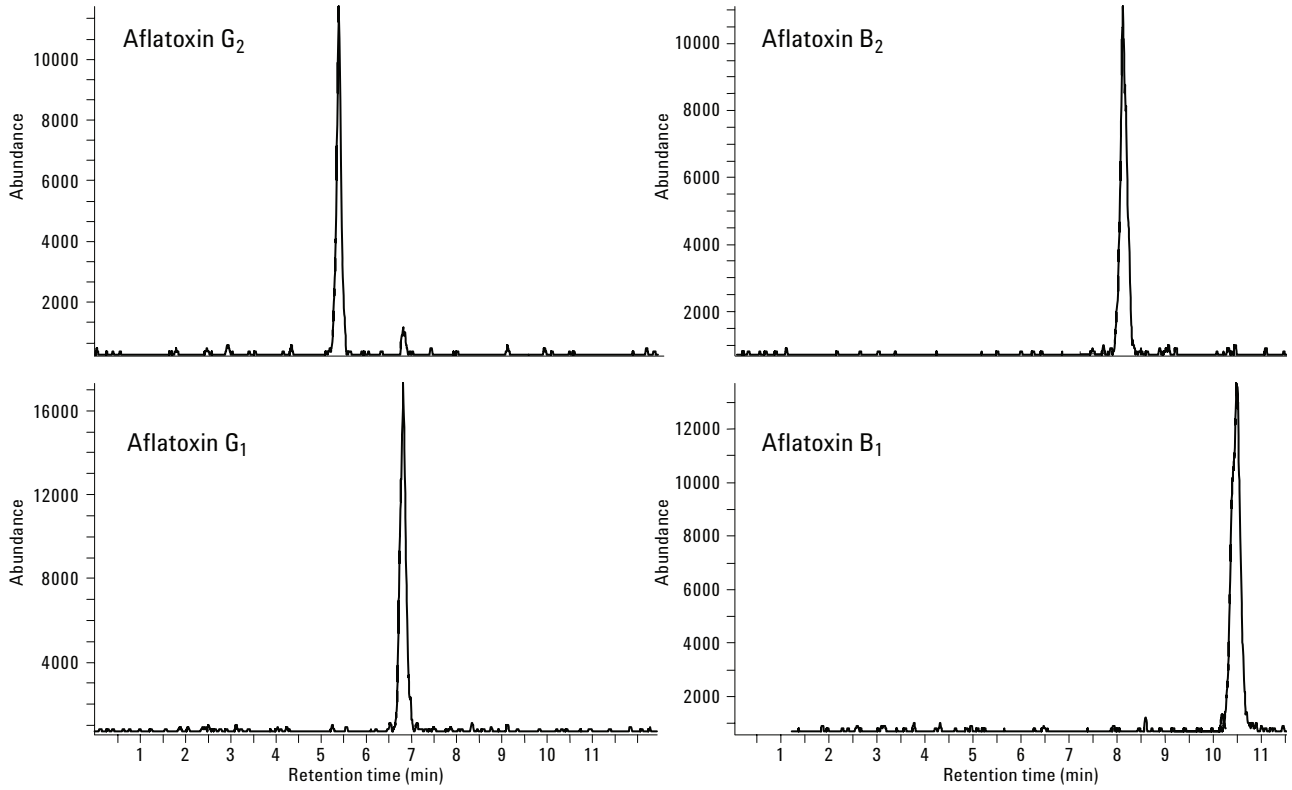

Figure 6. MRM of four aflatoxins in corn extract spiked at 0.2 ng/g.

Furthermore, the change on the peak intensity of each aflatoxin by the sample matrix was investigated by comparison with the peak intensity of aflatoxin standards. As these results show in Table 3, the relative intensity of each pesticide ranged from 88 to 96%. Thus, matrix effects such as ion suppression may be insignificant and it is possible to use external standards instead of matrix-matched standards.

Table 3. Relative Intensity of Each Aflatoxin in Sample Extracts

No	Mycotoxins	Relative intensity (%)	
		Cereal	Corn
1	Aflatoxin G_2	88	91
2	Aflatoxin G_1	92	94
3	Aflatoxin B_2	93	96
4	Aflatoxin B_1	97	95

Conclusions

The multi-aflatoxin method by LC/MS/MS described here was suitable for the determination of four aflatoxins in cereal and corn extract due to its high sensitivity and high selectivity. Another advantage of this method is that ion suppression was not observed for all food samples studied. Thus, it may eliminate the need for matrix-matched standards, which makes analysis more tedious for samples from different origins.

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Printed in the USA
January 4, 2008
5989-7615EN

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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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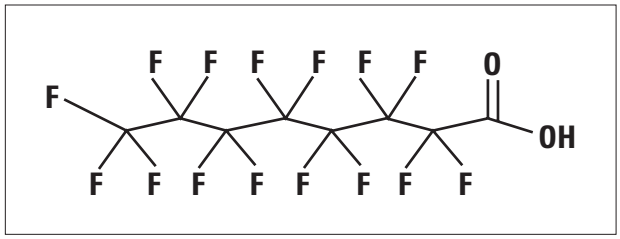
Abstract

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%.

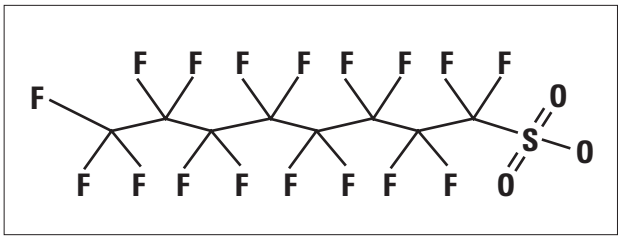
Introduction

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

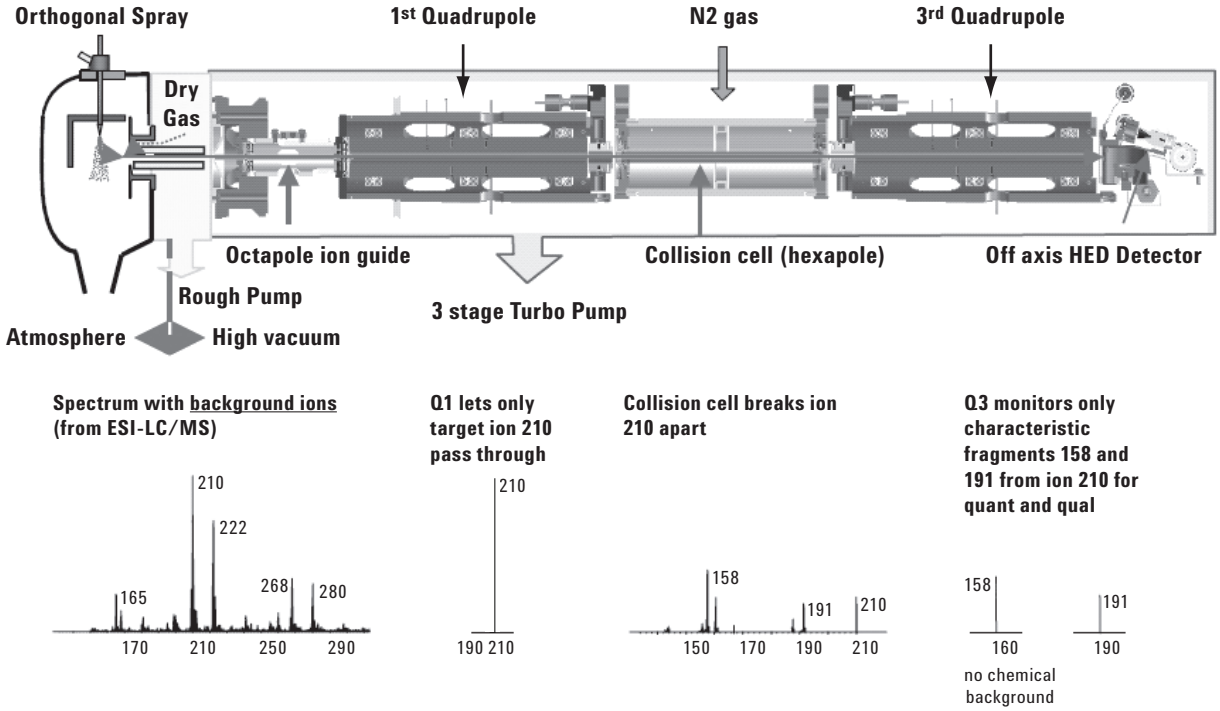


Figure 2. A cross-section of the Agilent 6410 QQQ above a sequence of spectra characteristic of ion transitions within the instrument for a hypothetical sample (*not* PFOA or PFOS). Note that the final spectrum is very clean, containing only the desired target ions. (HED = high-energy dynode electron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

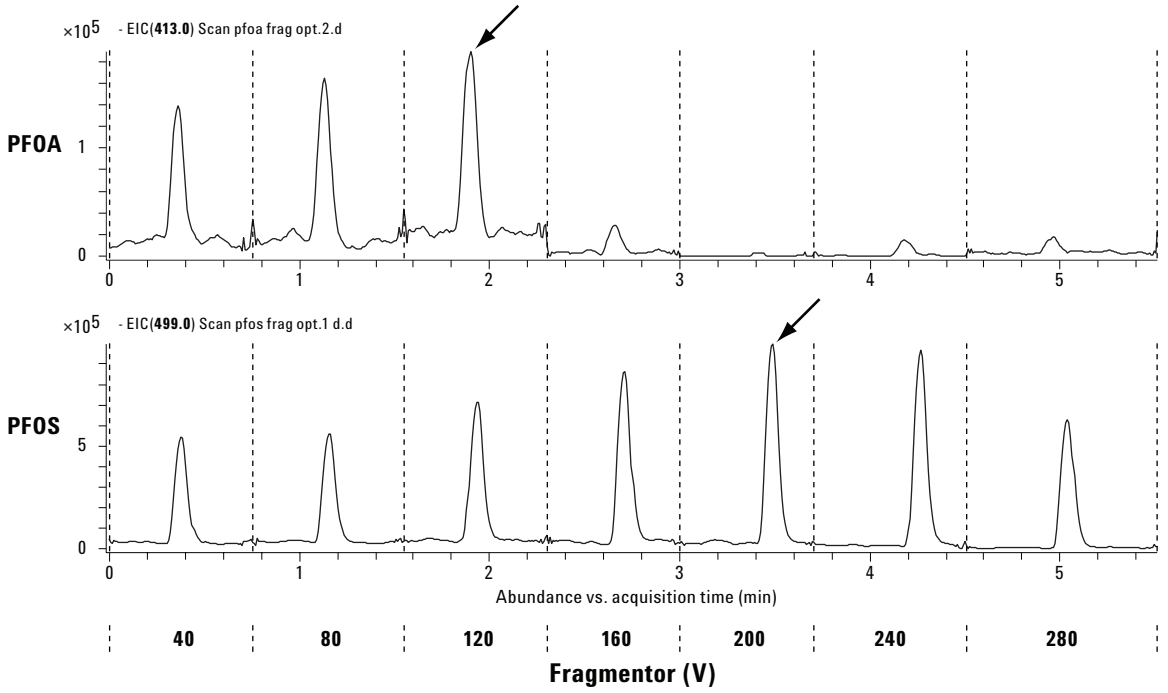


Figure 3. Determination of optimal fragmentor voltage using sequential plots of signal intensity versus applied voltage.

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.

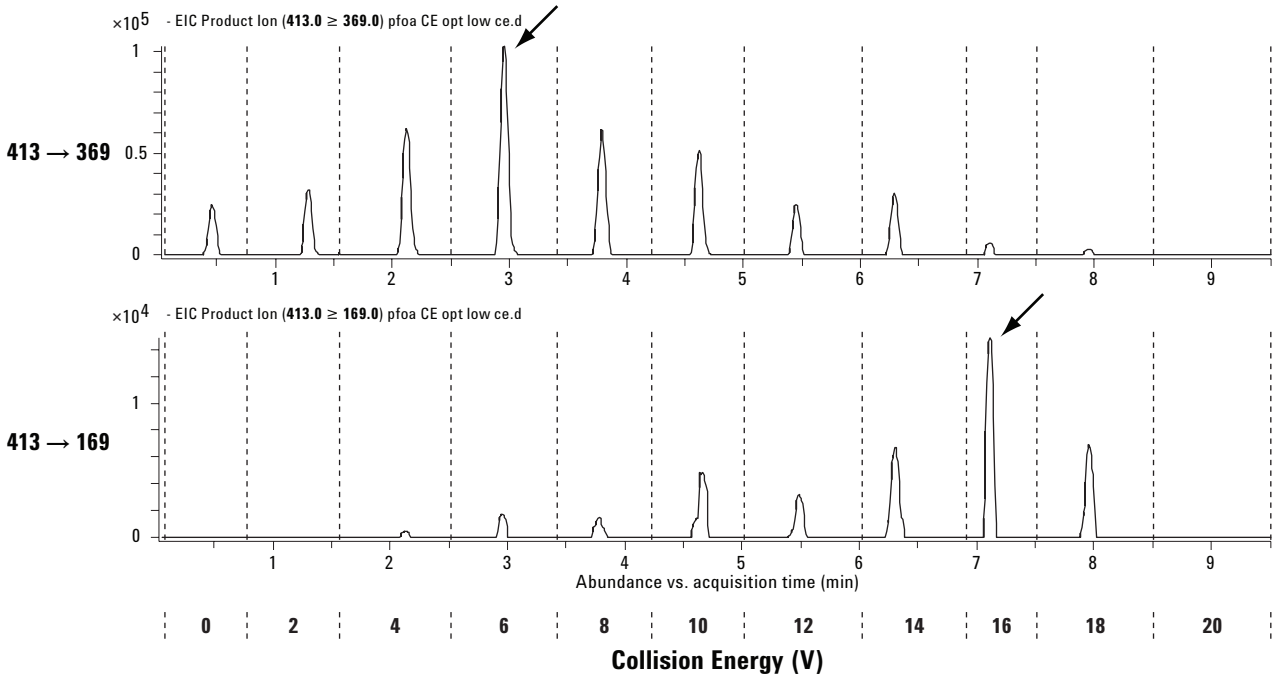


Figure 4. Signal intensity as a function of collision energy for PFOA product ions *m/z* 369 $[M\text{-}CO_2H]^-$ and *m/z* 169 $[C_3F_7]^+$.

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).

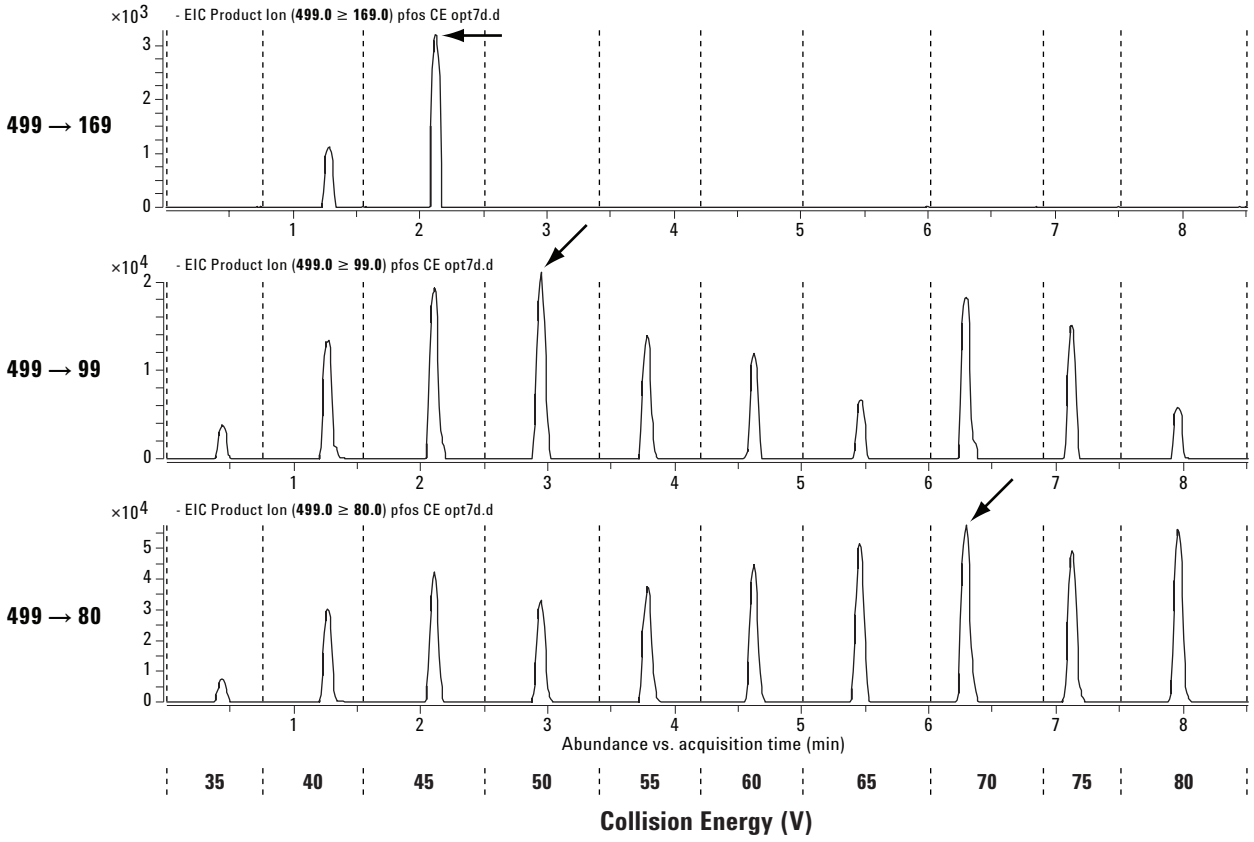
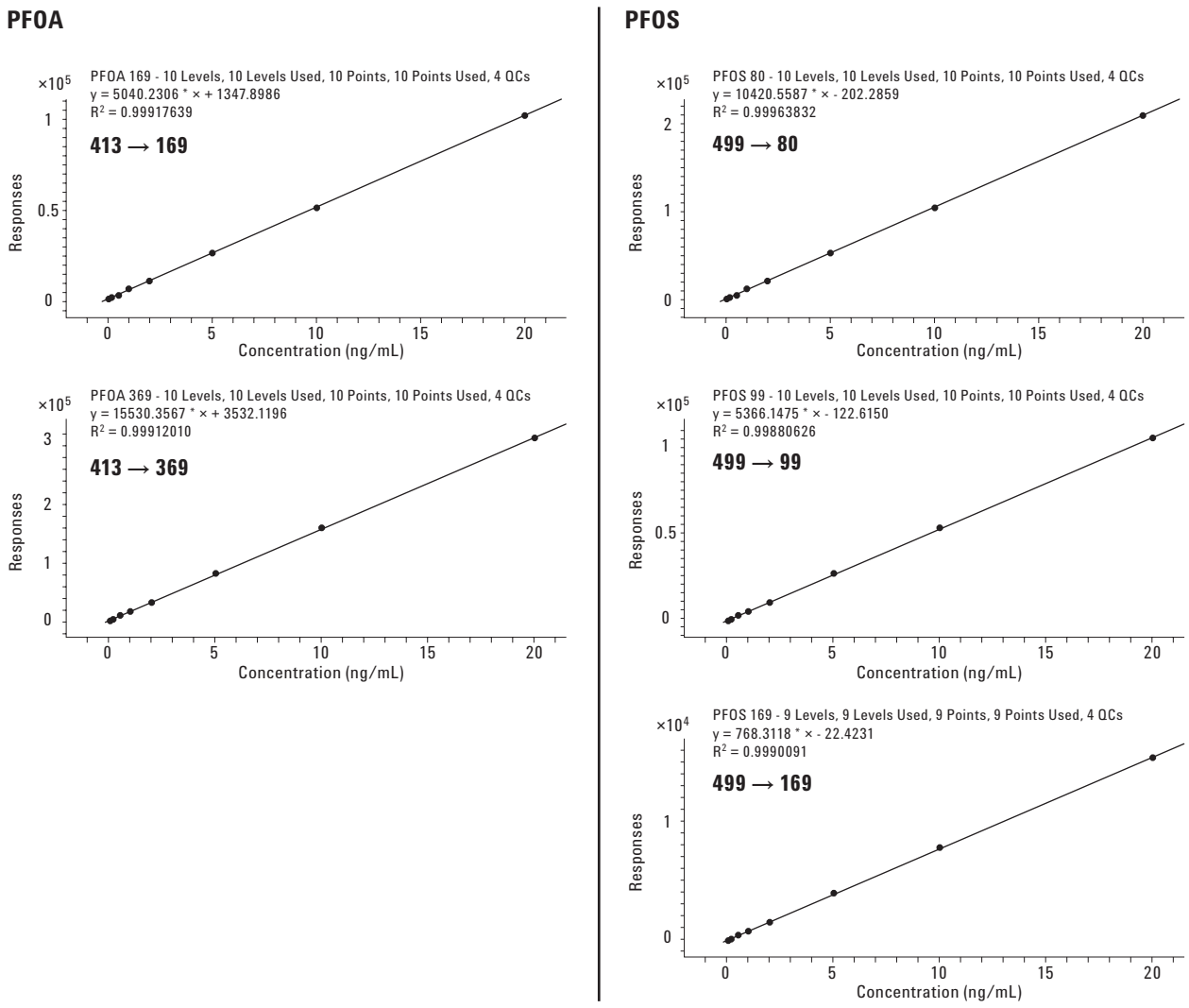


Figure 5. Signal intensity as a function of collision energy for PFOS product ions at *m/z* 169, 99, and 80.



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.

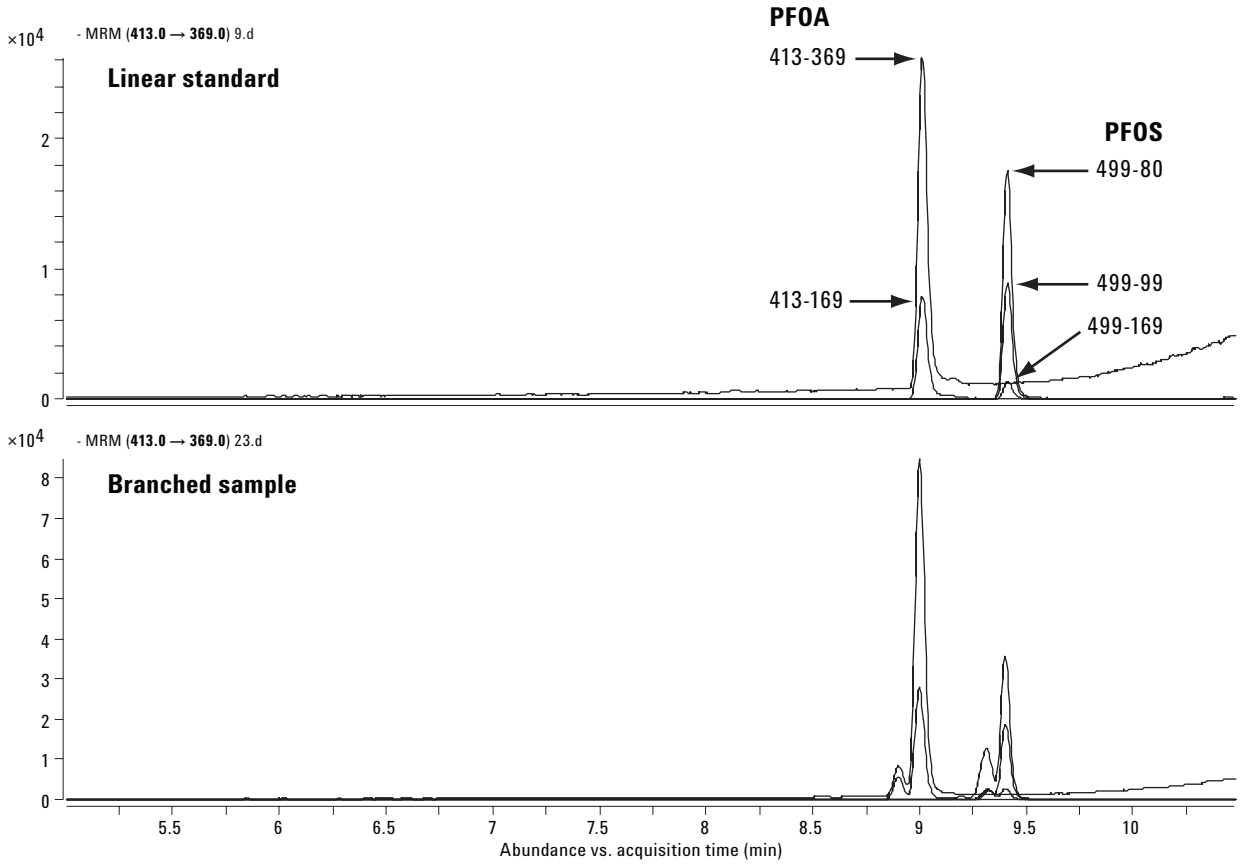


Figure 7. MRM chromatograms for PFOA and PFOS for both linear and branched samples.

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.

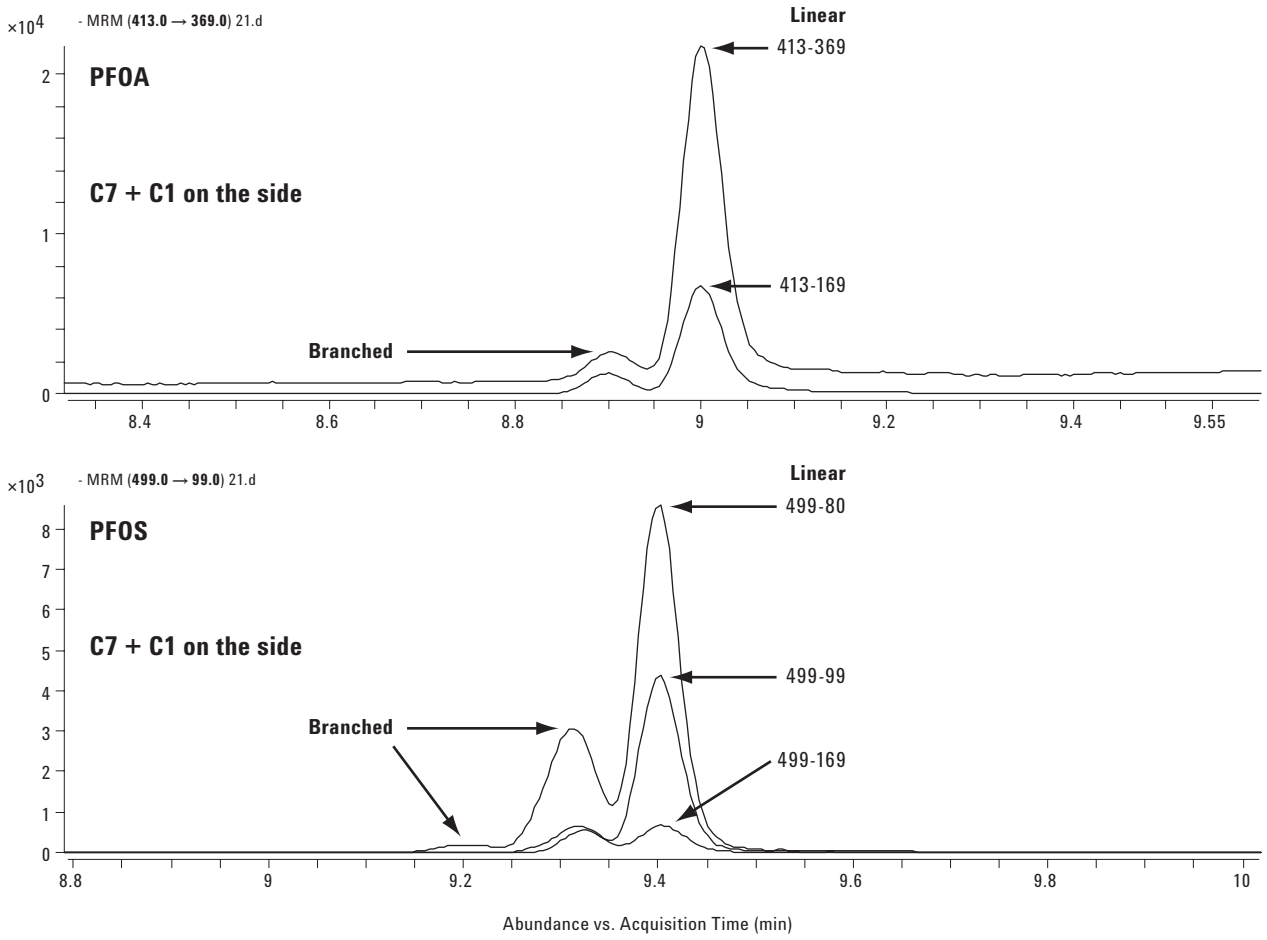


Figure 8. MRM chromatograms for PFOA and PFOS for both linear and branched samples.

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.

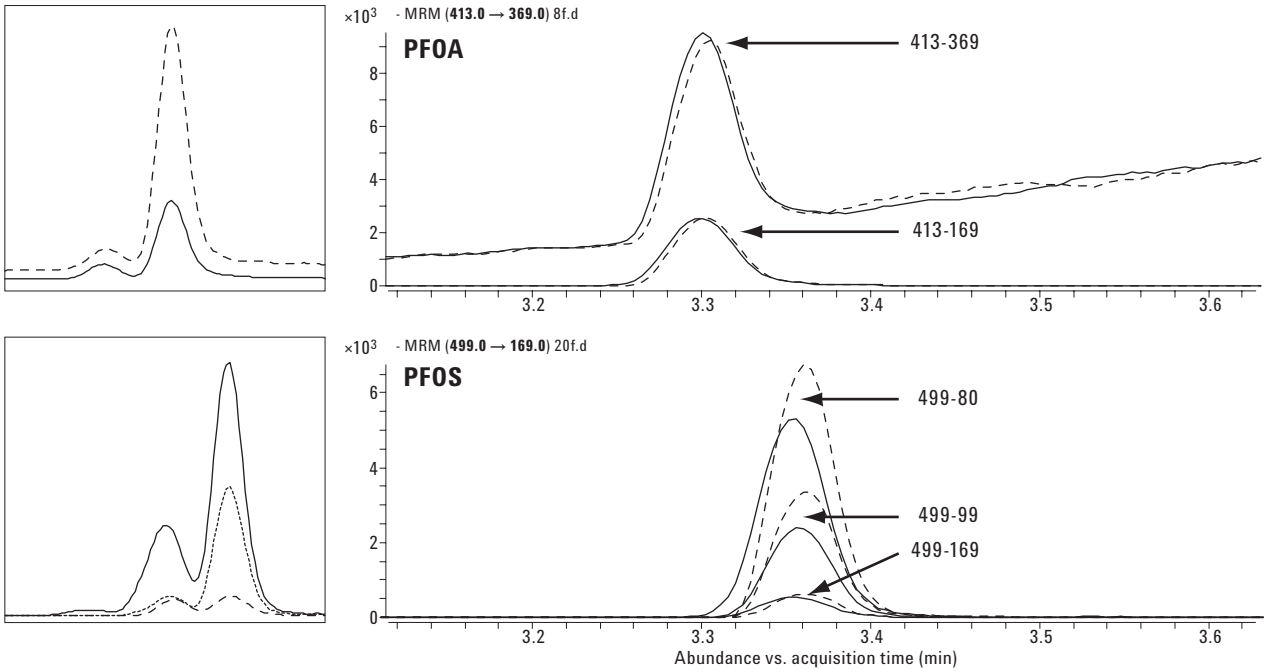


Figure 9. Comparison of PFOA and PFOS MRM chromatograms produced using both 10- and 3-minute gradients. The 3-minute gradient chromatograms are on the right.

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.

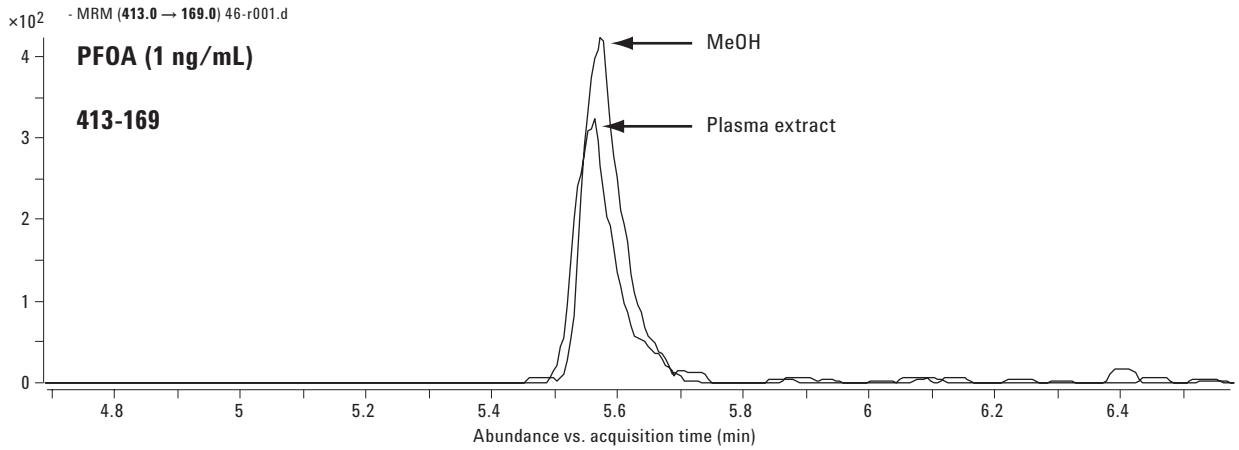


Figure 10. PFOA responses in MeOH and plasma extract at the same concentrations.

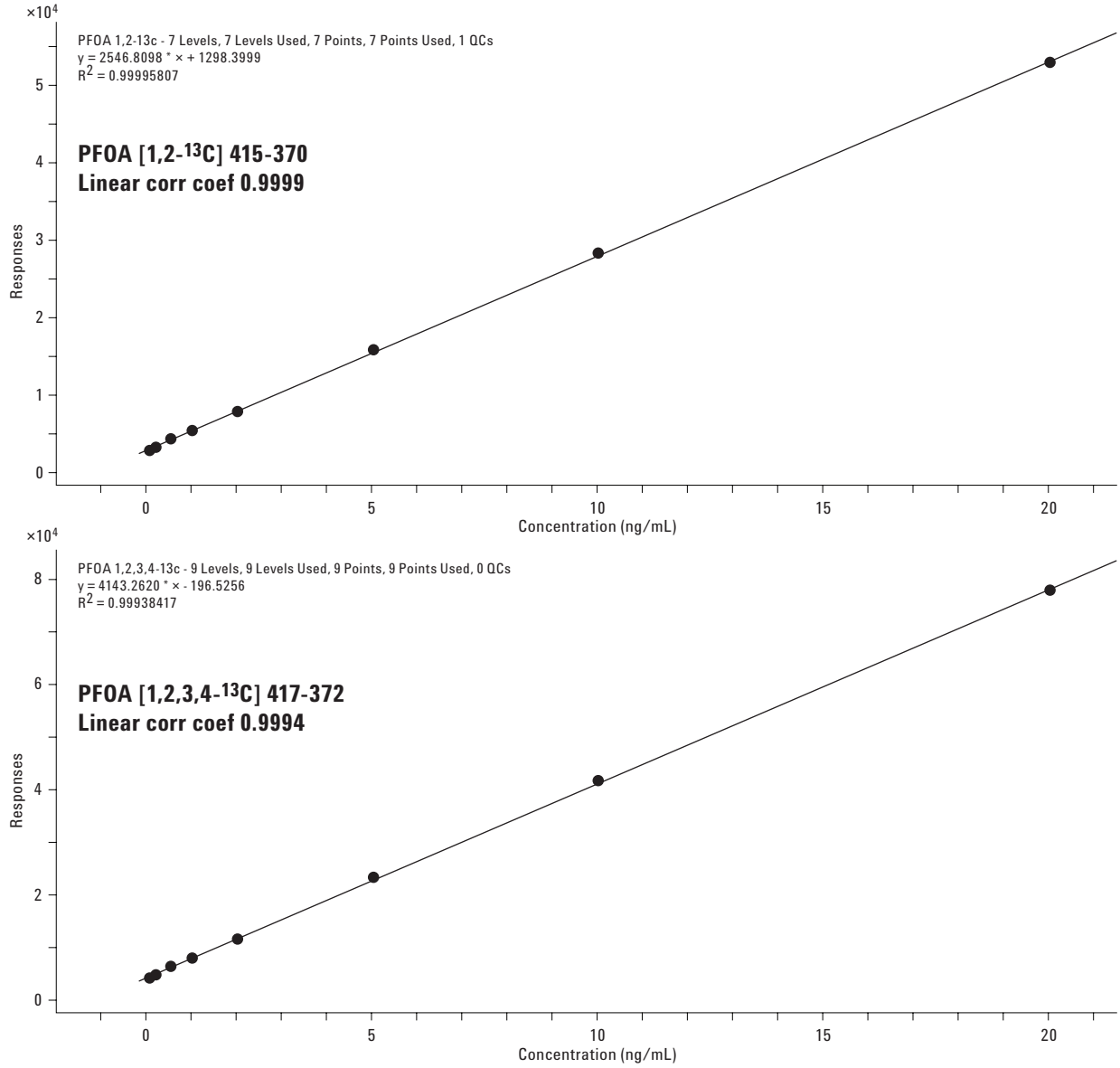


Figure 11. Linear correlations for PFOA using two different isotopically labeled calibration standards.

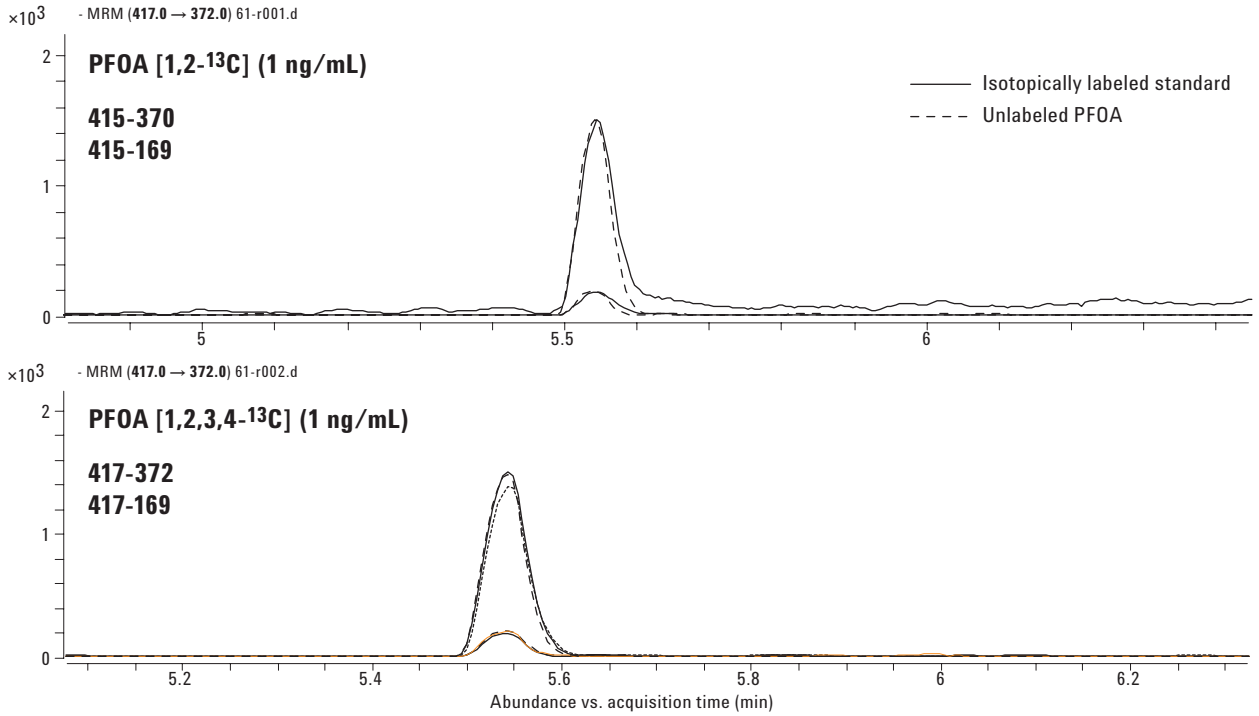


Figure 12. Both isotopically labeled PFOA compounds show good correlation to the unlabeled PFOA. The same transitions for the labeled and native forms of the PFOA were used.

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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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

Analysis of poisoned food by capillary electrophoresis

Application Note

Food

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Abstract

In cases of poisoning, analytical tools are needed to determine the identity of the toxins quickly and accurately. This enables healthcare professionals to administer appropriate treatment as quickly as possible and helps police to find those responsible. A rapid determination of anionic toxins in adulterated foods and beverages is possible using capillary electrophoresis (CE) with indirect UV detection. Cyanide, arsenite, arsenate, selenate, azide and other anions can be detected within 15 minutes, requiring only minimal sample preparation.

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Experimental

Anion analysis was performed using the Agilent Capillary Electrophoresis system equipped with diode-array detection and Agilent ChemStation software. The analysis is based on the Agilent Forensic Anion Solutions Kit (Agilent part number 5064-8208).

Prior to first use, a new capillary was flushed with run buffer for 15 minutes (at 1 bar). Between the analyses the capillary was flushed 2 minutes from the OutHome vial into waste, then 2 minutes from the InHome vial into waste. This procedure avoids baseline fluctuations as a result of buffer depletion. Buffer vials were replaced after 10 runs when using 2 mL vials, after 5 runs, when using 1 mL vials. Sample preparation consisted simply of dilution with water, or dilution and additional filtration through a 0.22 µm filter, as indicated in figure 1.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent ChemStation
- Agilent Forensic Anion Solutions Kit

Results and discussion

Figure 1 shows the analysis of food spiked with cyanide and arsenite. Depending on the results of this quick analysis, the sample can then undergo a more detailed analysis.

The assay was linear over the range 10–100 ppm with $r^2 > 0.999$. The method detection limit was 5–10 ppm.

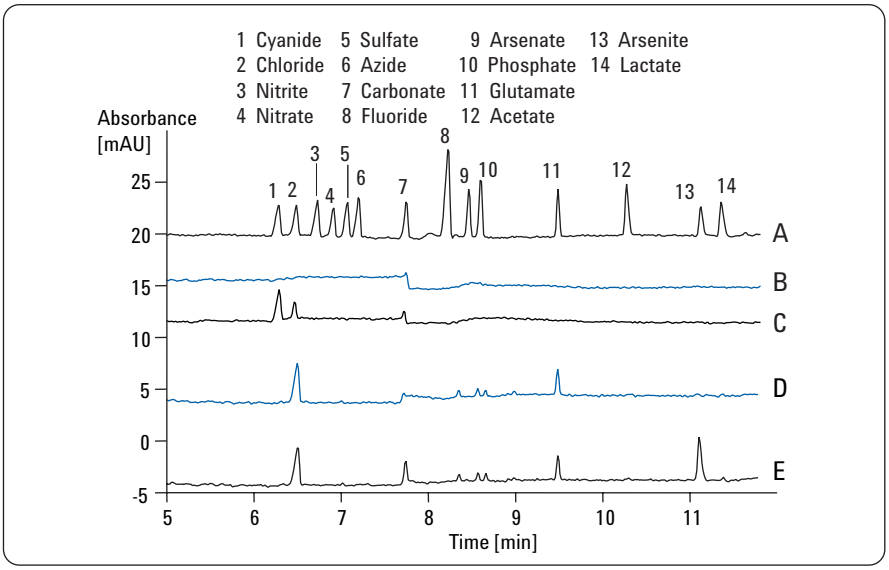

Figure 1
Analysis of cyanide and arsenite in food. A = anion standard (50 ppm each), B= Oolong tea (1:100 diluted with H_2O), C= Oolong tea as in B, spiked with 100 ppm NaCN, D=curry (1:100 diluted with H_2O, filtered through 0.22 µm filter), E=curry as in D, spiked with 100 ppm $NaAsO_2$.

Chromatographic conditions

Injection:	6 seconds at 50 mbar
Capillary:	Fused silica capillary, total length 112.5 cm, effective length 104 cm, internal diameter 50 µm (Agilent part number G1600-64211)
Buffer:	Agilent Basic Anion Buffer (Agilent part number 5064-8209)
Voltage:	-30 kV
Temperature:	30 °C
Detection:	Signal 350/20 nm, reference 275/10 nm

For the analysis of curry, the repeatability for arsenite (n = 6) was 0.06 % RSD for migration time and 2.7 % RSD for peak area. For cyanide in Oolong tea the respective values were 0.13 % RSD for migration time (n = 10) and 4 % for peak area (sample diluted in 0.01 N NaOH).

Other toxic anions that can be determined are arsenate, azide and selenate (which migrates between azide and carbonate). Compared to ion chromatography (IC), the advantages of CE for this type of analysis are the shorter analysis time and the minimal sample preparation needed for samples with a complex matrix (e.g. curry). Additionally, the analysis of azide and arsenate together with cyanide and arsenite is not possible in one run with IC.

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Published November 1, 2008
Publication Number 5989-9811EN

Agilent Technologies

Determination of Benzimidazole Fungicides in Apple Juice by SampliQ Polymer SCX Solid-Phase Extraction with High-Performance Liquid Chromatography

Application Note

Food Safety

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Abstract

Solid-phase extraction (SPE) coupled with high-performance liquid chromatography (HPLC) was optimized for extraction and quantification of two fungicides (carbendazim and thiabendazole) in apple juice. Results indicate that SPE using Agilent SampliQ SCX (60 mg, 3 mL) and HPLC using an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 100 mm, 3.5 µm) is an excellent combination for extraction and analysis of these compounds. Recoveries ranged from 92.1 to 99.4 percent with RSDs below 5 percent and limits of detection of 4 µg/kg.

Agilent Technologies

Introduction

Because of the indiscriminate use of pesticides for different applications, important environmental problems are emerging that are a risk to plant, animal, and human health. Fungicides are one group of these pesticides that are used primarily to control spoilage of crops as a result of fungal attack. Fungicides in general represent approximately 20 to 25 percent of all pesticides used. Benzimidazole fungicides are systemic pesticides, widely used in agriculture for pre- and post-harvest treatment for control of a wide range of pathogens. These substances are applied directly to the soil or sprayed over crop fields and hence are released to the environment. They readily penetrate plants through the roots and leaves and can directly enter natural waters by drainage from agricultural land. Most of these compounds persist in the environment after application; some even remain for years. Two of the main compounds in the benzimidazole family are carbendazim and thiabendazole (Table 1). Carbendazim has both protective and curative activity against a wide range of fungal diseases. It is toxic to humans, animals, and plants and also is very persistent in water, wastewater, soil, crops, and food. Thiabendazole is used to control fruit and vegetable diseases such as mold, rot, and blight, and is used as a veterinary drug to treat worms.

Agilent SampliQ SCX SPE cartridge was used to extract fungicides from apple juice. This application note describes the implementation and optimization of the method described in SN/T 1753-2006 and the results of validation.

Experimental

Materials and Chemicals

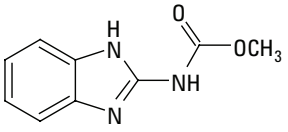
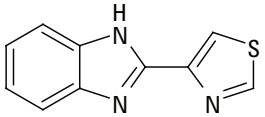
All reagents and solvents were HPLC or analytical grade. Fungicide standards were purchased from Sigma-Aldrich Trading Co. (Shanghai, China). Apple juice (food grade) was purchased from a local market.

Phosphate buffer: 1.38 g sodium dihydrogen phosphate and 1.41 g disodium hydrogen phosphate in 1,000 mL water, adjust pH to 3.0

Stock solution (0.1 mg/mL) was prepared in methanol and kept in the freezer (–20 °C). Working solutions were prepared using the stock solution diluted with methanol. The working solutions should be prepared every week and need to be stored at 4 °C.

The SPE cartridges were Agilent SampliQ SCX 3 mL, 60 mg (p/n 5982-3236). The analysis was performed on an Agilent 1200 HPLC with variable wavelength detector (VWD). The analytical column was an Agilent ZORBAX Eclipse Plus C18 3.5 µm 100 mm × 2.1 mm id (p/n 959793-902). Agilent 0.45-µm filter membranes (p/n 5185-5836) were used to filter sample solutions prior to HPLC analysis.

Table 1. Fungicides Used in this Study

No.	Name	pKa	Log P	Structure
1	Carbendazim CAS # 10605-21-7	4.48	1.45	
2	Thiabendazole CAS # 148-78-8	4.7	2.39	

HPLC Conditions

Column:	ZORBAX Eclipse Plus C18 100 mm × 4.6 mm 3.5 µm (p/n 959793-902)
Flow rate:	1.0 mL/min
Injection volume:	20 µL
Detection wavelength:	288 nm
Moblie phase:	Phosphate buffer-Acetonitrile (73:27)

Sample Preparation

Weigh 10 g apple juice, dilute to 100 mL with water, and mix with a glass rod for 1 minute. Transfer the diluted sample to a 250-mL Erlenmeyer flask and adjust pH to 10 with 2 mol/L NaOH solution. Divide the sample between two or three 50-mL polypropylene centrifuge tubes and centrifuge for 10 minutes at 4,000 rpm. Recombine the supernatants into a glass beaker.

SPE Purification

The procedure used for the SPE extraction is shown in Figure 1. The Agilent SampliQ SCX cartridges were conditioned with 3 mL of methanol, followed by 3 mL 0.15 mol/L NH_4OH solution with gravity flow (about 1 mL/min).

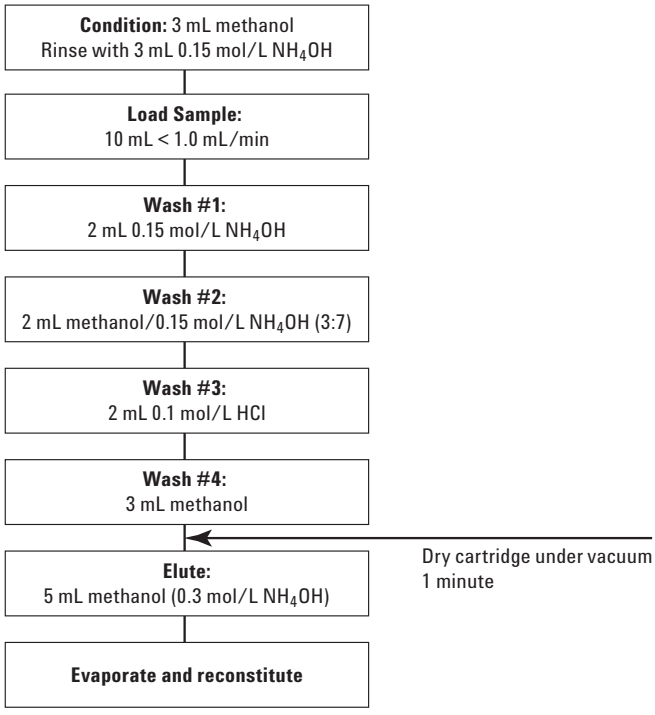


Figure 1. Fungicides in apple juice SPE procedure.

Load 10 mL supernatant liquid to SampliQ SCX cartridges at a speed about 1 mL/min. After the sample effuses completely, wash the cartridge with 2 mL of 0.15 mol/L NH_4OH, 2 mL of a solution of methanol and 0.15 mol/L NH_4OH (3:7), 2 mL of 0.1 mol/L HCl and 3 mL methanol. All three wash steps were under gravity flow. Discard all of the effluents. Dry the cartridge under negative pressure below 2.0 kPa for 1 minute. Finally, elute the cartridge with 5 mL of 0.5 mol/L NH_4OH in methanol, under gravity flow. Collect the eluent and dry it under nitrogen. Dissolve the resulting residue and bring it to a constant volume of 1 mL using the mobile phase. Then filter the residue through a 0.45-µm filter membrane and analyze.

Results and Discussion

Linearity, Limits of Detection

Stock solutions were diluted to different concentrations and analyzed by HPLC. Linear regressions were calculated for the tetracyclines using the areas and the solution concentrations. The limit of detection (LOD) was the injection concentration at which the signal-to-noise ratio was between 2 and 3. The linear range was between 25 and 500 µg/kg. The linearity and LOD are shown in Table 2.

Table 2. Linearity and LODs of Fungicides

Compound	Regression equation	Correlation coefficient	LOD (µg/kg)
Carbendazim	Y = 75.781× – 0.4018	0.9999	4
Thiabenzole	Y = 108.07× – 0.6984	0.9999	4

Recovery and Reproducibility

Recoveries were calculated for spiked fungicide standards in apple juice at 25, 50, and 100 µg/kg levels. The analysis was performed in replicates of six at each level. The chromatograms of the blank and spiked standard (100 µg/kg) are shown in Figure 2 and Figure 3. The recovery and reproducibility data are shown in Table 3.

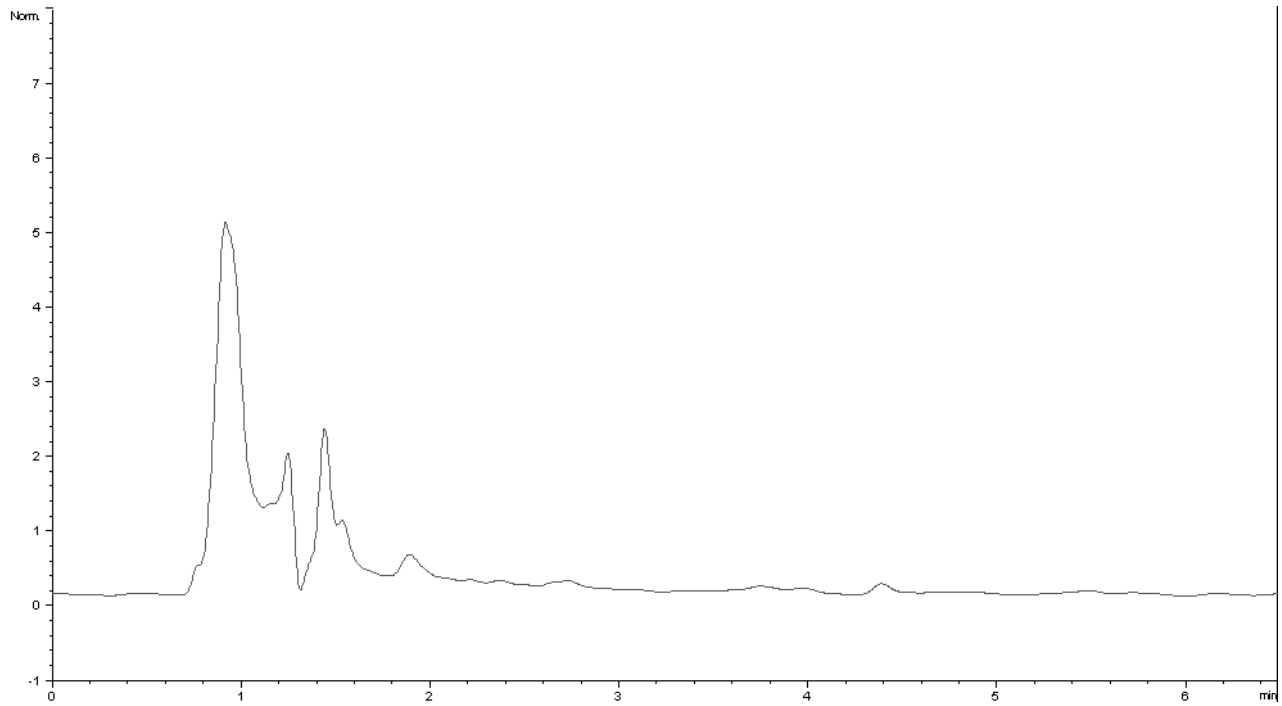


Figure 2. Chromatogram of apple juice blank.

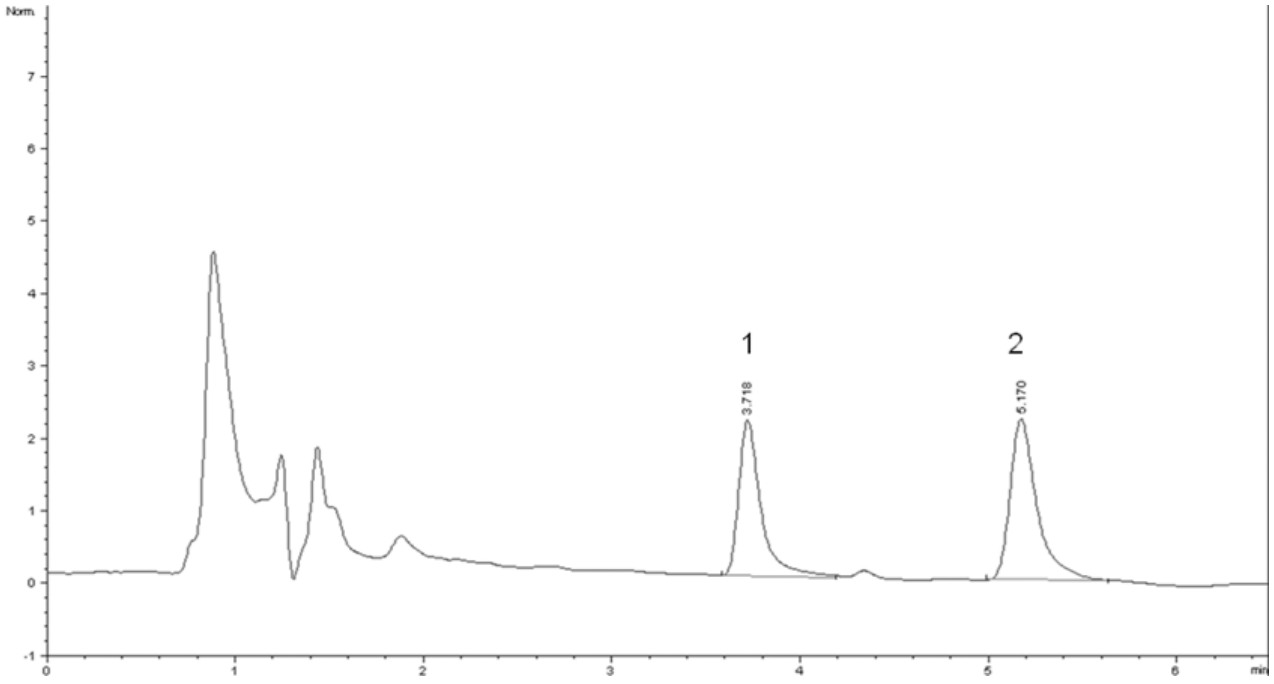


Figure 3. Chromatogram of apple juice sample spiked at 100 µg/kg (1 – Carbendazim, 2 – Thiabenzole).

Table 3. Recoveries and RSDs of Fungicides in Apple Juice by SPE

Compound	Spiked level (µg/kg)	Recovery (%)	% RSD (n = 6)
Carbendazim	25	98.6	3.99
	50	99.4	3.24
	100	95.9	3.27
Thiabenzole	25	99.0	2.38
	50	92.1	4.90
	100	93.0	3.79

Conclusions

Agilent SampliQ SCX provides a simple and effective single-cartridge SPE method for the purification and enrichment of fungicides in apple juice. The recovery and reproducibility results based on solution standards are acceptable for fungicide residue determination in apple juice under the Chinese regulation. The impurities from apple juice were minimal and did not interfere with any of the fungicides analyzed.

References

SN/T 1753-2006, Determination of thiabenzole and carbendazim residues in concentrated fruit juice for import and export – High Performance Liquid Chromatographic method.

For More Information

For additional information on Agilent SampliQ SPE products visit: www.agilent.com/chem/sampliq

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Published in the USA
December 30, 2008
5990-3235EN

Agilent Technologies

An Application Kit for Multi-Residue Screening of Pesticides using LC/TOF or Q-TOF with a Pesticide Personal Compound Database

Application Note

Food Safety and Environmental

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USA

Abstract

An application kit for pesticide screening has been developed for the Agilent time-of-flight (TOF) and quadrupole time-of-flight (Q-TOF) mass spectrometers using a database with almost 1600 entries. It can be quickly and easily used for both food and environmental samples where the ability to detect and identify a large number of pesticides is necessary. The system allows the user to create custom databases containing retention times of compounds of interest for targeted analysis. Screening with this database thus provides both targeted and non-targeted pesticide detection. A test mix for both positive ion and negative ion modes is provided to demonstrate the functionality of the kit. An example of a general method for pesticide screening is given along with an example of a spinach extraction using the Agilent SampliQ extraction and dispersive SPE kits for complete food analysis.

Agilent Technologies

Introduction

Because over 1000 pesticides have been in use over the last century and new pesticides are being developed, there is a great need to perform both targeted and non-targeted screening in food and the environment. The Agilent time-of-flight (TOF) mass spectrometers provide both high mass resolution and mass accuracy that allow comparison of the measured mass to the exact mass of an ionized compound. In addition, the tandem hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer provides the capability of both screening and confirming compounds in one instrument.[1] Both liquid chromatography (LC) combined with TOF MS and Q-TOF MS provide a robust and sensitive means to perform this type of screening at levels required by the international community. Because TOF is a pulsing instrument the resulting data is always full spectra, which allows the screening of compounds that are sought (targeted) and those that may not be expected (non-targeted).[2] In contrast LC/MS/MS with a triple quadrupole in its most sensitive mode, multi-reaction monitoring (MRM), provides targeted screening and confirmation only.[3]

Recently Agilent has introduced the Pesticide Personal Compound Database (PCD) consisting of 1600 compounds and pesticides. With PCD the analyst can use the pesticide database as is for non-targeted screening or create custom databases from the read-only supplied database. The custom database can be edited by changing entries, adding, and deleting entries. In addition, a powerful feature of updating retention times allows the users' custom database to be modified with retention times from the users' chromatographic conditions.[4] The analyst can create as many custom databases with LC-dependent retention times as needed. This allows easy targeted (compounds verified with standards run with specific conditions) and non-target analysis (compounds in the database that have not been verified). The ability to detect and identify compounds not being sought in food and environmental samples can be very important. However, this ability must not be confused with affirmation that compounds not detected are not present. This can only be done by validation studies showing that the specific LC/MS method employed on specific matrices can detect the compounds reported as not present at the levels of concern. As an example, the pesticide database contains compounds not amenable to LC/MS such as hexachlorobenzene. These are included for the added information of the user. In addition, confirmation of positives would always require standards run with chromatographic conditions that would provide indicative retention times and additional structural information that can be obtained from fragments generated by MS/MS. Even with these analytical considerations, screening for a large list of pesticides as enabled by the LC/TOF or Q-TOF with the Agilent Pesticide PCD can be very valuable in detecting and identifying compounds that should not be present.

Experimental

Reagents and Chemicals

Pesticide standards were from a variety of sources: Sigma, Ultra-Scientific, ChemService, and Dr. Ehrenstorfer. For trace analysis the highest purity mobile phases are recommended. B&J LC/MS grade acetonitrile and methanol are used here. Buffers should be prepared from the highest quality chemicals such as GFS doubly distilled acetic acid, formic acid and ammonium hydroxide. If solid ammonium acetate and ammonium formate is used it should be prepared in a concentrated solution and then any particulates removed with 0.2-µm filters. Agilent Pesticide Test Mix, p/n 5190-0469 acid and base diluted separately as instructed to 10 ppb in 10% acetonitrile/90% water. An Agilent SampliQ QuEChERS AOAC Extraction kit, p/n 5982-5755. Agilent SampliQ QuEChERS AOAC Dispersive SPE kits for Highly Pigment Fruits and Vegetables, p/n 5982-5321 (2 mL) and p/n 5982-5356 (15 mL).

LC/MS methods are given in the Appendices:

Appendix I, LC/MS/MS Conditions for Test Mix Positive and Negative Ion Samples.

Appendix II. Agilent 1200 Series SL LC Parameters.

Appendix III, Agilent 1290 Infinity LC Parameters.

Spinach sample preparation

- Weigh 15 g (±0.1 g) of homogenized spinach sample.
- Spike standards or IS solution if necessary.
- Vortex 30 s.
- Add 15 mL of 1% acetic acid in acetonitrile.
- Add 1 bag of extraction kit (p/n 5982-5982-5755) buffered QuEChERS extraction tubes, AOAC Method 2007.01 with 6 g $MgSO_4$, 1.5 g NaAcetate.
- Cap and hand shake vigorously for 1 min.
- Centrifuge at 4000 rpm for 5 min.
- Transfer 1 mL or 8 mL of the upper layer to the dispersive SPE kit (p/n 5982-5321 or p/n 5982-5356) for highly pigmented fruits and vegetables.
- Vortex 1 min.
- Centrifuge 2-mL tubes at 13000 rpm for 2 min, or 15-mL tubes at 4000 rpm for 5 min.

- Transfer 200 µL of the upper layer to the autosampler vial.
- Add 800 µL of water or appropriate standard spiking solution.
- Vortex 1 min, and prepare for LC/MS/MS analysis.

Results and Discussion

Fast and easy startup with Agilent test mix

To facilitate fast startup for pesticide screening, a positive and negative ion compound test mix is included with the Agilent Application Kit. This type of screening depends on obtaining accurate mass results and the TOF or Q-TOF should be operated with appropriate reference ions so that the best results will be obtained. Each of these test mixtures are prepared with a final injection concentration at 10 ppb, the accepted limit for pesticides worldwide. The extracted ion chromatogram (EIC) for each of the pesticides in the positive ion mix is shown in Figure 1. A method is provided with the kit that will allow the user to repeat this analysis. This method is an acquisition only method. Similar results demonstrate that the system is working properly. There are also two methods provided for work list automation data analysis that will generate the summary report of a database search of the Pesticide PCD. One method is the MFE_pesticide and this uses the “find compounds by molecular feature extraction (MFE)” algorithm in MassHunter Qualitative Analysis, a powerful data mining tool. This unique data mining program searches the data for all ions that can be associated with a real chromatographic peak and that may represent a "feature" of a molecule. This excludes reference ions and constant background ions and "spikes" that do not represent real compounds in the data file. MFE will create a compound list of all peaks in the data file that it has determined to represent real molecules. This algorithm is fast and generates good results with appropriate settings. The resulting report is shown in Table 1 for the positive test mix. This mixture contains only the compounds highlighted in the report. Please note that the database search screen does not confirm the presence of compounds and that compounds listed in the results does not indicate that they are conclusively present. Compounds listed could be from the blank, carry over or other sources.

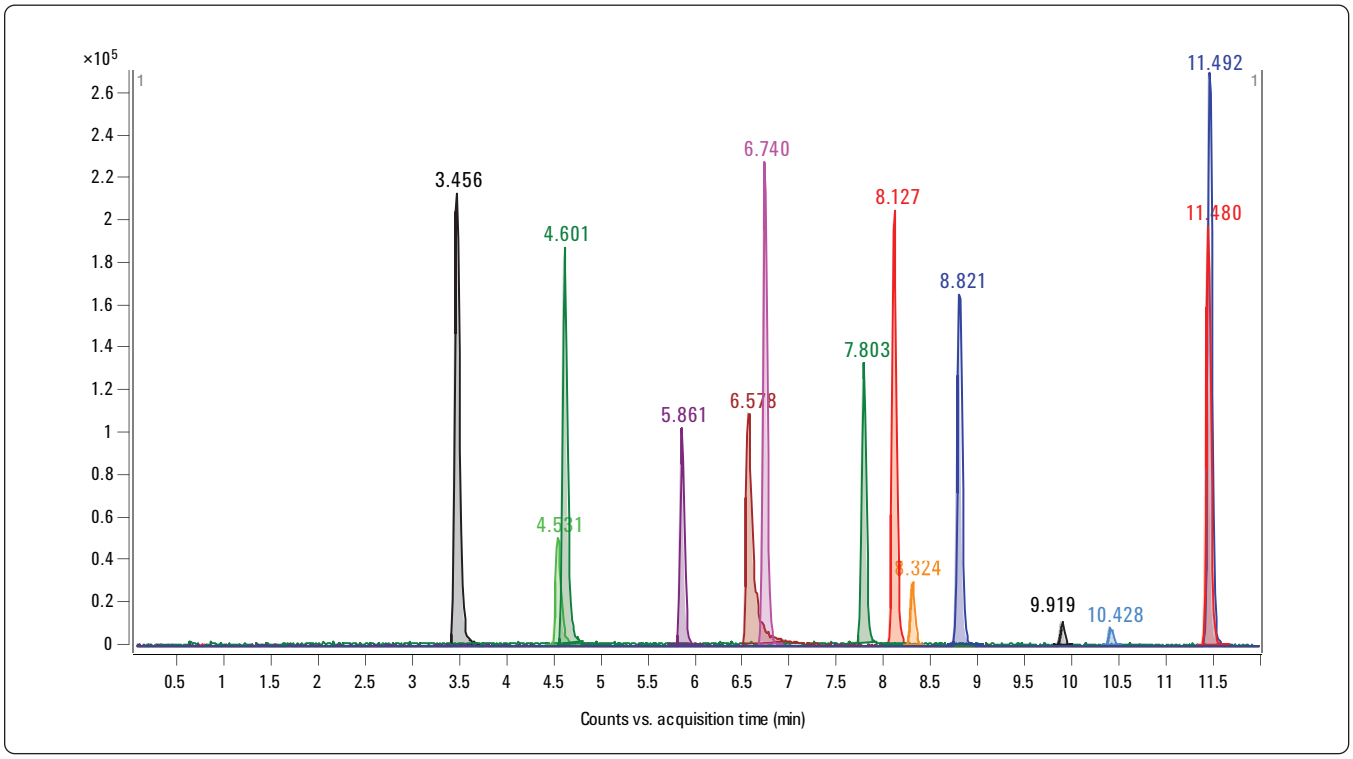


Figure 1. Extracted ion chromatogram of the positive ion test mix .

Table 1. Find Compounds by Molecular Feature Extractor with Pesticide Database Search Report for Positive Ion Test Mix

Data File	TestMix_pos_1.d	**Sample Name**	Test_Mix_pos_1
Sample Type	Sample	**Position**	P1-F2
Instrument Name	CAS6530_1	**User Name**	
Acq Method	Test_Mix_Pos.m	**Acquired Time**	6/1/2009 3:28:51 PM
IRM Calibration Status	Success	**DA Method**	MFE_Pesticide.m
Comment			

Compound Table

Compound Label		RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 19:	Aminocarb	3.472	208.1213	Aminocarb	$C_{11}H_{16}N_2O_2$	–0.44
Cpd 40:	Imazapyr	4.543	261.1113	Imazapyr	$C_{13}H_{15}N_3O_3$	–0.03
Cpd 41:	Thiabendazole	4.612	201.036	Thiabendazole	$C_{10}H_7N_3S$	0.2
Cpd 52:	Ethiofencarb sulfoxide	5.176	241.0777	Ethiofencarb sulfoxide	$C_{11}H_{15}NO_3S$	–1.91
Cpd 62:	Dimethoate	5.866	228.9998	Dimethoate	$C_5H_{12}NO_3PS_2$	–0.75
Cpd 65:	Imazalil	6.549	296.0488	Imazalil	$C_{14}H_{14}Cl_2N_2O$	–1.58
Cpd 66:	Imazalil	6.579	296.0485	Imazalil	$C_{14}H_{14}Cl_2N_2O$	–0.65
Cpd 68:	Metoxuron	6.746	228.0666	Metoxuron	$C_{10}H_{13}ClN_2O_2$	–0.09
Cpd 85:	Carbofuran	7.805	221.1054	Carbofuran	$C_{12}H_{15}NO_3$	–1.05
Cpd 88:	Atrazine	8.138	215.094	Atrazine	$C_8H_{14}ClN_5$	–0.92
Cpd 89:	DEET	8.2	191.1309	DEET	$C_{12}H_{17}NO$	0.53
Cpd 90:	Tibenzate	8.323	228.0607	Tibenzate	$C_{14}H_{12}OS$	1
Cpd 91:	Metosulam	8.33	417.0069	Metosulam	$C_{14}H_{13}Cl_2N_5O_4S$	–0.98
Cpd 92:	Fluoroglycofen	8.33	419.0033	Fluoroglycofen	$C_{16}H_9ClF_3NO_7$	–3.28
Cpd 93:	Tibenzate	8.433	228.0608	Tibenzate	$C_{14}H_{12}OS$	0.39
Cpd 97:	Tibenzate	8.527	228.0609	Tibenzate	$C_{14}H_{12}OS$	–0.12
Cpd 99:	Metazachlor	8.837	277.0983	Metazachlor	$C_{14}H_{16}ClN_{30}$	–0.53
Cpd 107:	Molinate	9.927	187.1027	Molinate	$C_9H_{17}NOS$	2.02
Cpd 111:	Malathion	10.448	330.036	Malathion	$C_{10}H_{19}O_6PS_2$	0.2
Cpd 113:	Phenylacrylicacid	10.558	148.0522	Phenylacrylicacid	$C_9H_8O_2$	1.59
Cpd 121:	Tri-n-butyl phosphate	11.177	266.1645	Tri-n-butyl phosphate	$C_{12}H_{27}O_4P$	0.58
Cpd 123:	Tri-n-butyl phosphate	11.272	266.1646	Tri-n-butyl phosphate	$C_{12}H_{27}O_4P$	0.32
Cpd 125:	Pyraclostrobin	11.477	387.0989	Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$	–0.9
Cpd 127:	Diazinon	11.497	304.1012	Diazinon	$C_{12}H_{21}N_2O_3PS$	–0.56

Database Search Results

Compound	Hits
Aminocarb	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Aminocarb	TRUE	$C_{11}H_{16}N_2O_2$	208.1213	208.1212	–0.44	3.472

Database Search Results

Compound	Hits
Imazapyr	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Imazapyr	TRUE	$C_{13}H_{15}N_3O_3$	261.1113	261.1113	–0.03	4.543

Database Search Results

Compound	Hits
Thiabendazole	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Thiabendazole	TRUE	$C_{10}H_7N_3S$	201.036	201.0361	0.2	4.612

Database Search Results

Compound	Hits
Ethiofencarb sulfoxide	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Thiabendazole	TRUE	$C_{10}H_7N_3S$	201.036	201.0361	0.2	4.612

Database Search Results

Compound	Hits
Ethiofencarb sulfoxide	2

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Ethiofencarb sulfoxide	TRUE	$C_{11}H_{15}NO_3S$	241.0777	241.0773	–1.91	5.176
Methiocarb sulfoxide	FALSE	$C_{11}H_{15}NO_3S$	241.0777	241.0773	–1.91	5.176

Database Search Results

Compound	Hits
Dimethoate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Dimethoate	TRUE	$C_5H_{12}N0_3PS_2$	228.9996	228.9996	–0.75	5.866

Database Search Results

Compound	Hits
Imazalil	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Imazalil	TRUE	$C_{14}H_{14}Cl_2N_2O$	296.0488	296.0483	–1.58	6.549

Database Search Results

Compound	Hits
Imazalil	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Imazalil	TRUE	$C_{14}H_{14}Cl_2N_2O$	296.0485	296.0483	–0.65	6.579

Database Search Results

Compound	Hits
Metoxuron	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Metoxuron	TRUE	$C_{10}H_{13}ClN_2O_2$	228.0666	228.0666	–0.09	6.746

Database Search Results

Compound	Hits
Carbofuran	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Carbofuran	TRUE	$C_{12}H_{15}NO_3$	221.1054	221.1052	–1.05	7.805

Database Search Results

Compound	Hits
Atrazine	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Atrazine	TRUE	$C_8H_{14}ClN_5$	215.094	215.0938	–0.92	8.138

Database Search Results

Compound	Hits
DEET	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
DEET	TRUE	$C_{12}H_{17}NO$	191.1309	191.131	0.53	8.2

Database Search Results

Compound	Hits
Tibenzate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Tibenzate	TRUE	$C_{14}H_{12}OS$	228.0607	228.0609	1	8.323

Database Search Results

Compound	Hits
Metosulam	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Metosulam	TRUE	$C_{14}H_{13}Cl_2N_5O_4S$	417.0069	417.0065	–0.98	8.33

Database Search Results

Compound	Hits
Fluoroglycofen	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Fluoroglycofen	TRUE	$C_{16}H_9ClF_3NO_7$	419.0033	419.002	–3.28	8.33

Database Search Results

Compound	Hits
Tibenzate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Tibenzate	TRUE	$C_{14}H_{12}OS$	228.0608	228.0609	0.39	8.433

Database Search Results

Compound	Hits
Tibenzate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Tibenzate	TRUE	$C_{14}H_{12}OS$	228.0609	228.0609	–0.12	8.527

Database Search Results

Compound	Hits
Metazachlor	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Metazachlor	TRUE	$C_{14}H_{16}ClN_3O$	277.0983	277.0982	-0.53	8.837

Database Search Results

Compound	Hits
Molinate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Molinate	TRUE	$C_9H_{17}NOS$	187.1027	187.1031	2.02	9.927

Database Search Results

Compound	Hits
Malathion	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Malathion	TRUE	$C_{10}H_{19}O_6PS_2$	330.036	330.0361	0.2	10.448

Database Search Results

Compound	Hits
Phenylacrylicacid	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Phenylacrylicacid	TRUE	$C_9H_8O_2$	148.0522	148.0524	1.59	10.558

Database Search Results

Compound	Hits
Tri-n-butyl phosphate	2

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Tri-n-butyl phosphate	TRUE	$C_{12}H_{27}O_4P$	266.1645	266.1647	0.58	11.177
Tri-iso-butyl phosphate		$C_{12}H_{27}O_4P$	266.1645	266.1647	0.58	11.177

Database Search Results

Compound	Hits
Tri-n-butyl phosphate	2

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Tri-n-butyl phosphate	TRUE	$C_{12}H_{27}O_4P$	266.1646	266.1647	0.32	11.272
Tri-iso-butyl phosphate		$C_{12}H_{27}O_4P$	266.1646	266.1647	0.32	11.272

Database Search Results

Compound	Hits
Pyraclostrobin	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Pyraclostrobin	TRUE	$C_{19}H_{18}ClN_3O_4$	387.0989	387.0986	–0.9	11.477

Database Search Results

Compound	Hits
Diazinon	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Diazinon	TRUE	$C_{12}H_{21}N_2O_3PS$	304.1012	304.1011	–0.56	11.497

The second method is Find_formula_pesticide. This method uses the “find by formula” algorithm of MassHunter Qualitative Analysis. This algorithm searches the data for the ions specified for each molecule in the database. For the supplied database this would entail generating extracted ion chromato-grams for each entry times each adduct (1600 for H^+, 1600 for Na^+, etc.). This is thorough but slower. However, if these searches are done automatically in a worklist, the processing time is reasonable. The analyst must determine what is the best fit-for-purpose procedure. Note that automatic database searching can be done during the worklist acquisition or after. Using the “worklist run” parameter of MassHunter acquisition, acquisition and data analysis can be selected, or data analysis only after the data has been collected. The data analysis methods can be added to the worklist by adding the column "Override DAMethod" to the MassHunter worklist and inserting the method to be used. (The qualitative analysis methods can be saved to the name of the acquisition method eliminating the need to add the "Override" column. (However, keeping acquisition methods and data analysis methods separate provides more flexibility.) All methods can be customized to meet the needs of a particular analysis.

Figure 2 shows the chromatogram of the negative ion compound test mix. Table 2 shows the results automatically generated for the negative ion mixture using the MFE_pesticide method. The report is generated using "Find and Identify" selection of compound automation and the 1600-compound pesticide database, pesticides.mtl, is searched. The worklist automation uses the "Compound automation and report" selection. To obtain the report shown, the "CompoundReportwithIdentificationHits.xltx" template of the "Common Reporting Options" in the General Navigation bar of MassHunter Qualitative analysis must be selected. This is important because as shown in the compound list of Table 2, the wrong isomer, dinoprop, is listed. This is the first isomer found in the database. The selected report template then lists the results of each database hit and the three isomers in the database are shown under this heading in the report. If the data were analyzed with the "Find by Formula" algorithm, the report would include all the isomers in the database in the main body of the report. If a retention time that matched the compound were in a custom database, only that isomer would be reported (targeted analysis). (Note that for the find by formula method to work within a worklist the Worklist Actions of the method should separately list "Compound Automation without report" and then "Generate Compound Report.")

The compound actually present is dinoseb and if this were a non-targeted analysis the analyst would need to confirm which one was present.

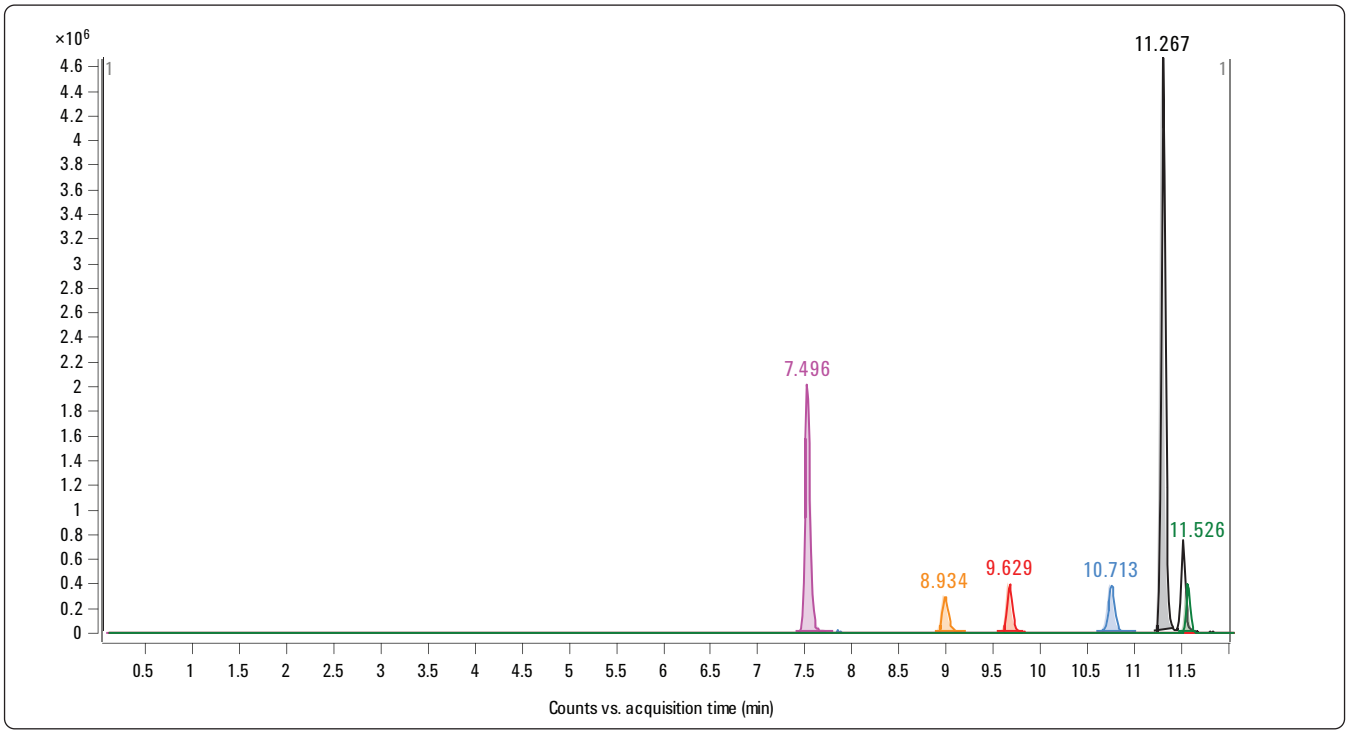


Figure 2. Extracted ion chromatogram of the negative ion test mix.

Table 2. Find compounds by Molecular Feature Extractor with Pesticide Database Search Report for Negative Ion Test Mix

Data File	Test_mix_neg_01.d	**Sample Name**	Test Mix Neg 1
Sample Type	Sample	**Position**	P1-F1
Instrument Name	CAS6530_1	**User Name**	
Acq Method	Test_mix_neg.m	**Acquired Time**	6/1/2009 1:33:54 PM
IRM Calibration Status	Success	**DA Method**	MFE_Pesticide.m
Comment			

Compound Table

Compound Label		RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 12:	Bentazone	7.491	240.0573	Bentazone	$C_{10}H_{12}N_2O_3S$	–1.69
Cpd 15:	Dibutyl succinate	7.904	230.1517	Dibutyl succinate	$C_{12}H_{22}O_4$	0.5
Cpd 24:	2,4-D Methyl ester	8.768	233.9847	2,4-D Methyl ester	$C_9H_8Cl_2O_3$	1.7
Cpd 26:	2,4,5-T	8.934	253.9306	2,4,5-T	$C_8H_5Cl_3O_3$	–0.72
Cpd 32:	Silvex	9.623	267.9465	Silvex	$C_9H_7Cl_3O_3$	–1.6
Cpd 37:	Citronellal hydrate	10.219	172.1465	Citronellal hydrate	$C_{10}H_{20}O_2$	–1.25
Cpd 39:	Citronellal hydrate	10.37	172.1464	Citronellal hydrate	$C_{10}H_{20}O_2$	–0.47
Cpd 41:	Acifluorfen	10.716	360.9967	Acifluorfen	$C_{14}H_7ClF_3NO_5$	–0.55
Cpd 42:	Citronellal hydrate	10.736	172.1466	Citronellal hydrate	$C_{10}H_{20}O_2$	–1.37
Cpd 51:	Alantolactone	11.249	232.1462	Alantolactone	$C_{15}H_{20}O_2$	0.35
Cpd 52:	Dinoprop (see below)	11.267	240.075	Dinoprop	$C_{10}H_{12}N_2O_5$	–1.72
Cpd 56:	Hexaflumuron	11.53	459.982	Hexaflumuron	$C_{16}H_8Cl_2F_6N_2O_3$	–0.76

Database Search Results

Compound	Hits
Bentazone	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Bentazone	TRUE	$C_{10}H_{12}N_2O_3S$	240.0573	240.0569	–1.69	7.491

Database Search Results

Compound	Hits
Dibutyl succinate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Dibutyl succinate	TRUE	$C_{12}H_{22}O_4$	230.1517	230.1518	0.5	7.904

Database Search Results

Compound	Hits
2,4,5-T	2

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
2,4,5-T	TRUE	$C_8H_5Cl_3O_3$	253.9306	253.9304	–0.72	8.934
Tricamba		$C_8H_5Cl_3O_3$	253.9306	253.9304	–0.72	8.934

Database Search Results

Compound	Hits
Silvex	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Silvex	TRUE	$C_9H_7Cl_3O_3$	267.9465	267.9461	–1.6	9.623

Database Search Results

Compound	Hits
Citronellal hydrate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Citronellal hydrate	TRUE	$C_{10}H_{20}O_2$	172.1465	172.1463	–1.25	10.219

Database Search Results

Compound	Hits
Citronellal hydrate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Citronellal hydrate	TRUE	$C_{10}H_{20}O_2$	172.1464	172.1463	–0.47	10.37

Database Search Results

Compound	Hits
Acifluorfen	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Acifluorfen	TRUE	$C_{14}H_7ClF_3NO_5$	360.9967	360.9965	–0.55	10.716

Database Search Results

Compound	Hits
Citronellal hydrate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Citronellal hydrate	TRUE	$C_{10}H_{20}O_2$	172.1466	172.1463	–1.37	10.736

Database Search Results

Compound	Hits
Alantolactone	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Alantolactone	TRUE	$C_{15}H_{20}O_2$	232.1462	232.1463	0.35	11.249

Database Search Results (Note that the following are isomers with the same formula even though the compound present is listed as "FALSE.")

Compound	Hits					
Dinoprop	3					
Compound	**Best**	**Formula**	**Mass**	**Tgt Mass**	**Diff (ppm)**	**RT**
Dinoprop	TRUE	$C_{10}H_{12}N_2O_5$	240.075	240.0746	–1.72	11.267
Dinoseb	FALSE	$C_{10}H_{12}N_2O_5$	240.075	240.0746	–1.72	11.267
Dinoterb		$C_{10}H_{12}N_2O_5$	240.075	240.0746	–1.72	11.267

Database Search Results

Compound	Hits					
Hexaflumuron	1					
Compound	**Best**	**Formula**	**Mass**	**Tgt Mass**	**Diff (ppm)**	**RT**
Hexaflumuron	TRUE	$C_{16}H_8Cl_2F_6N_2O_3$	459.982	459.9816	–0.76	11.53

Customized databases with user added retention times

One of the powerful benefits of the supplied database is that it can be saved to a user customized database. To create a read-write customizable database, the user selects the "File" menu item and the "New Database." The software then allows selection of an existing database and then the naming of a new database. A description can also be given. When "Create" is selected the database with the new name contains all the entries of the selected database. In this way multiple custom databases can be created. The technical note on the Pesticide PCD [4] shows how the user can run standards with unique chromatographic conditions and easily update retention times in their custom database. This is shown in Figures 3 and 4 for the positive and negative ion test mix respectively.

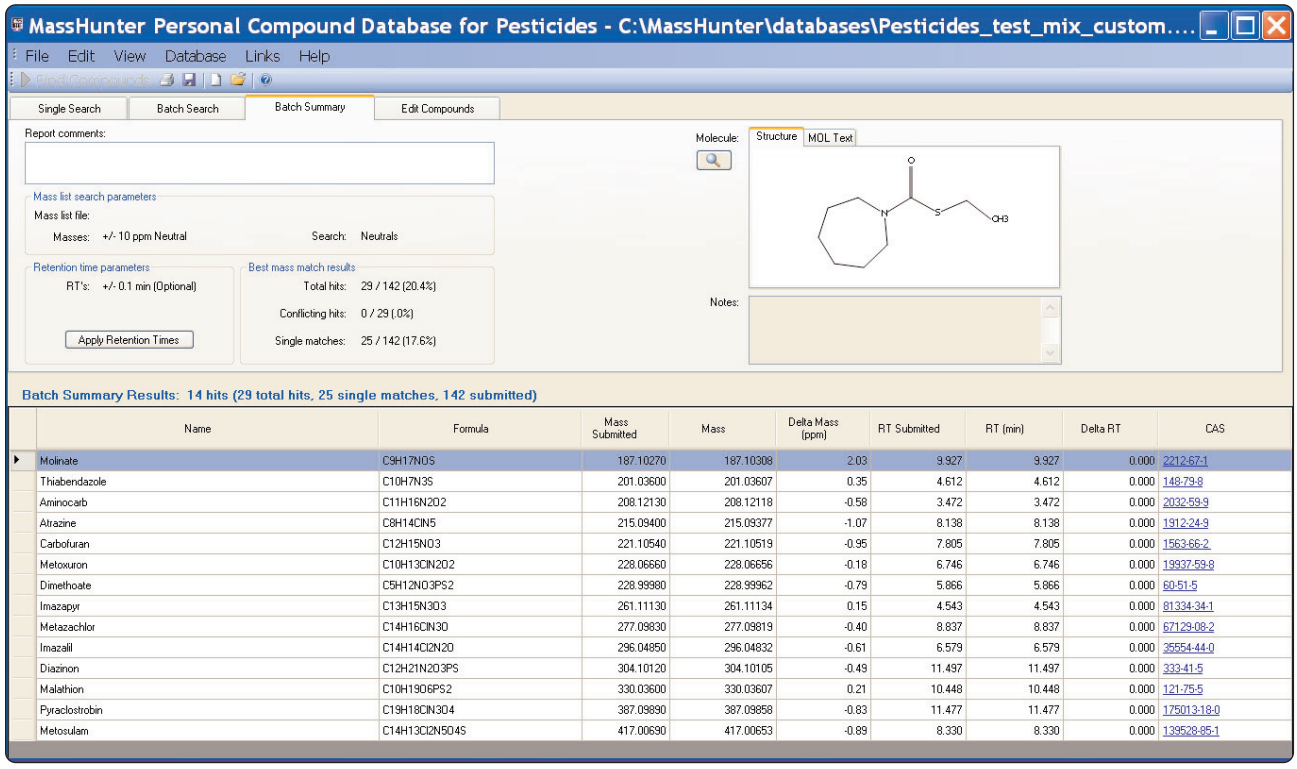


Figure 3. Pesticide Personal Compound Database (now with Library –PCDL, not shown) customized with retention times from positive ion test mix.

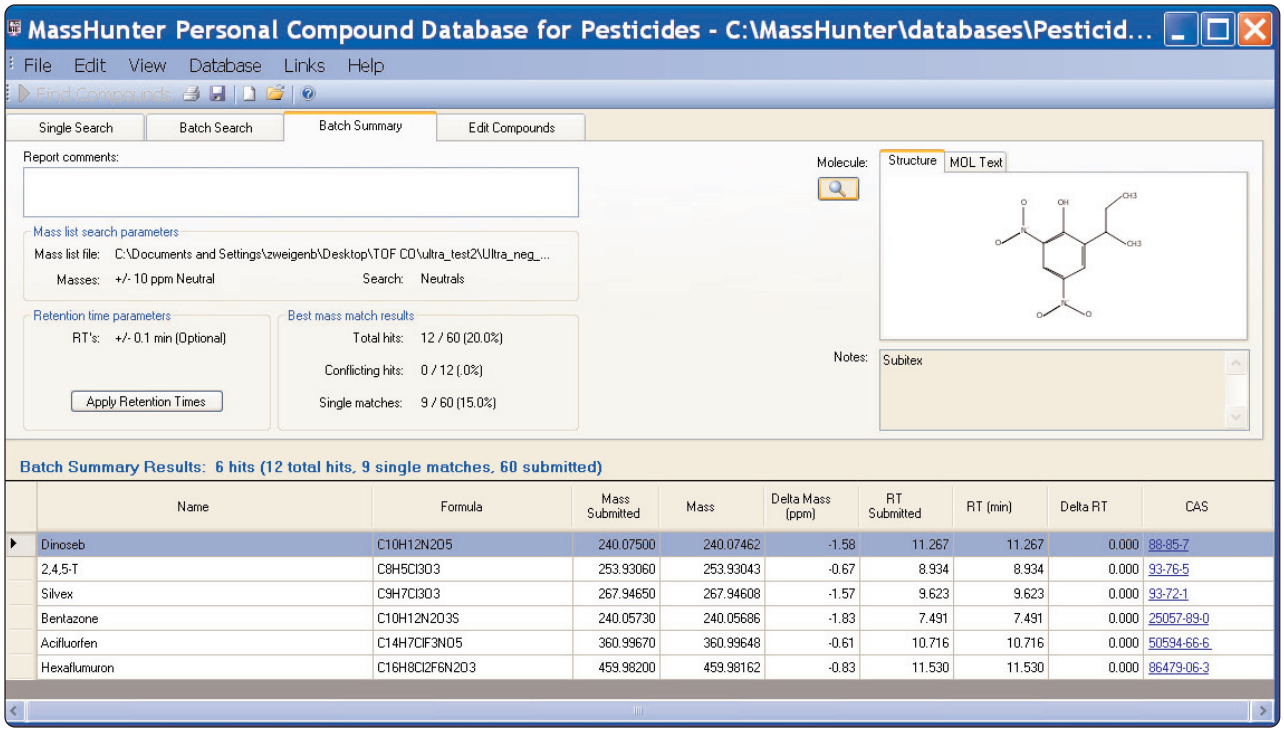


Figure 4. Pesticide Personal Compound Database (PCDL) customized with retention times for negative ion test mix.

If the analysis were for targeted compounds where retention times are known, dinoseb would be chromatographically separated from the other isomers. It is a simple exercise to take the results of the test mix, create a custom database from the provided pesticide database and update retention times. This would now create a targeted analysis. Either data analysis method can be modified for a targeted and non-targeted analysis by selecting "mass and retention time (optional)" for the search criteria. Targeted only analysis would be performed if "mass and retention time (required)" was checked. A report for a targeted and non-targeted analysis of the negative test mix with the method Find_by Formula and a custom database with the retention time for dinoseb would only list that compound. In this result, only dinoseb is reported because it is the compound in the custom database that matches the retention time. Even with retention times, identified compounds in the database must be confirmed. Both screening and confirmation can be done with the LC/Q-TOF.

Table 3. Find compounds by Formula with Pesticide Database Search Report for Negative ion Test Mix

Data File	Test_mix_neg_01.d	**Sample Name**	Test Mix Neg 1
Sample Type	Sample	**Position**	P1-F1
Instrument Name	CAS6530_1	**User Name**	
Acq Method	Test_mix_neg.m	**Acquired Time**	6/1/2009 1:33:54 PM
IRM Calibration Status	Success	**DA Method**	find_by_formula_pesticids.m
Comment			

Compound Table

Compound Label	RT	Mass	Abund	Name	Formula	Tgt Mass	DB Diff (ppm)
Cpd 1: Dichloromethoxybenzene	5.583	175.9796	9712	Dichloromethoxybenzene	$C_7H_6Cl_2O$	175.9796	0.11
Cpd 2: Bentazone	7.492	240.0573	108523	Bentazone	$C_{10}H_{12}N_2O_3S$	240.0569	1.69
Cpd 3: Dibutyl succinate	7.904	230.1517	7790	Dibutyl succinate	$C_{12}H_{22}O_4$	230.1518	–0.5
Cpd 6: Dichloroprop	8.764	233.9845	33463	Dichloroprop	$C_9H_8Cl_2O_3$	233.985	–2.39
Cpd 7: Disugran	8.764	233.9845	33463	Disugran	$C_9H_8Cl_2O_3$	233.985	–2.39
Cpd 4: Dichlorophenol 2,4-	8.764	161.9633	6051	Dichlorophenol 2,4-	$C_6H_4Cl_2O$	161.9639	–3.59
Cpd 5: 2,4-D Methyl ester	8.764	233.9845	33463	2,4-D Methyl ester	$C_9H_8Cl_2O_3$	233.985	–2.39
Cpd 9: Tricamba	8.941	253.9306	15646	Tricamba	$C_8H_5Cl_3O_3$	253.9304	0.75
Cpd 8: 2,4,5-T	8.941	253.9306	15646	2,4,5-T	$C_8H_5Cl_3O_3$	253.9304	0.75
Cpd 10: Trichlorophenol, 2,4,6-	9.613	195.9245	5877	Trichlorophenol, 2,4,6-	$C_6H_3Cl_3O$	195.9249	–2.12
Cpd 11: Silvex	9.624	267.9468	18804	Silvex	$C_9H_7Cl_3O_3$	267.9461	2.64
Cpd 14: Acifluorfen	10.708	360.9966	18261	Acifluorfen	$C_{14}H_7ClF_3NO_5$	360.9965	0.43
Cpd 13: Nitrofluorfen	10.708	317.0062	10928	Nitrofluorfen	$C_{13}H_7ClF_3NO_3$	317.0067	–1.41
Cpd 12: Azinphos-methyl	10.708	317.0061	9536	Azinphos-methyl	$C_{10}H_{12}N_3O_3PS_2$	317.0058	1
Cpd 15: Citronellal hydrate	10.732	172.1466	132453	Citronellal hydrate	$C_{10}H_{20}O_2$	172.1463	1.39
Cpd 16: Alantolactone	11.251	232.1462	10414	Alantolactone	$C_{15}H_{20}O_2$	232.1463	–0.54
Cpd 20: Ethiofencarb sulfoxide	11.262	241.078	23056	Ethiofencarb sulfoxide	$C_{11}H_{15}NO_3S$	241.0773	2.86
Cpd 21: Methiocarb sulfoxide	11.262	241.078	23056	Methiocarb sulfoxide	$C_{11}H_{15}NO_3S$	241.0773	2.86
Cpd 19: Dinoprop	11.262	240.0752	249379	Dinoprop	$C_{10}H_{12}N_2O_5$	240.0746	2.22
Cpd 17: Dinoseb	11.262	240.0752	249379	Dinoseb	$C_{10}H_{12}N_2O_5$	240.0746	2.22
Cpd 18: Dinoterb	11.262	240.0752	249379	Dinoterb	$C_{10}H_{12}N_2O_5$	240.0746	2.22
Cpd 22: Hexaflumuron	11.533	459.9823	19824	Hexaflumuron	$C_{16}H_8Cl_2F_6N_2O_3$	459.9816	1.44

The power of Q-TOF for screening and confirmation

As an example of the power of this technique, a strawberry extract was spiked and analyzed using an Agilent 1200 Series SL LC with an Agilent 6520 Q-TOF. The extracted ion chromatogram of the over 100 pesticides spiked into this sample is shown in Figure 5. A pesticide screen with a Q-TOF is the same as with a TOF. However, LC/Q-TOF MS offers the highly selective MS/MS with accurate mass measurement that provides a workflow for both screening and confirmation. [1]

Screening hundreds of target and non-target pesticides using the Agilent 1200 Series SL with 6230 TOF

A standard of over 200 pesticides is run in a similar fashion and the EIC generated from the pesticides detected in a "find compounds by molecular feature" extractor with database search is shown in Figure 4. This method employs the Agilent 1200 Series SL and the Agilent 6230 TOF with Jet Stream Technology. This is the preferred configuration as it provides additional sensitivity to meet the demanding needs of multi-residue analysis. The method for this analysis is also provided with the Agilent Pesticide Screen Application Kit for TOF and Q-TOF.

The highest quality results are obtained with good chromatographic and mass spectral resolution. The ability to detect and identify thousands of compounds lies in both these parameters and accurate mass measurement. However, for any given real food sample only a few pesticides will be found. This may not be the case for environmental samples but the possibility of no more than 10 to 20 per site would be realistic. Given this reality, the need to be able to validate that hundreds of compounds can be detected in a fast analysis would provide this capability. Figure 6 shows a 3-minute run of over 100 pesticides using the new Agilent 1290 Infinity LC connected to the new Agilent 6540 Q-TOF. Given the chromatographic resolution achieved and the mass spectral resolution obtained, this analysis is reasonable for screening pesticides in food and environmental samples. The quality of the mass spectral data is shown in Figure 7 and this was collected at rate of 10 spectra per second.

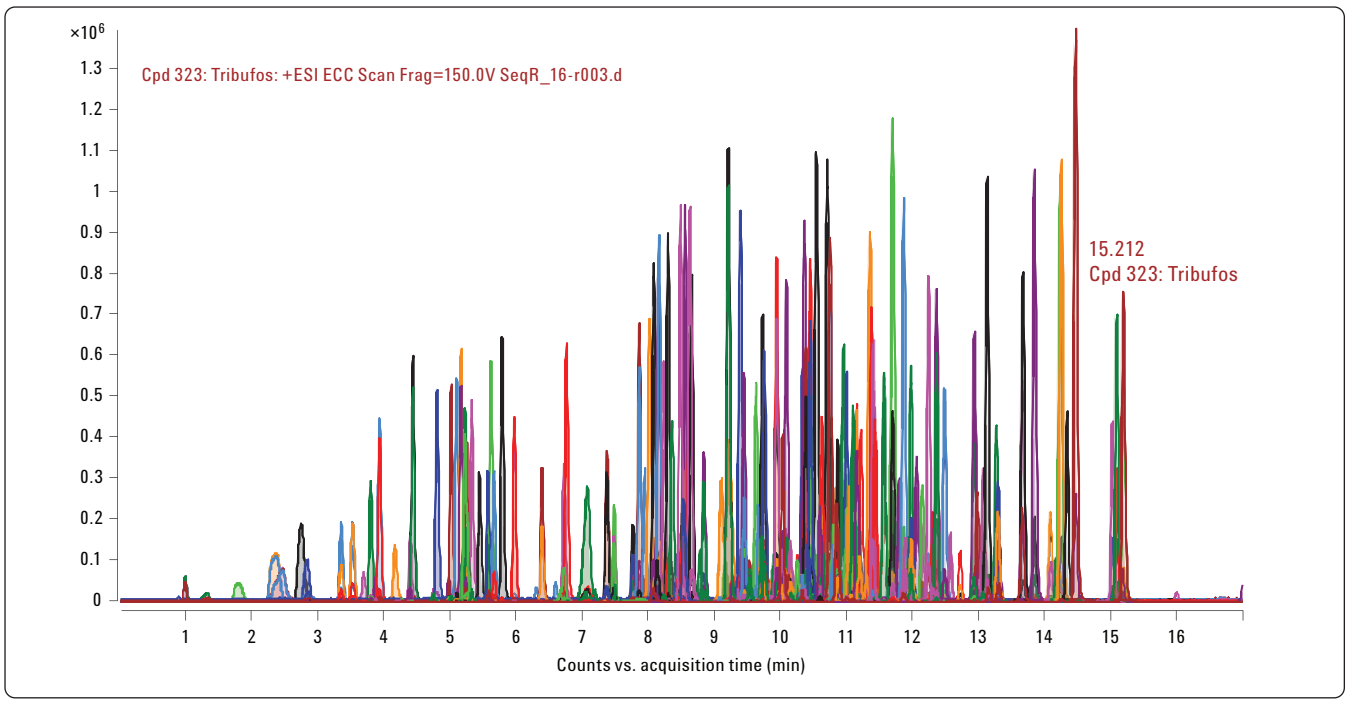


Figure 5. Extracted compound chromatogram (from compounds found by MFE) of 200 pesticides using the Agilent 1200 Series SL LC with the Agilent 6230 TOF.

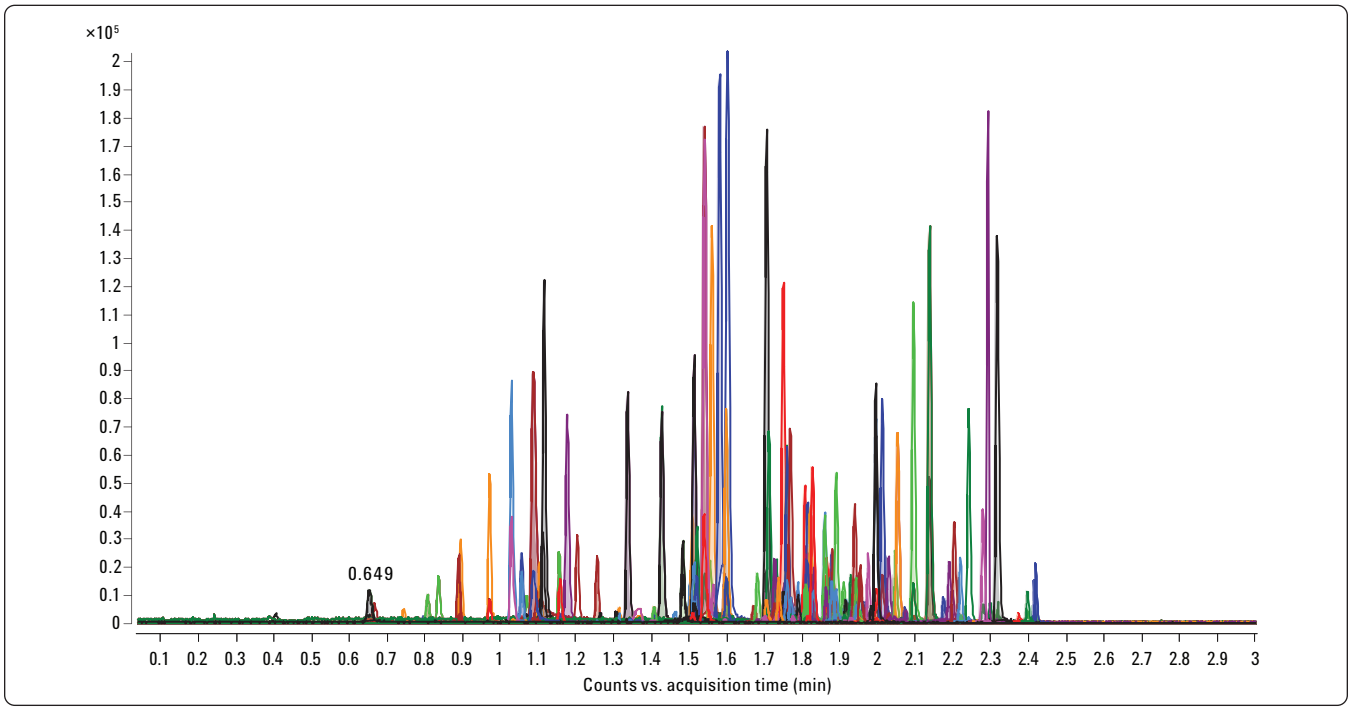
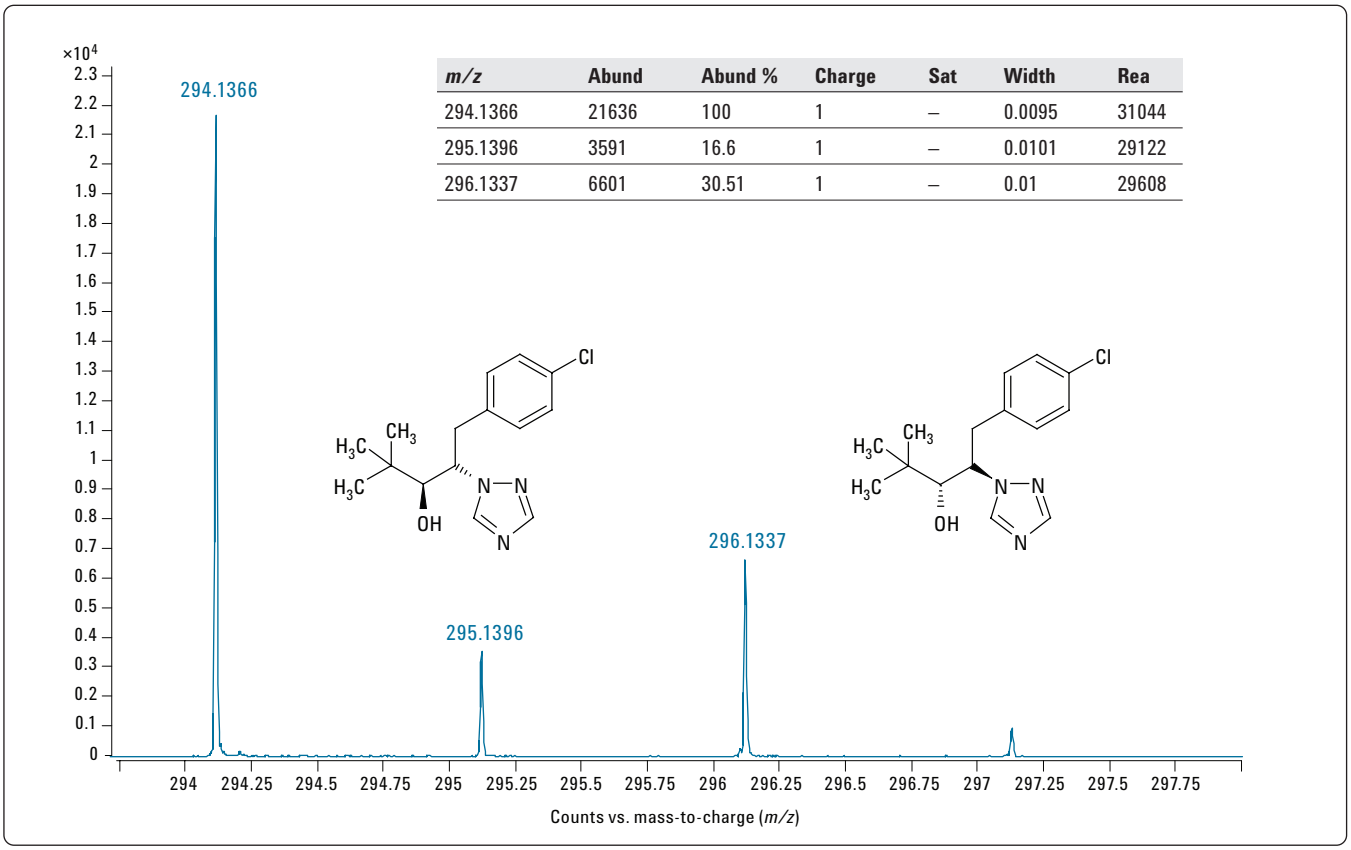


Figure 6. Extracted compound chromatogram of 100 pesticides in 3 min using the new Agilent 1290 Infinity LC with the new Agilent 6540 Q-TOF.



m/z	Abund	Abund %	Charge	Sat	Width	Rea
294.1366	21636	100	1	–	0.0095	31044
295.1396	3591	16.6	1	–	0.0101	29122
296.1337	6601	30.51	1	–	0.01	29608

Figure 7. Example mass spectrum from data on 3 min run with Agilent 1290 Infinity LC and Agilent 6540 Q-TOF. Note the mass resolution at 10 spectra per second.

Extraction to analysis with SampliQ extraction and SPE Kits

Finally, as an example of a complete analysis a spinach sample was spiked with pesticides at the 10-ppb level and extracted using the SampliQ QuEChERS Kit p/n 5982-5755. Then the Agilent SampliQ QuEChERS AOAC Dispersive SPE kit for highly pigmented fruits and vegetables, p/n 5982-5356 (15 mL), was used for clean-up. In addition, a reagent blank was prepared and run using an Agilent 1200 Series SL/6530 LC/Q-TOF and the standard screened with "find by molecular feature extractor" and the Pesticide database (not customized). The resulting mass list from the reagent blank was placed in the MFE settings by exporting the mass list to a .csv file, selecting "exclude these masses" under "Filter Mass", and using the exported .csv file as the database. In this way all the ions in the reagent blank will be removed from standards and samples processed with this method. The spiking solution (neat standard) was analyzed using the same acquisition method and the Worklist Automation. The results are given in Table 4 and represent the pesticides in the standard. It should be noted that if background removal is performed, the mass list should be searched by the database to make sure that compounds of concern will not be excluded. The .csv file is editable in Excel and masses can be removed from the exclusion list if necessary (for example, if pesticides are found in the blank).

Table 4. Neat Pesticide Standard for Spinach Extract

Data File	2ppb neat std .d	**Sample Name**	2ppb neat in 20:80 ACN/H2O
Sample Type	Sample	**Position**	P1-F1
Instrument Name	CAS6530_1	**User Name**	Jaz
Acq Method	MFE_Compound_report.m	**Acquired Time**	7/10/2009 12:43:56 PM
IRM Calibration Status	Success	**DA Method**	MFE_Pesticide_report.m
Comment			

Compound Table

Compound Label	RT	Mass	Name	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 12: Methamidophos	2.053	141.0012	Methamidophos	$C_2H_8NO_2PS$	0.96	1
Cpd 15: Acephate	2.467	183.0115	Acephate	$C_4H_{10}NO_3PS$	2.24	1
Cpd 18: Acephate	2.632	183.0119	Acephate	$C_4H_{10}NO_3PS$	–0.02	1
Cpd 24: Pymetrozine	3.242	217.0967	Pymetrozine	$C_{10}H_{11}N_5O$	–1.54	1
Cpd 25: Pymetrozine	3.361	217.0965	Pymetrozine	$C_{10}H_{11}N_5O$	–0.5	
Cpd 29: Carbendazim	4.259	191.0695	Carbendazim	$C_9H_9N_3O_2$	0.06	1
Cpd 35: Thiabendazole	4.633	201.0359	Thiabendazole	$C_{10}H_7N_3S$	0.81	1
Cpd 44: Imidacloprid	5.564	255.0527	Imidacloprid	$C_9H_{10}ClN_5O_2$	–1.43	1
Cpd 51: Imazalil	6.587	296.0489	Imazalil	$C_{14}H_{14}Cl_2N_2O$	–1.92	1
Cpd 54: Dicyclanil	7.172	190.0968	Dicyclanil	$C_8H_{10}N_6$	–0.77	1
Cpd 55: Thiophanate-methyl	7.426	342.0463	Thiophanate-methyl	$C_{12}H_{14}N_4O_4S_2$	–1.97	1
Cpd 86: Propoxur	7.621	209.1053	Propoxur	$C_{11}H_{15}NO_3$	–0.35	1
Cpd 87: Pyrocatechol	7.621	110.0368	Pyrocatechol	$C_6H_6O_2$	–0.59	1
Cpd 89: Norethynodrel	7.631	298.192	Norethynodrel	$C_{20}H_{26}O_2$	4.2	1
Cpd 91: Carbaryl	7.994	201.079	Carbaryl	$C_{12}H_{11}NO_2$	–0.07	7
Cpd 92: Naphthol, 1-	7.995	144.0575	Naphthol, 1-	$C_{10}H_8O$	0.01	1
Cpd 97: Ethoprop	9.908	242.0569	Ethoprop	$C_8H_{19}O_2PS_2$	–2	1
Cpd 99: Penconazole	10.219	283.0649	Penconazole	$C_{13}H_{15}Cl_2N_3$	–2.26	1
Cpd 101: Cyprodinil	10.482	225.1268	Cyprodinil	$C_{14}H_{15}N_3$	–0.68	1
Cpd 105: Kresoxim methyl	10.924	313.132	Kresoxim methyl	$C_{18}H_{19}NO_4$	–1.93	1

Figure 8 shows the extracted compound chromatogram from the "Molecular Feature Extractor" of the spinach extract. Even with the clean-up procedure and background ions removed, this is a complex sample. Table 5 shows the database search result for the spinach extract and all compounds detected in the standards were detected in the extract. If this were an analysis done for targeted and non-targeted analysis, all non-target positives (those without matching retention times) should be examined in MassHunter Qualitative Analysis before further analysis.

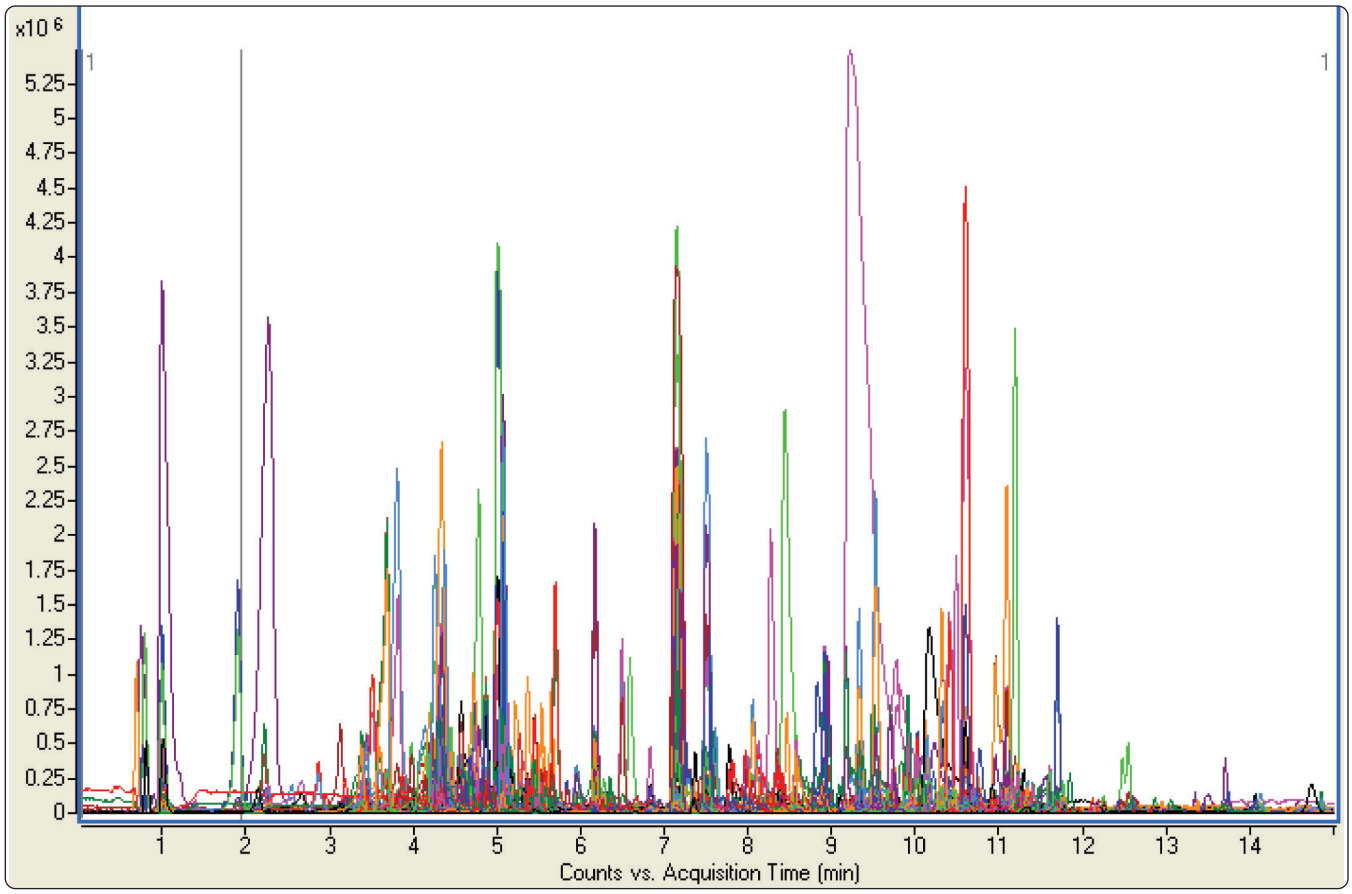


Figure 8. Extracted compound chromatogram of spinach sample with over 1200 compound features found .

Table 5. Results of Spinach Screen using Molecular Feature Extractor

Data File	Spinach AOAC 10ppb.d	**Sample Name**	Spinach AOAC 10 ppb (2 ppb in sample)
Sample Type	Sample	**Position**	P1-A4
Instrument Name	CAS6530_1	**User Name**	Jaz
Acq Method	MFE_Compound_report.m	**Acquired Time**	7/10/2009 12:40:59 PM
IRM Calibration Status	Some Ions Missed	**DA Method**	MFE_Pesticide_report.m
Comment			

Compound Table

Compound Label		RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 36:	Methamidophos	2.042	141.001	Methamidophos	$C_2H_8NO_2PS$	2.37
Cpd 42:	Carbofuran-3-OH-7-phenol	2.226	180.0786	Carbofuran-3-OH-7-phenol	$C_{10}H_{12}O_3$	0.12
Cpd 44:	Metolcarb	2.233	165.0789	Metolcarb	$C_9H_{11}NO_2$	0.43
Cpd 74:	Acephate	2.614	183.0116	Acephate	$C_4H_{10}NO_3PS$	1.72
Cpd 94:	Decarbofuran	3.127	207.0896	Decarbofuran	$C_{11}H_{13}NO_3$	–0.45
Cpd 97:	Quinacetol sulfate	3.14	187.0631	Quinacetol sulfate	$C_{11}H_9NO_2$	1.39
Cpd 103:	3,5-Xylyl methylcarbamate	3.197	179.0945	3,5-Xylyl methylcarbamate	$C_{10}H_{13}NO_2$	0.78
Cpd 119:	Carbofuran, - 3 hydroxy	3.323	237.1003	Carbofuran, - 3 hydroxy	$C_{12}H_{15}NO_4$	–0.88
Cpd 121:	Pymetrozine	3.36	217.0963	Pymetrozine	$C_{10}H_{11}N_5O$	0.44
Cpd 136:	Propoxur	3.445	209.1053	Propoxur	$C_{11}H_{15}NO_3$	–0.42
Cpd 140:	3,5-Xylyl methylcarbamate	3.471	179.0953	3,5-Xylyl methylcarbamate	$C_{10}H_{13}NO_2$	–3.52
Cpd 161:	8-Hydroxyquinoline	3.59	145.0526	8-Hydroxyquinoline	C_9H_7NO	0.82
Cpd 198:	Metalaxyl	3.805	279.1478	Metalaxyl	$C_{15}H_{21}NO_4$	–2.52
Cpd 207:	Phenyl isocyanate	3.817	119.0373	Phenyl isocyanate	C_7H_5NO	–1.32
Cpd 221:	Aspidinol	3.946	224.1049	Aspidinol	$C_{12}H_{16}O_4$	–0.24
Cpd 284:	Phenoxyacetic acid	4.155	152.0472	Phenoxyacetic acid	$C_8H_8O_3$	1.13
Cpd 303:	Dimethyl phthalate	4.188	194.0579	Dimethyl phthalate	$C_{10}H_{10}O_4$	0.28
Cpd 310:	Trinexapac	4.21	224.0685	Trinexapac	$C_{11}H_{12}O_5$	0.08
Cpd 316:	Carbendazim	4.254	191.0695	Carbendazim	$C_9H_9N_3O_2$	–0.21
Cpd 323:	Geraniol	4.282	154.1357	Geraniol	$C_{10}H_{18}O$	0.55
Cpd 338:	Dimethyl phthalate	4.316	194.0579	Dimethyl phthalate	$C_{10}H_{10}O_4$	–0.1
Cpd 368:	Propoxur	4.372	209.1054	Propoxur	$C_{11}H_{15}NO_3$	–1.02
Cpd 386:	Aldicarb	4.443	190.0776	Aldicarb	$C_7H_{14}N_2O_2S$	–0.09
Cpd 455:	Phenoxyacetic acid	4.617	152.0474	Phenoxyacetic acid	$C_8H_8O_3$	–0.33
Cpd 461:	Thiabendazole	4.628	201.0362	Thiabendazole	$C_{10}H_7N_3S$	–0.66
Cpd 492:	Butopyronoxyl	4.723	226.1207	Butopyronoxyl	$C_{12}H_{18}O_4$	–0.65
Cpd 584:	Tiocarbazil	4.904	279.167	Tiocarbazil	$C_{16}H_{25}NOS$	–4.82

Compound Label		RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 587:	Kresoxim methyl	4.905	313.132	Kresoxim methyl	$C_{18}H_{19}NO_4$	–1.89
Cpd 641:	Pyrethrin I	5.022	328.2041	Pyrethrin I	$C_{21}H_{28}O_3$	–0.8
Cpd 642:	Allethrin	5.022	302.1884	Allethrin	$C_{19}H_{26}O_3$	–0.79
Cpd 644:	Spiromesifen	5.022	370.2148	Spiromesifen	$C_{23}H_{30}O_4$	–1.01
Cpd 720:	Phosfon	5.19	396.1312	Phosfon	$C_{19}H_{32}Cl_3P$	–1.33
Cpd 721:	Santonin	5.197	246.1259	Santonin	$C_{15}H_{18}O_3$	–1.43
Cpd 740:	Dimethyl phthalate	5.255	194.0576	Dimethyl phthalate	$C_{10}H_{10}O_4$	1.68
Cpd 743:	Metaldehyde	5.265	176.1044	Metaldehyde	$C_8H_{16}O_4$	2.75
Cpd 804:	Phosfon	5.439	396.1301	Phosfon	$C_{19}H_{32}Cl_3P$	1.47
Cpd 816:	Allethrin	5.454	302.1882	Allethrin	$C_{19}H_{26}O_3$	–0.08
Cpd 830:	Buthiobate	5.498	372.1696	Buthiobate	$C_{21}H_{28}N_2S_2$	–0.67
Cpd 858:	Imidacloprid	5.57	255.0527	Imidacloprid	$C_9H_{10}ClN_5O_2$	–1.47
Cpd 976:	Kresoxim methyl	6.15	313.1321	Kresoxim methyl	$C_{18}H_{19}NO_4$	–2.14
Cpd 1047:	Kresoxim methyl	6.366	313.132	Kresoxim methyl	$C_{18}H_{19}NO_4$	–1.78
Cpd 1063:	Alantolactone	6.463	232.1463	Alantolactone	$C_{15}H_{20}O_2$	0.23
Cpd 1075:	Santonin	6.521	246.1258	Santonin	$C_{15}H_{18}O_3$	–0.98
Cpd 1089:	Imazalil	6.595	296.049	Imazalil	$C_{14}H_{14}Cl_2N_2O$	–2.29
Cpd 1124:	Salbuterol	6.838	239.1522	Salbuterol	$C_{13}H_{21}NO_3$	–0.34
Cpd 1144:	Butopyronoxyl	6.93	226.1206	Butopyronoxyl	$C_{12}H_{18}O_4$	–0.52
Cpd 1212:	Cinmethylin	7.152	274.1937	Cinmethylin	$C_{18}H_{26}O_2$	–1.56
Cpd 1242:	Dicyclanil	7.22	190.0968	Dicyclanil	$C_8H_{10}N_6$	–0.54
Cpd 1274:	Thiophanate-methyl	7.419	342.0457	Thiophanate-methyl	$C_{12}H_{14}N_4O_4S_2$	–0.24
Cpd 1331:	Bisphenol A	7.596	228.1141	Bisphenol A	$C_{15}H_{16}O_2$	4.06
Cpd 1335:	Propoxur	7.614	209.1052	Propoxur	$C_{11}H_{15}NO_3$	–0.1
Cpd 1337:	Pyrocatechol	7.615	110.0369	Pyrocatechol	$C_6H_6O_2$	–1.11
Cpd 1348:	Cinmethylin	7.7	274.1933	Cinmethylin	$C_{18}H_{26}O_2$	–0.16
Cpd 1357:	Bisphenol A	7.778	228.1147	Bisphenol A	$C_{15}H_{16}O_2$	1.28
Cpd 1394:	Naphthol, 1-	7.996	144.0574	Naphthol, 1-	$C_{10}H_8O$	0.69
Cpd 1395:	Carbaryl	7.996	201.0791	Carbaryl	$C_{12}H_{11}NO_2$	–0.39
Cpd 1410:	Spinosyn B	8.128	717.4462	Spinosyn B	$C_{40}H_{63}NO_{10}$	–1.38
Cpd 1429:	Spinosyn A	8.278	731.4619	Spinosyn A	$C_{41}H_{65}NO_{10}$	–1.43
Cpd 1438:	Spiroxamine	8.364	297.2675	Spiroxamine	$C_{18}H_{35}NO_2$	–2.27
Cpd 1477:	Spinosyn D	8.621	745.4775	Spinosyn D	$C_{42}H_{67}NO_{10}$	–1.29
Cpd 1494:	Embelin	8.752	294.1838	Embelin	$C_{17}H_{26}O_4$	–2.25
Cpd 1501:	Cinmethylin	8.797	274.1935	Cinmethylin	$C_{18}H_{26}O_2$	–0.9
Cpd 1506:	Santonin	8.81	246.1261	Santonin	$C_{15}H_{18}O_3$	–2.01
Cpd 1526:	Cinmethylin	8.908	274.1935	Cinmethylin	$C_{18}H_{26}O_2$	–0.82
Cpd 1574:	Cinmethylin	9.184	274.1936	Cinmethylin	$C_{18}H_{26}O_2$	–1.21
Cpd 1578:	Bromophos	9.22	363.8501	Bromophos	$C_8H_8BrCl_2O_3PS$	–2.55
Cpd 1604:	Bromophos	9.279	363.8502	Bromophos	$C_8H_8BrCl_2O_3PS$	–2.57
Cpd 1715:	Imazethapyr	9.883	289.1417	Imazethapyr	$C_{15}H_{19}N_3O_3$	3.08

Compound Label	RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 1728: Ethoprop	9.911	242.0566	Ethoprop	$C_8H_{19}O_2PS_2$	–0.93
Cpd 1746: Imazethapyr	10.027	289.1419	Imazethapyr	$C_{15}H_{19}N_3O_3$	2.59
Cpd 1782: Penconazole	10.224	283.0649	Penconazole	$C_{13}H_{15}Cl_2N_3$	–1.99
Cpd 1797: Cinmethylin	10.325	274.1931	Cinmethylin	$C_{18}H_{26}O_2$	0.68
Cpd 1829: Cyprodinil	10.49	225.1271	Cyprodinil	$C_{14}H_{15}N_3$	–2.07
Cpd 1833: Chenodeoxycholic acid	10.526	392.291	Chenodeoxycholic acid	$C_{24}H_{40}O_4$	4.36
Cpd 1838: Embelin	10.541	294.1837	Embelin	$C_{17}H_{26}O_4$	–2.12
Cpd 1885: Chenodeoxycholic acid	10.749	392.2909	Chenodeoxycholic acid	$C_{24}H_{40}O_4$	4.45
Cpd 1908: Cinmethylin	10.852	274.1936	Cinmethylin	$C_{18}H_{26}O_2$	–1.24
Cpd 1927: Kresoxim methyl	10.933	313.1321	Kresoxim methyl	$C_{18}H_{19}NO_4$	–2.18
Cpd 1933: Cinmethylin	10.967	274.1934	Cinmethylin	$C_{18}H_{26}O_2$	–0.53
Cpd 1951: Cinmethylin	11.089	274.1939	Cinmethylin	$C_{18}H_{26}O_2$	–2.13
Cpd 1978: Carbofuranphenol	11.235	164.0841	Carbofuranphenol	$C_{10}H_{12}O_2$	–2.38
Cpd 2017: Spiroxamine	11.536	297.2666	Spiroxamine	$C_{18}H_{35}NO_2$	0.5
Cpd 2044: Spiroxamine	11.731	297.2663	Spiroxamine	$C_{18}H_{35}NO_2$	1.49
Cpd 2054: Spiroxamine	11.846	297.2673	Spiroxamine	$C_{18}H_{35}NO_2$	–1.65
Cpd 2062: Spiroxamine	11.955	297.2672	Spiroxamine	$C_{18}H_{35}NO_2$	–1.26
Cpd 2096: Etacelasil	12.24	316.1098	Etacelasil	$C_{11}H_{25}ClO_6Si$	3.42
Cpd 2205: Spiroxamine	12.797	297.267	Spiroxamine	$C_{18}H_{35}NO_2$	–0.58
Cpd 2206: Ivermectin B1b	12.806	860.488	Ivermectin B1b	$C_{47}H_{72}O_{14}$	4.9
Cpd 2253: Ivermectin B1b	13.128	860.4891	Ivermectin B1b	$C_{47}H_{72}O_{14}$	3.61
Cpd 2314: Ivermectin B1b	13.372	860.4896	Ivermectin B1b	$C_{47}H_{72}O_{14}$	3.04
Cpd 2474: Ivermectin B1b	14.122	860.4906	Ivermectin B1b	$C_{47}H_{72}O_{14}$	1.85

Conclusions

The Agilent TOF and Q-TOF Pesticide Application Kit has been developed to provide comprehensive screening of pesticides for both targeted and non-targeted compounds. The database includes almost 1600 compounds and gives the user great flexibility in its use.

The kit offers:

- Fast and easy startup of complex analyses
- A comprehensive pesticide database of almost 1600 compounds including:
    - Chemical structures, formulas and exact masses
    - Direct Chemical Internet links to PUBCHEM and Chemspider
    - IUPAC Names
    - The ability to create spectral libraries
    - Completely customizable additions/deletions and retention time additions for chromatographic conditions developed by the user
- Results can be searched directly from the PCDL software
- Results can be data-mined with powerful searching tools such as, the Molecular Feature Extractor and Find by Formula
- Searches of the database can be partially or completely automated using MassHunter Qualitative Analysis and the MassHunter Acquisition Worklist

References

1. Agilent Technologies publication 5990-3935EN, "Q-TOF LC/MS Screening and Confirming of Non-Targeted Pesticides in a Strawberry Extract."
2. Agilent Technologies publication 5989-5496EN, "Automated Screening of 600 Pesticides in Food by LC/TOF MS Using a Molecular-Feature Database Search."
3. Agilent Technologies publication 5990-4253EN, "Multi-Residue Pesticide Analysis with Dynamic Multiple Reaction Monitoring and Triple Quadrupole LC/MS/MS."
4. Agilent Technologies publication 5990-3976EN, "Pesticide Personal Compound Database for Screening and Identification."

For More Information

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

Appendix I

LC/MS/MS Conditions for Test mix Positive and Negative Ion Samples

Agilent 1200 Series SL LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	35 °C
Injection volume:	5
Autosampler temperature:	Ambient
Needle wash:	5 s with methanol
Mobile phase:	A = 5 mM acetic acid in water B = 100% acetonitrile
Flow rate:	0.3 mL/min
Gradient:	5% B at t = 0 to 95% B at t = 12 min
Stop time:	12 min
Post time:	3 min

Agilent 6530 Q-TOF Parameters

Jet Stream Conditions

Gas temperature:	250 °C
Gas flow:	7 L/min
Nebulizer:	40 psi
Sheath gas temperature:	325 °C
Sheath gas flow:	11 L/min
Capillary + ion:	3500 V
Nozzle voltage:	0 V
Capillary – ion:	2500 V
Nozzle voltage:	1500 V
Acquisition Mode:	MS1
Min Range	100 *m/z*
Max Range	1100 *m/z*
Scan Rate	1.4 per s
Reference Masses:	Positive ion 121.050873 ($M+H^+$ for purine) 922.009798 ($M+H^+$ for HP-921)
Reference Masses:	Negative ion 119.0362 ($M-H^-$ for purine) 980.016375 ($M+C_2H_3O_2^-$ for HP-921 acetate adduct)

Appendix II

Agilent 1200 Series SL LC Parameters

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	55 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.3 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
14	No change	600	95
17	No change	600	95

Stop time	17 min
Post time	3 min

Agilent 6230 TOF Parameters

Jet Stream Conditions

Drying gas temperature:	225 °C
Drying gas flow (nitrogen):	9 L/min
Nebulizer gas pressure (nitrogen):	25 psig
Capillary voltage:	4500 V
Sheath gas temperature:	350 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	500 V
Acquisition Mode MS1	
Min Range	25 m/z
Max Range	3200 m/z
Scan Rate	3
Reference Masses:	Positive ion 121.050873 ($M+H^+$ for purine) 922.009798 ($M+H^+$ for HP-921)

Appendix III

Agilent 1290 Infinity LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18 HD, 2.1 mm × 100 mm, 1.8 µm Agilent p/n
Column temperature:	60 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25) 5 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
LC flow rate:	1.0 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.15	No change	600	6
2.1	No change	600	95
3	No change	600	95

Stop time	3 min
Post time	1 min

6540 Q-TOF Parameters

Jet stream conditions

Drying gas temperature:	325 °C
Drying gas flow (nitrogen):	8 L/min
Nebulizer gas pressure (nitrogen):	60 psig
Capillary voltage:	4000 V
Sheath gas temperature:	350 °C
Sheath gas flow:	12 L/min
Nozzle voltage:	500
Acquisition Mode:	MS1
Min Range	100 *m/z*
Max Range	1000 *m/z*
Scan Rate	10 per s
Reference Masses:	Positive ion 121.050873 ($M+H^+$ for purine) 922.009798 ($M+H^+$ for HP-921)

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Printed in the USA
August 5, 2009
5990-4251EN

Agilent Technologies

Multi-Residue Pesticide Analysis with Dynamic Multiple Reaction Monitoring and Triple Quadrupole LC/MS/MS

Fast and Effective Method Development Using an Application Kit and a Pesticides Compound Parameter Database

Application Note

Food Safety and Environmental

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Abstract

The analysis of pesticide residues in food and environmental samples is challenging due to the low concentrations and large number of analytes that need to be monitored and quantified. In addition, method development for Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS) with a triple quadrupole instrument is laborious and time consuming because of the compound dependent parameters that need to be optimized. This application note describes how pesticide residue LC/MS/MS methods can be set up quickly and efficiently using the Agilent Pesticides Application Kit. This Application Kit contains a pesticide test mix, a 600-compound pesticide MRM database, a quick start guide and several dynamic Multiple Reaction Monitoring (MRM) methods, which can easily be incorporated into a specific method for pesticide residue analysis. The Pesticides Dynamic MRM database contains compounds commonly monitored around the world and provides fast, customized method development of the analysts' list of pesticides. Results from a 100 and 300-compound mixture are demonstrated with an Agilent 1200 SL Series Rapid Resolution LC and the Agilent 6460 Series Triple Quadrupole LC/MS System with Agilent Jet Stream Technology. The 300-compound mixture was also analyzed using an Agilent 1290 Infinity Ultra High Pressure Liquid Chromatograph (UHPLC) and a 6460 LC/MS. With the higher pressure capabilities of the Agilent 1290 Infinity UHPLC, rapid separations with higher peak capacity and less peak overlap than the Agilent 1200 Series RRLC were produced. Using a spinach matrix spiked with 16 pesticides, the performance of a complete method with the SampliQ extraction and dispersive SPE kits and the Agilent LC/MS/MS triple quadrupole on a typical food matrix was

Agilent Technologies

Introduction

The analysis of target pesticide residues has traditionally been performed using Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Mass Spectrometry (LC/MS) methods. Because of the number of pesticides used and the sensitivity needed for monitoring hundreds of pesticides in a single analysis, both techniques are a requirement. GC/MS is needed for the less polar, more volatile pesticides and LC/MS for pesticides that are more polar or thermally labile and there is much overlap between them. However, many of the pesticides developed over the last 20 years are most amenable to LC/MS. The method of choice for trace analysis in complex matrices uses a triple quadrupole (QQQ) mass spectrometer incorporating multiple reaction monitoring (MRM). During an MRM analysis the QQQ monitors the product ions produced by collisions of precursor ions in the central quadrupole (the collision cell) of the mass spectrometer, as seen in Figure 1. An MRM analysis can generate a very sensitive and specific analysis of target compounds.

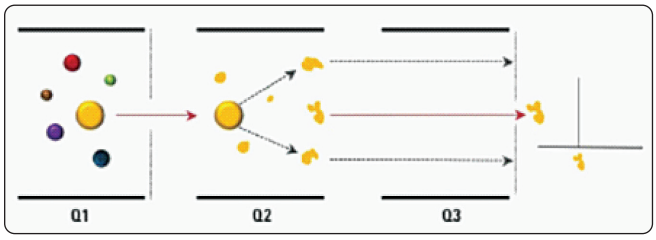


Figure 1. A schematic diagram of MRM mode on a triple quadrupole instrument. The precursor ion is selected in Q1, fragmentation occurs in Q2, and the product is selected by Q3. Since two stages of mass selectivity are used, there is very little interference from background matrix resulting in excellent sensitivity.

Over time regulating agencies have continually increased the number of pesticides and residues that must be monitored. It is now common that hundreds of residues need to be analyzed in a single LC/MS analysis. To address this challenge the MRM transitions that need to be monitored are switched using programmed time segments. This is called time segmented MRM. It is accomplished by programming the QQQ to monitor specific product ions in time segments during the LC/MS analysis. However, the method requires well defined elution time boundaries and must avoid time segment switches when compounds elute from the LC. If a time segmented MRM analysis is generated for a sample that contains hundreds of residues, the time segmented MRM analysis becomes subject to cycle and dwell time limitations that affect the sensitivity and specificity of the analysis. A new technique, Dynamic Multiple Reaction Monitoring (MRM) alleviates these limitations and also allows easier method development and future modifications of the method, such as the addition of new pesticides to be analyzed. Using Dynamic MRM, analyte ions are only monitored while they are eluting from the LC. This significantly improves the MS duty cycle time for very complex samples when compared with the time segment method and improves the sensitivity and specificity of an analysis.[1]

One of the challenges in developing an MRM method, whether it is a time segment or Dynamic MRM, is creating the time sequence of MS/MS events and mass spectrometer conditions necessary to maximize sensitivity and specificity. It is essential to generate a list of two or more MRM transitions and compound specific parameters, fragmentor voltage and collision energy for each compound being analyzed. The availability of a database containing over 600 pesticides with the MS/MS instrumental information that can be used with all Agilent triple quadrupoles eliminates the need to create this information via tedious manual procedures. The database allows easy import of selected compounds into the user's analytical method. A portion of this database is shown in Figure 2. In addition to creating custom methods, the read-only database allows the user to copy their customized database to meet his or her specific needs. A technical note describes this database in detail. [2] The Agilent Pesticides Application Kit also includes a pesticide test mixture that is used to demonstrate the performance of the system and pre-tested methods, allowing faster method development. Neither the kit nor the test mixture diminishes the need for each laboratory to define suitable QC/QA procedures and perform validation. Each laboratory must have QC tests fit-for-purpose and run analytical standards to validate analytical results.

This application note will demonstrate the use of the Agilent Pesticide Application Kit with a 600-compound parameter database and Dynamic MRM for the analysis of complex pesticide mixtures. The liquid chromatographic separations are performed using an Agilent 1200 SL Series RRLC or an Agilent 1290 Infinity UHPLC with an Agilent 6460 QQQ incorporating Jet Stream technology.[3] The methods described in the note are straightforward to generate using the Agilent MassHunter data analysis software and the Pesticide Dynamic MRM Database. Some limits of detection (LOD) of 100 fg or less were achieved using these methods with the Agilent 6460 Series QQQ LC/MS system. These methods are also compatible with all Agilent 6400 series LC/MS systems.

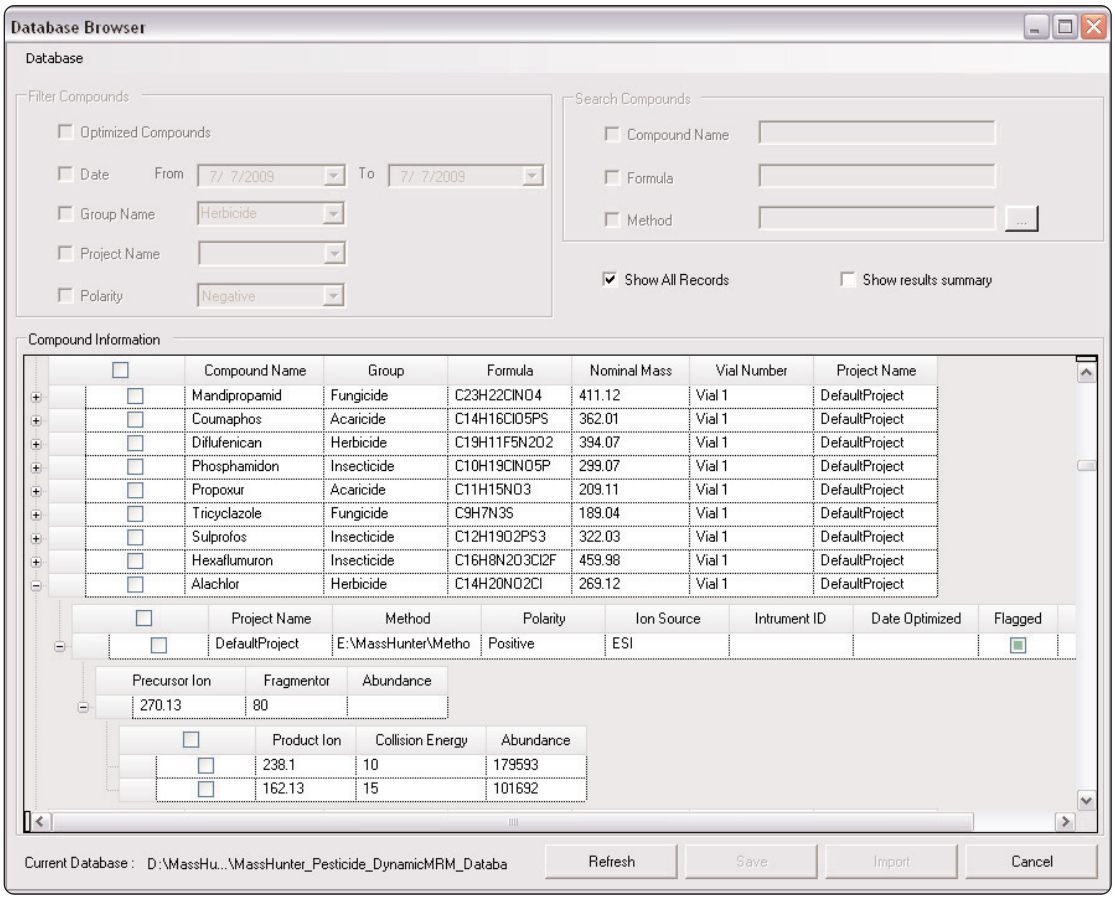


Figure 2. Compound Parameter Database with over 600 pesticides entries.

Experimental

Reagents and Chemicals

- Agilent Pesticide Test Mix, p/n 5190-0469 acid and base diluted separately as instructed to 10 ppb in 10% acetonitrile/90% water
- An Agilent SampliQ QuEChERS AOAC Extraction kit, p/n 5982-5755. Agilent SampliQ QuEChERS AOAC Dispersive SPE kits for Highly Pigmented Fruits and Vegetables, p/n 5982-5321 (2 mL) and p/n 5982-5356 (15 mL)
- Multiple pesticide standards were obtained from Sigma, Chemservice, and Dr. Erhenstofer

Instrument Settings

- *Appendix I: LC/MS/MS Conditions for Test mix Positive and Negative Ion Samples
- Appendix II: LC/MS/MS Conditions for a 100 Pesticide Methods
- *Appendix III: LC/MS/MS Conditions for 300-Pesticide Methods using the Agilent 1200 Series SL
- Appendix IV: LC/MS/MS Conditions for the 300-Pesticide Methods using the Agilent 1290 Infinity LC
- Appendix V: LC/MS/MS Conditions for Pesticides in Spinach using QuEChERS Extraction.
- *Appendix VI: LC/MS/MS Conditions for the 165-Pesticide Methods using the Agilent 1200 Series SL
- *Appendix VII: LC/MS/MS Conditions for the 224-Pesticide Methods using the Agilent 1200 Series SL
- Appendix VIII: LC/MS/MS Conditions for the 224-Pesticide Methods using Agilent 1290 Infinity LC

*Each of these methods are included with the Application Kit

Spinach Sample Preparation

- Weigh 15 g (±0.1 g) of homogenized spinach sample.
- Spike standards or IS solution if necessary.
- Vortex 30 s.
- Add 15 mL of 1% acetic acid in acetonitrile.
- Add 1 bag of extraction kit (p/n 5982-5982-5755) buffered QuEChERS extraction tubes, AOAC Method 2007.01 to 6 g $MgSO_4$ and 1.5 g NaAc.
- Cap and hand-shake vigorously for 1 min.
- Centrifuge at 4000 rpm for 5 min.
- Transfer 1 mL or 8 mL upper layer to the dispersive SPE kit (p/n 5982-5321 or p/n 5982-5356) for highly pigmented fruits and vegetables.
- Vortex 1 min.
- Centrifuge 2-mL tubes at 13000 rpm for 2 min, or 15 mL tubes at 4000 rpm for 5 min.
- Transfer 200 µL of the upper layer to the autosampler vial.
- Add 800 µL of water or appropriate standard spiking solution.
- Vortex 1 min, to prepare for LC/MS/MS analysis.

Results and Discussion

Positive and Negative Ion Test Mix

In addition to the 600-compound database, the Agilent Application Kit for pesticide residue analysis also includes a positive and negative ion test mix, with their analysis methods shown in Appendix I. The methods contain compound names, MRM transitions, fragmentor voltages, collision energies, and retention times for the Dynamic MRM. The test mix and the supplied method allow the analyst to demonstrate that the system is operating properly for pesticide analysis immediately after installation. The LC/MS/MS extracted ion chromatograms (EIC) from the test mix analyzed in the positive and negative ion mode using Dynamic MRM is shown in Figures 3 and 4.

The Application Kit Quick Start Guide [4] shows the analyst how to run the test mixes and create a Dynamic MRM method. To create new methods, standards are analyzed at higher concentrations with a one segment MRM method. The data is processed using the Agilent MassHunter Quantitative Data Analysis software to generate a custom report that now includes analyte retention times. A Dynamic MRM method is generated by importing the results from the custom report and specifying a delta retention time window. This process will be automated in the near future. Table 1 shows a partial listing of

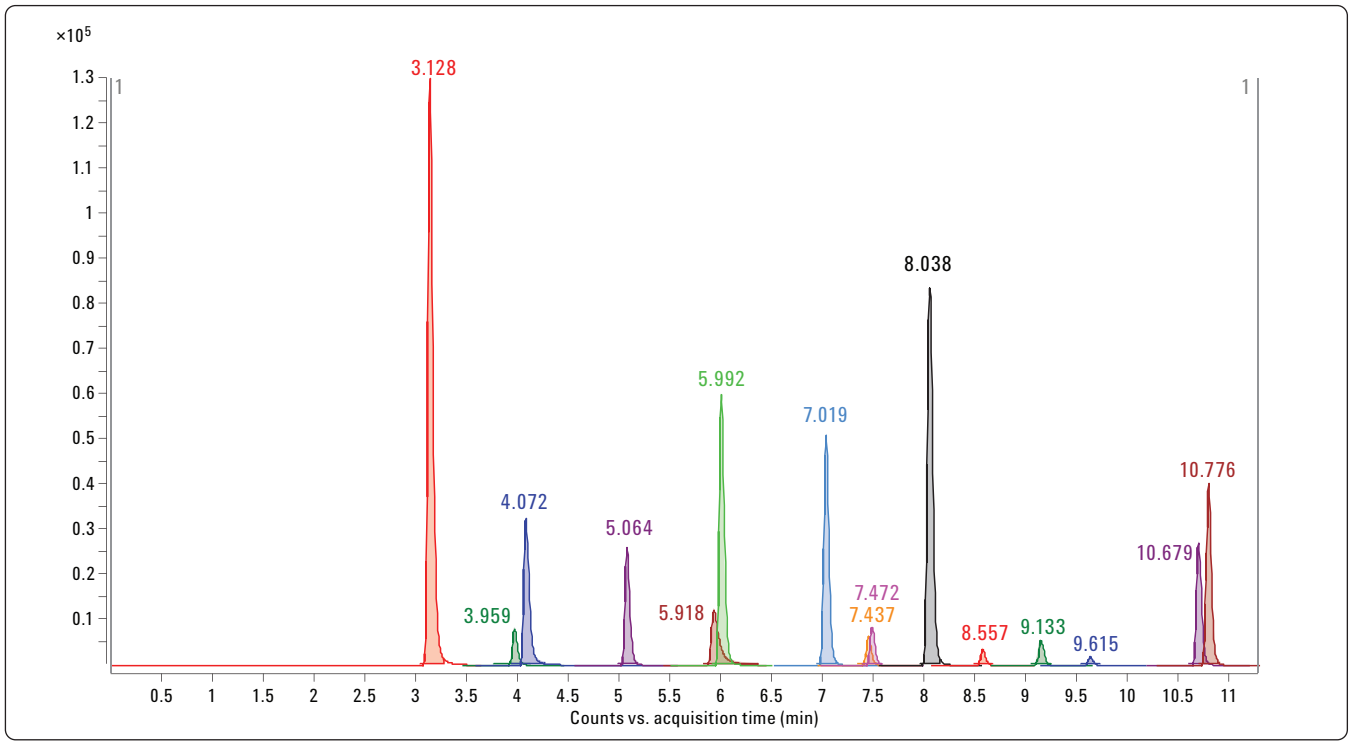


Figure 3. Positive ion test mix extracted ion chromatogram (see Appendix 1 for list of compounds matching retention times given in chromatogram).

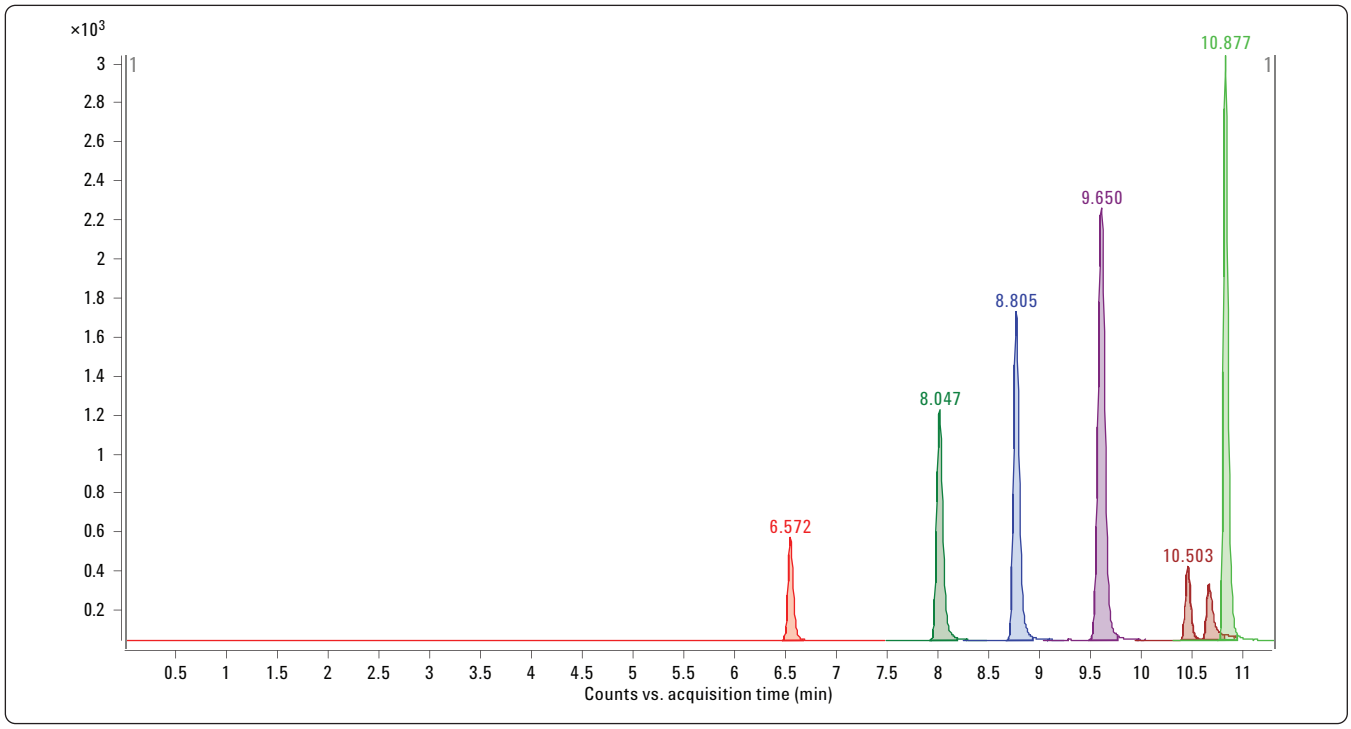


Figure 4. Negative Ion Test Mix extracted ion chromatogram (see Appendix 1 for list of compounds matching retention times given in chromatogram).

the acquisition parameters from a Dynamic MRM method. Note in this example the retention time window (Delta RT) is 2 min which is large for narrow peaks. A window this wide can be used to run standards where retention times have shifted and need to be updated in the users' customized method.

Table 1. Dynamic MRM Screen Capture of Acquisition Parameters

Acquisition | Source | Chromatogram | Instrument | Diagnostics

Scan segments

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time
Acephate	☐	184	Unit	125	Unit	80	10	1.212	2
Aminocarb	☐	209	Unit	137	Unit	120	20	1.251	2
Atrazine	☐	216	Unit	132	Unit	120	20	7.602	2
Azinphos-methyl	☐	318	Unit	132	Unit	80	10	9.346	2
Carbofuran	☐	222	Unit	123	Unit	120	15	7.13	2
Chlorpyrifos methyl	☐	322	Unit	125	Unit	80	15	12.168	2
Diazinon	☐	305	Unit	153	Unit	160	20	11.822	2
Dimethoate	☐	230	Unit	171	Unit	80	10	4.645	2
Imazalil	☐	297	Unit	159	Unit	160	20	6.498	2

Dynamic MRM Parameters

Cycle Time 500 ms

Fast and effective screening of a 100-compound pesticide mix using Dynamic MRM

A 100-compound mix of pesticides was used to demonstrate the effectiveness of the Dynamic MRM. Appendix II contains the LC/MS/MS conditions and a partial listing of the Dynamic MRM method used to analyze a 100-pesticide mixture at the 100 pg/compound level. Note that the column used was 50 mm in length so faster analysis and less efficiency is obtained. The LC/MS/MS extracted ion chromatogram shown in Figure 5 illustrates the performance of the system. The complete LC analysis took less than 15 minutes. Figure 6 shows a 1-min time window where 11 compounds (22 MRM's) are eluting. Figure 7 shows the 1-min delta retention time window for each Dynamic MRM transition. Note the many peak overlaps in the chromatograms. This necessitates the use of dynamic transitions instead of time segmented transitions in order to achieve the needed cycle time so that each peak can have enough data points to adequately describe the peak for quantitation. Furthermore time segmented MRM has an inherent "dead time" data loss when monitoring analyte peaks eluting near or between time segment boundaries. Time segmented MRM methods may require duplicate monitoring of specific analytes which elute over adjoining time segments. In addition, Dynamic MRM maximizes the dwell times for overlapping peaks enhancing the signal-to-noise while maintaining constant cycle time. Note that the cycle time selected should ideally provide about 20 data points across a peak with a minimum of 64 data points in the retention time window (Delta Ret Window).

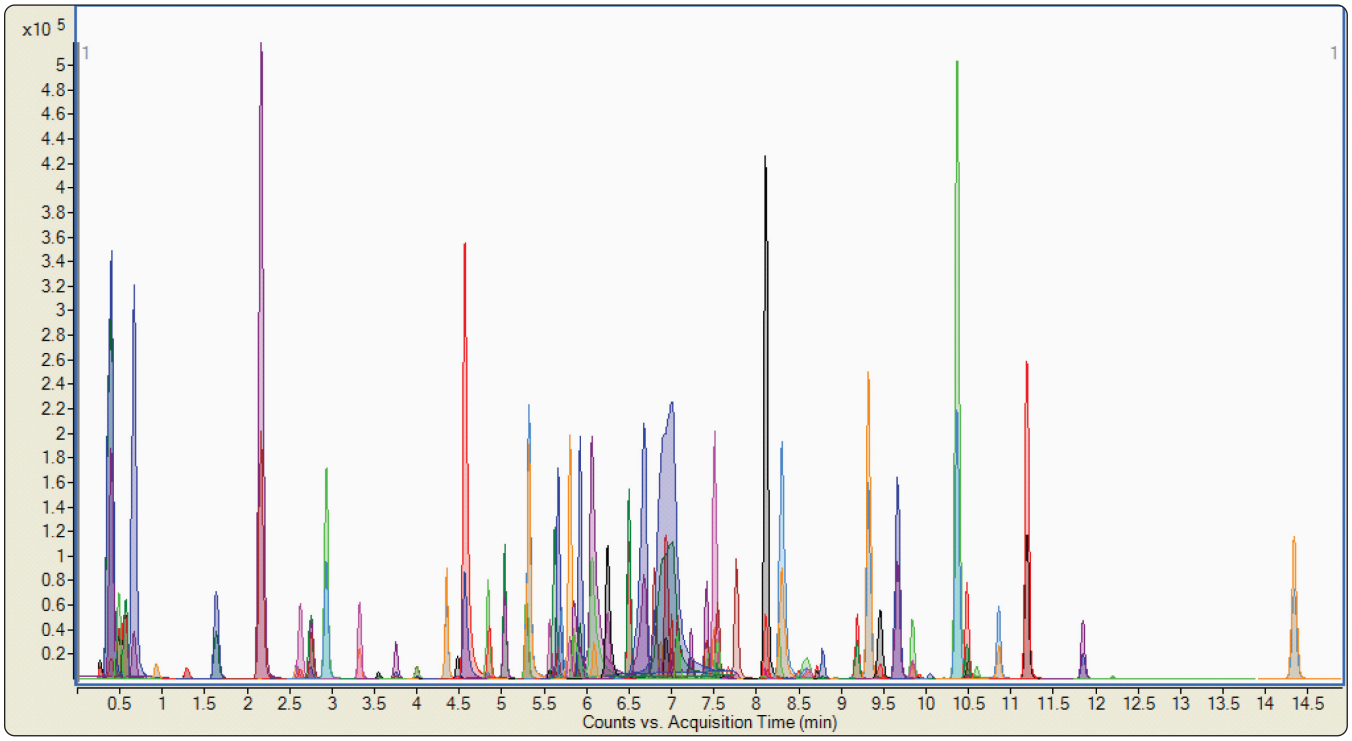


Figure 5. Extracted Ion Chromatograms of 100 compound pesticide mixture (100 pg level).

Compound name	Precursor ion	Product ion	Retention time
Cinosulfuron	414.1	183	5.579
Cinosulfuron (Q)	414.1	157	5.579
Chlorotoluron	213.1	72	5.642
Chlorotoluron (Q)	213.1	140	5.642
Atrazine	216.1	174	5.682
Atrazine (Q)	216.1	132	5.682
Carbaryl	202.1	145	5.736
Carbaryl (Q)	202.1	117	5.734
Carboxin	236.1	143	5.836
Carboxin (Q)	236.1	87	5.836
Chlorsulfuron	358.0	167	5.896
Chlorsulfuron (Q)	358.0	141	5.896
Ethiofencarb	226.1	107	5.937
Ethiofencarb (Q)	226.1	164	5.936
Dodemorph	283.3	116	6.073
Dodemorph (Q)	282.3	98	6.074
Diuron (Q)	233.0	160	6.101
Cyprodinil	226.1	108	6.245
Cyprodinil (Q)	226.1	93	6.246
Difenoxurone	287.1	123	6.509
Difenoxurone (Q)	287.1	72	6.509

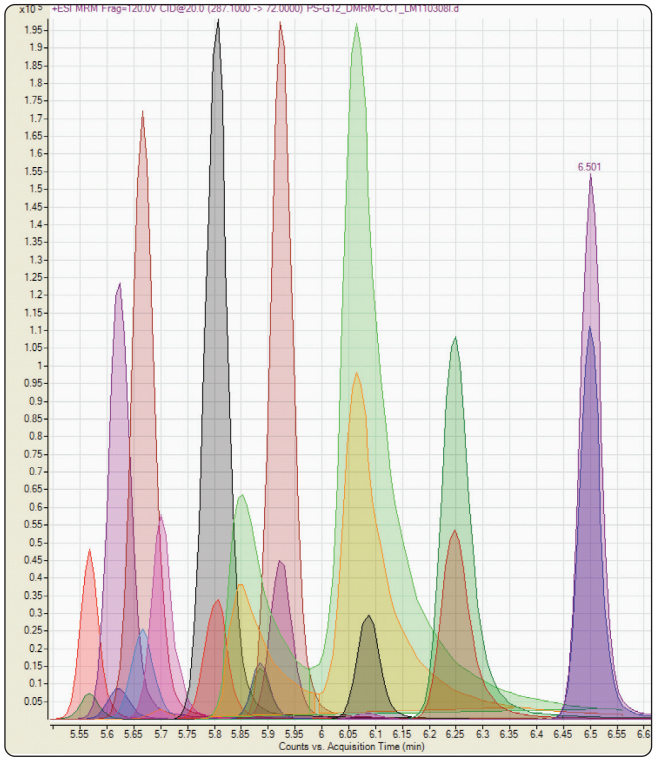


Figure 6. Left: Table of 11 compounds monitored during a 1 minute time window. Right: Dynamic MRM of compounds being monitored.

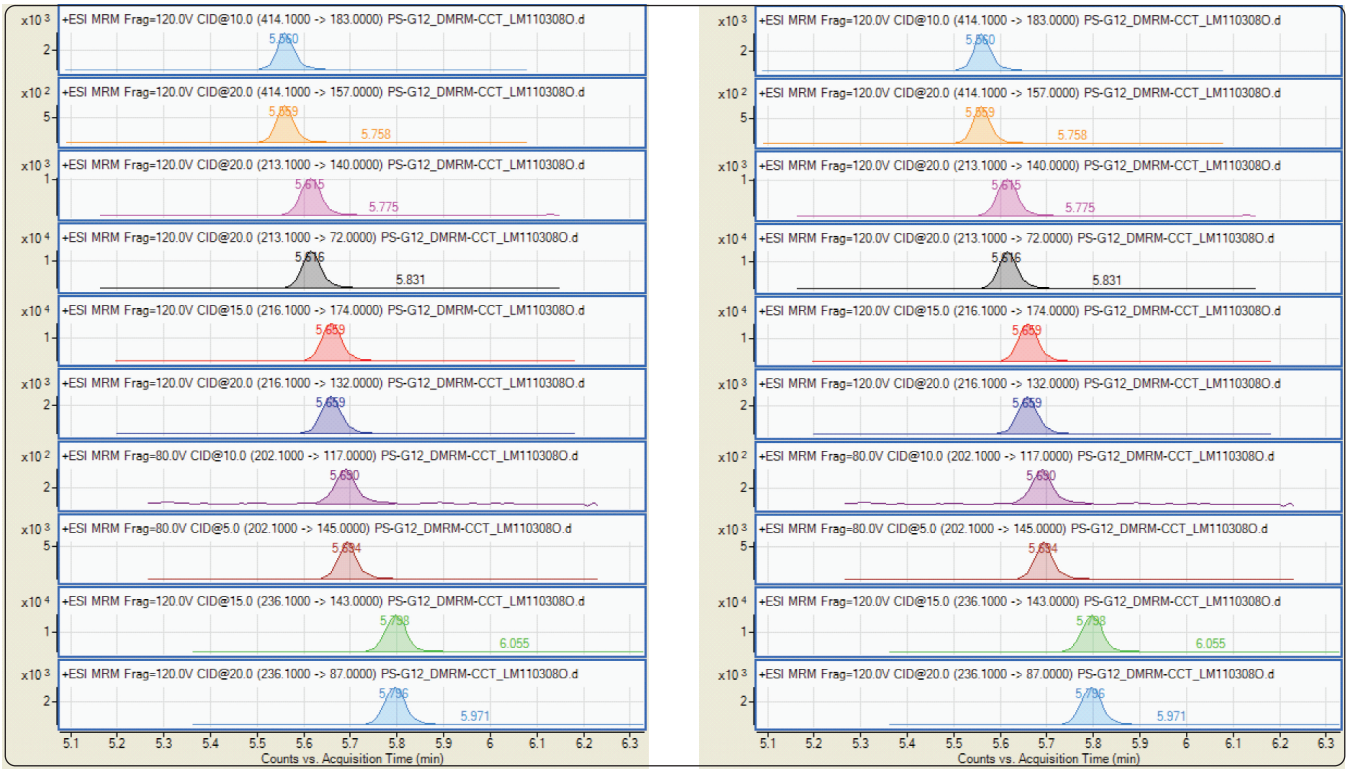


Figure 7. Dynamic MRM windows for each MRM transition.

Typical results achieved with the method are shown in Figure 8. It illustrates the results from one of the compounds, atrazine, in the 100-compound mixture. Note the 20 data points that were collected during the elution of atrazine. This provides a sufficient number of data points to assure quantitative accuracy and shows the effectiveness of Dynamic MRM.

Average signal height:	15,650
Average signal area:	50,966
RSD:	3.2%
Estimated LOQ:	100 fg or less

6–7 data points above FWHM

3 sec FWHM, 6 sec at 10% valley

20 data points baseline-to-baseline

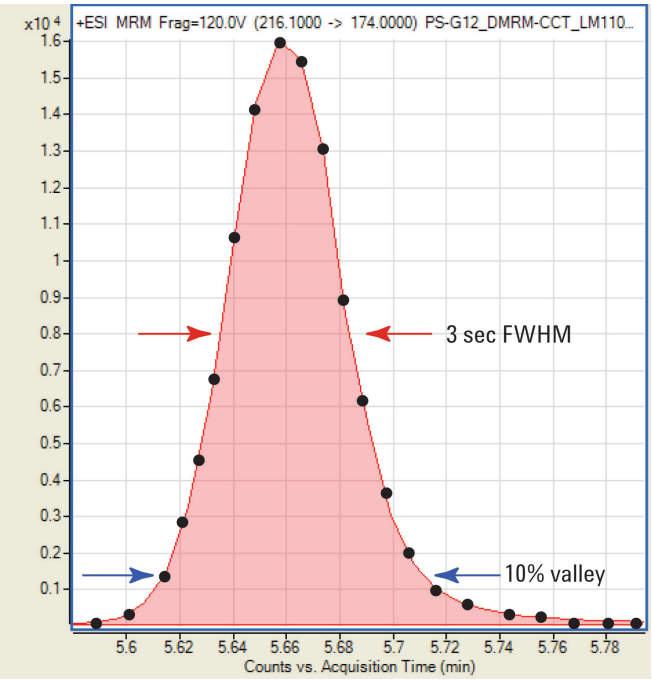


Figure 8. Typical analytical results shown with 10 pg of atrazine visualizing the effectiveness of Dynamic MRM.

The calibration data from four compounds in the mixture are illustrated in Figure 9. R^2's = 0.0998 are achieved for each pesticide. With constant cycle time maintained, the quantitative results with Dynamic MRM are excellent.

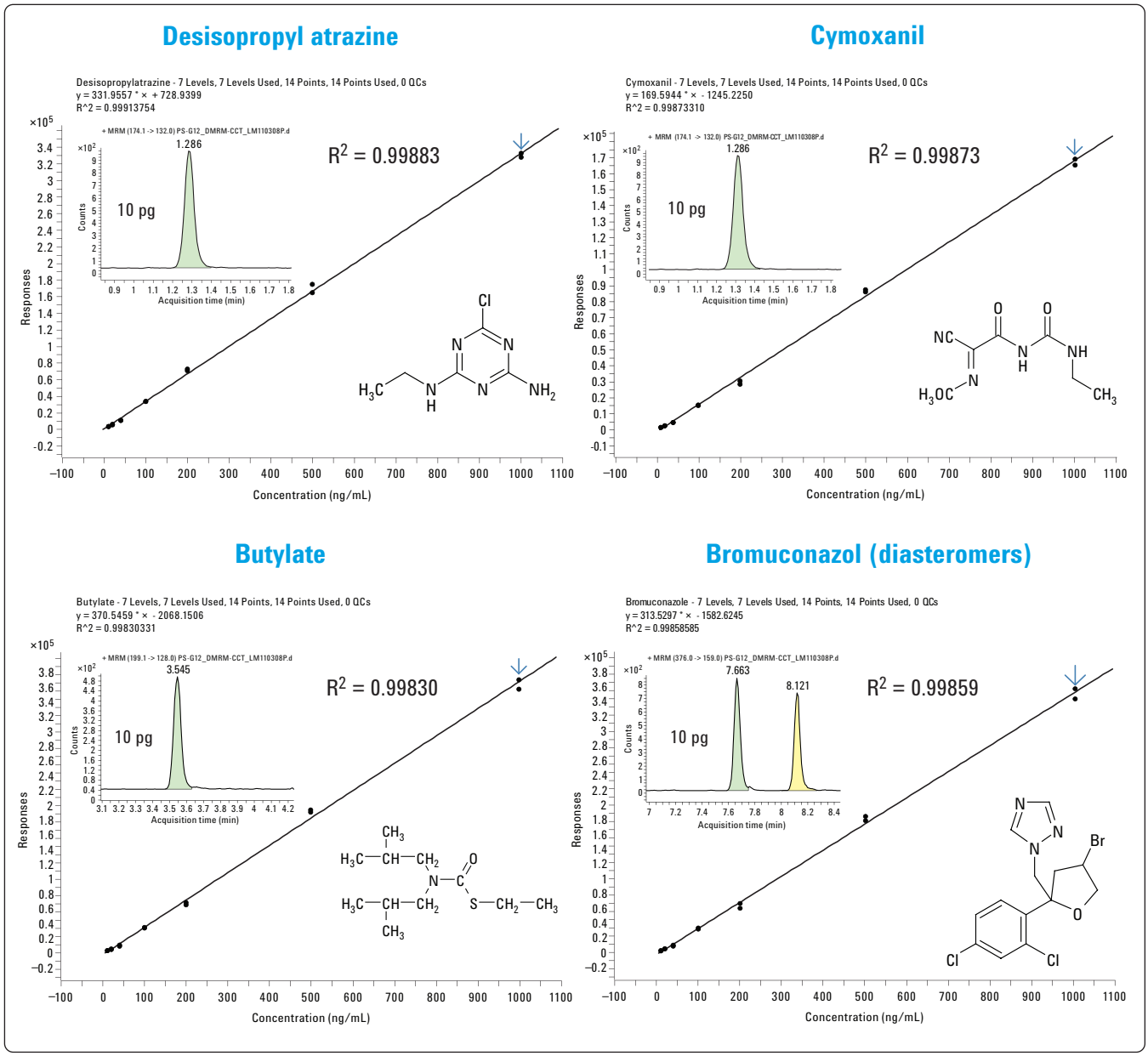


Figure 9. Dynamic MRM Calibration Plots, 10 pg–1 ng (7 levels).

Sharper peaks are produced with a 300-pesticide mix using the new Agilent 1290 Infinity LC

Appendix III contains a partial listing of the Dynamic MRM method used to analyze a 300-pesticide mixture at the 100 pg/compound level. The LC/MS/MS extracted ion chromatogram is shown in Figure 10. The analysis took less than 20 minutes using the Agilent 1200 Series SL RRLC and an Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm column at a flow rate of 0.5 mL/min. The same mixture was separated using an Agilent 1290 Infinity UHPLC with an Eclipse-Plus C18, 2.1 mm × 150 mm, 1.8 µm column. Figure 11, an extracted ion chromatogram and Figure 12, an expanded portion of the chromatogram, demonstrate that this complex mixture has been analyzed in about 15 minutes which is approximately 25% faster than with the Agilent 1200 Series SL RRLC. The Agilent 1290 Infinity UHPLC also produced a separation with higher peak capacity and less peak overlap than the Agilent 1200 Series SL RRLC. Typical peak ½ heights using atrazine as an example with the Agilent 1290 Infinity UHPLC are 1.8 s. This is because the longer column provides higher efficiency and the Agilent 1290 Infinity LC can operate at the pressure these conditions incurred (~900 bar).

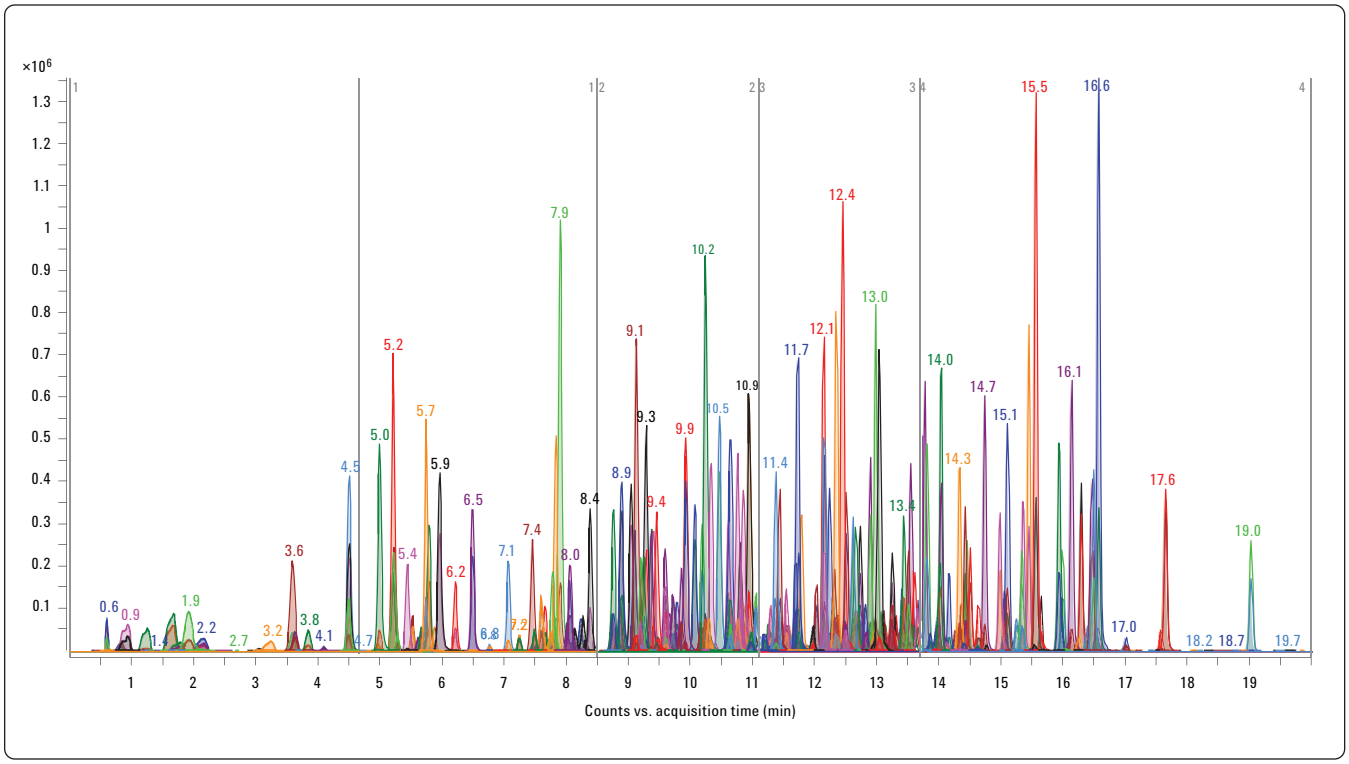


Figure 10. EIC of 300 compound pesticide mixture using an Agilent 1200 Series SL RRLC.

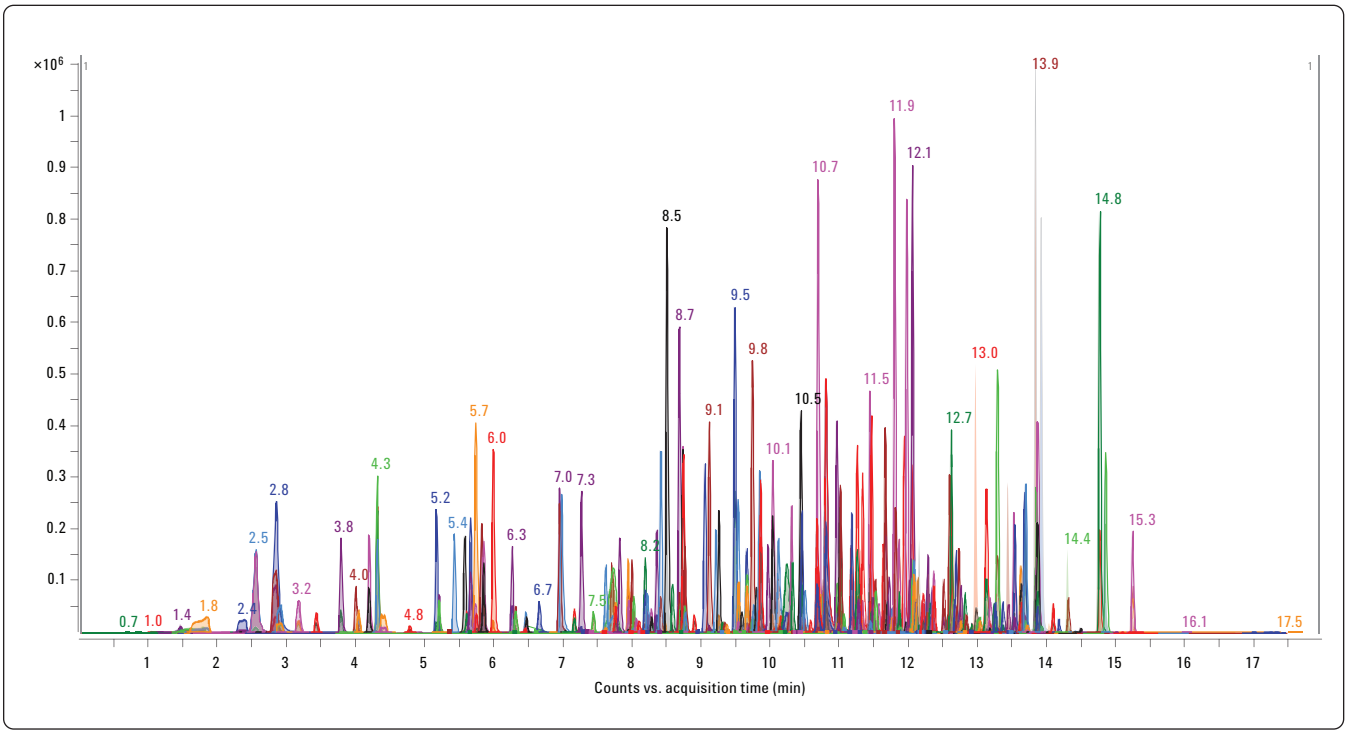


Figure 11. EIC of 300-compound pesticide mixture using the Agilent 1290 Infinity UHPLC.

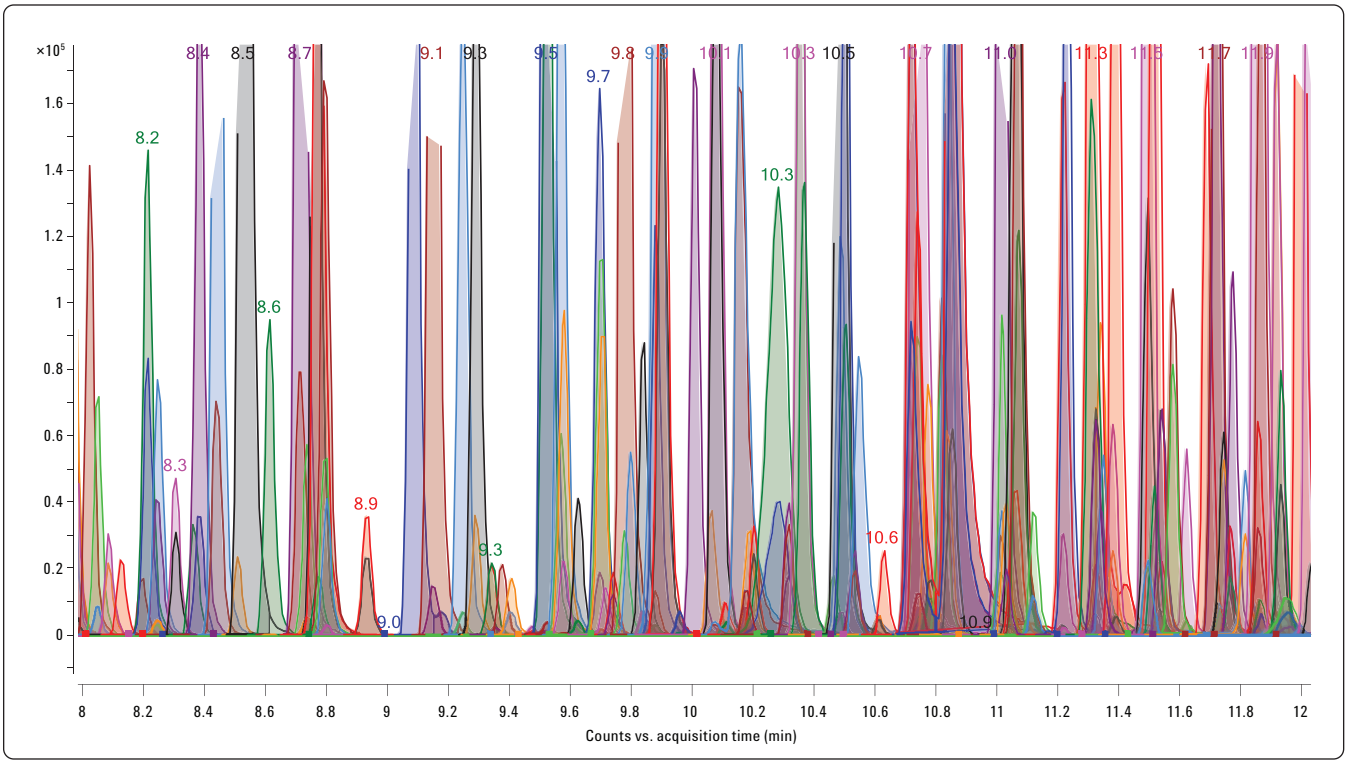


Figure 12. Expanded EIC of 300-compound pesticide mixture using an Agilent 1290 Infinity UHPLC illustrating the high peak capacity of the Agilent 1290 Infinity.

Faster analysis with a 224-pesticide mix using the new Agilent 1290 Infinity LC

Another advantage of the Agilent 1290 Infinity LC with the Agilent 6460 Series QQQ LC/MS is the ability to increase flow and decrease analysis time. Using the 1200 Series SL the analysis of 225 pesticides is performed in 15 min and shown in Figure 13. The method for this analysis is given in Appendix IV. With the Agilent 1290 Infinity LC the flow can be doubled and the gradient completed in half the time. This provides the same separation in less than 7 min as shown in Figure 14. The method for this analysis is given in Appendix V. Analyzing hundreds of pesticides in one run, it is best to obtain the highest peak capacity as shown in the 300-pesticide example. However, if speed of analysis is absolutely necessary, it is shown that the higher pressure capability of the Agilent 1290 Infinity LC and the higher pressure capability of the HD columns provide the performance needed.

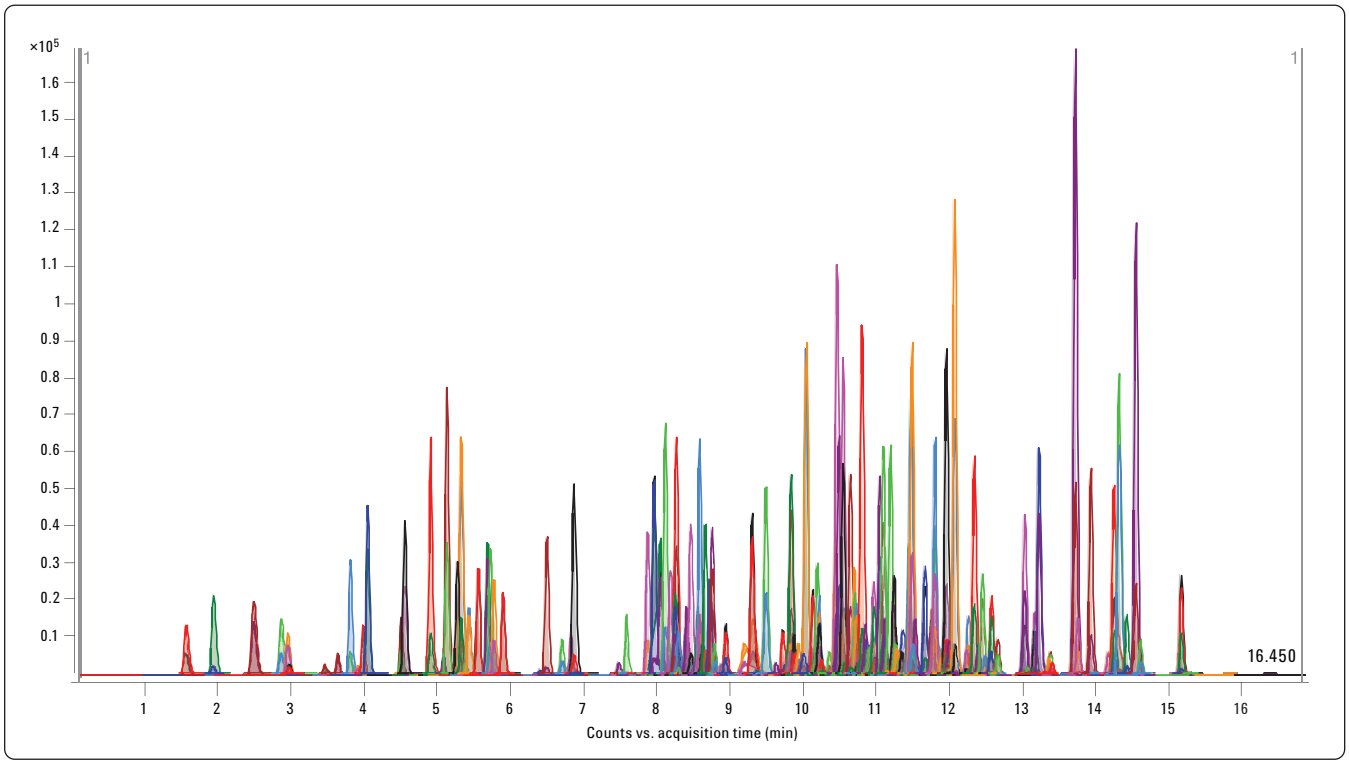


Figure 13. EIC of 224 pesticides using the Agilent 1200 Series SL LC and the Agilent 6460 QQQ LC/MS.

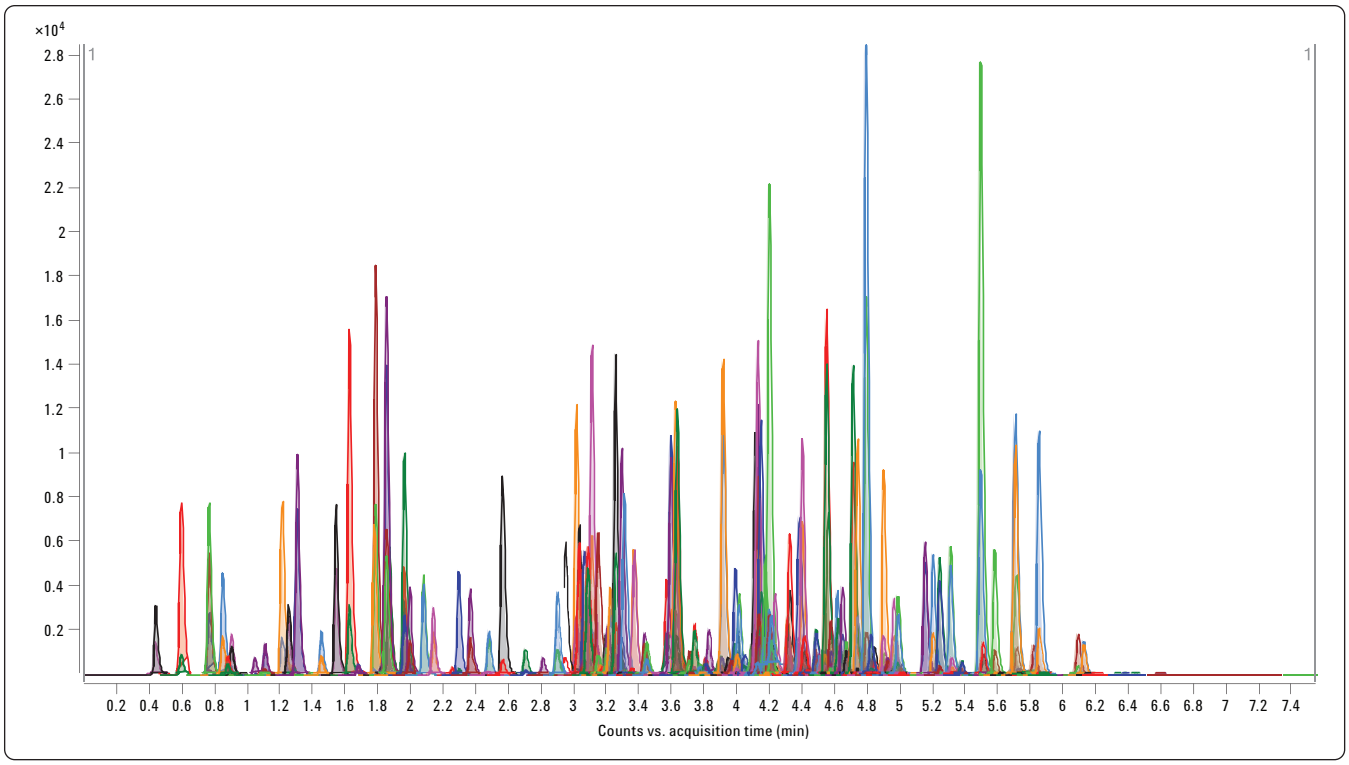


Figure 14. EIC of 224-pesticide mix analyzed with Agilent 1290 Infinity LC and the Agilent 6460 QQQ LC/MS.

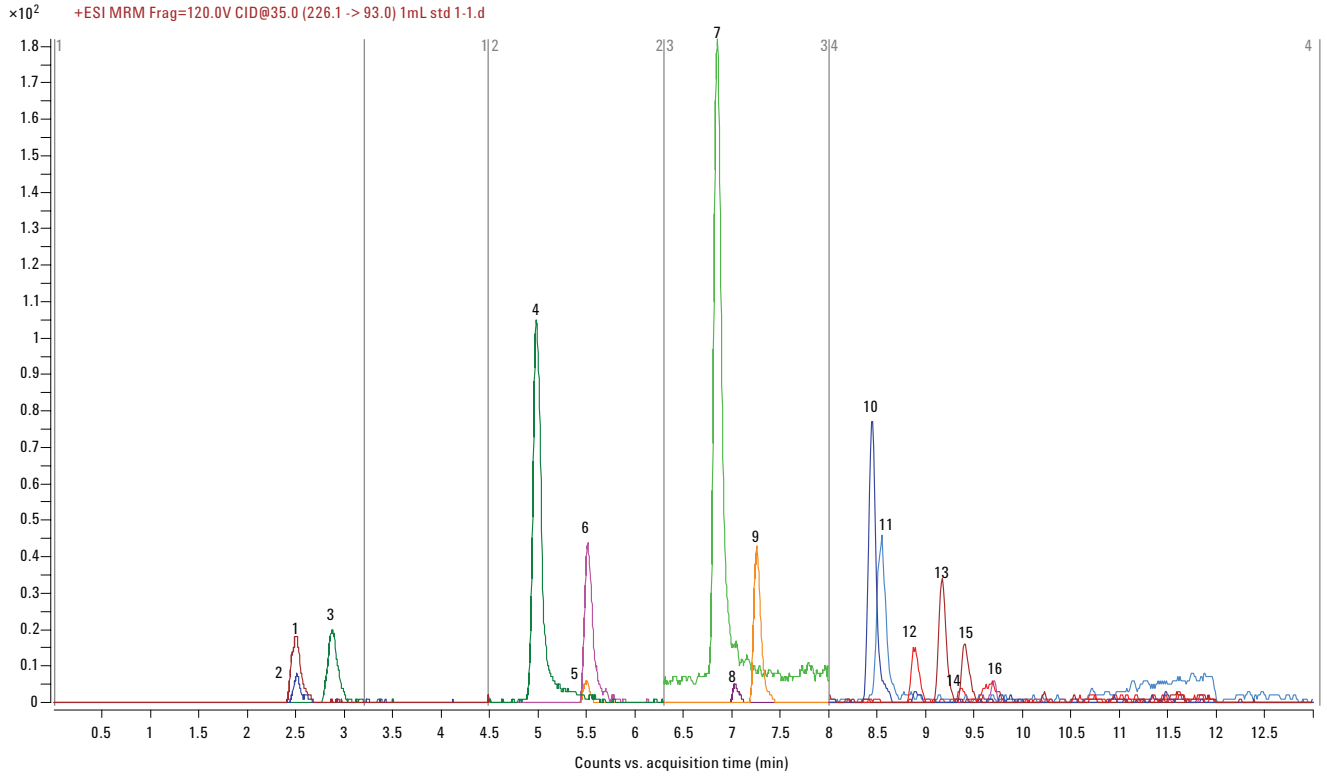
Pesticides Application Kit in a food matrix-Spinach using SampliQ Extraction and Dispersive SPE Kits

To demonstrate the use of the Agilent application kit for the analysis of a typical food product with Agilent's easy to use SampliQ extraction and dispersive SPE kits, a spinach matrix was spiked with 10 ppb of the 16 pesticides listed in Table 2. Triphenylphosphate (TPP) is the internal standard.

Table 2. List of 16 Pesticides and Instrument Parameters Spiked into Spinach Matrix at 10 ppb

Analyte	MRM channel (*m/z*) Quantifier	Qualifier	Fragmentor (V)	Collision energy (V) Quantifier	Qualifier	Retention Time (min)
Acephate	184.0 > 94.9	184.0 > 110.0	60	3	15	2.55
Methamidophos	142.0 > 94.0	142.0 > 124.9	60	8	8	2.54
Pymetrozine	218.1 > 105.0	218.1 > 78.0	115	20	50	2.97
Carbendazim	192.1 > 160.0	192.1 > 105.0	95	18	40	5.07
Imidacloprid	256.1 > 209.1	256.1 > 175.0	60	12	18	5.53
Thiabendazole	202.1 > 175.0	202.1 > 131.0	110	27	38	5.65
Propoxur	210.1 > 111.0	210.1 > 92.9	50	12	15	6.89
Thiophanate methyl	343.1 > 151.0	343.1 > 117.9	105	17	65	7.08
Carbaryl	202.0 > 145.0	202.0 > 115.0	50	3	40	7.30
Ethoprophos	243.1 > 130.9	243.1 > 172.9	80	15	15	8.50
Imazalil	297.1 > 158.9	297.1 > 200.9	80	22	15	8.52
Penconazole	284.1 > 158.9	284.1 > 172.9	80	32	32	8.95
Cyprodinil	226.1 > 93.0	226.1 > 108.0	120	35	35	9.23
Dichlorfluanid	333.0 > 123.0	333.0 > 223.9	85	28	5	9.40
Kresoxim methyl	314.0 > 222.1	314.0 > 235.0	70	10	10	9.44
Tolyfluanid	347.0 > 136.9	347.0 > 238.0	60	25	3	9.73
TPP (IS)	327.1 > 77.0	327.1 > 151.9	70	45	45	9.49

Figure 15 shows the EIC of the spinach sample spiked at the 10-ppb pesticide level. All the pesticides are easily detected at this level with a total analysis time less than ten minutes.



10 ppb of pesticides spiked into the spinach matrix.

Peak identification:

1. Acephate
2. Methamidophos
3. Pymetrozine
4. Carbendazim
5. Imidacloprid
6. Thiabendazole
7. Propoxur
8. Thiophanate methyl
9. Carbaryl
10. Ethoprophos
11. Imazalil
12. Penconazole
13. Cyprodinil
14. Dichlorfluanid
15. Kresoxim methyl,
16. Tolyfluanid

Internal standard peak (TPP) is not shown to get clear peak profile of other small peaks.

Figure 15. EIC of 10 ppb pesticides into spinach matrix..

An example of the linearity achieved for the spiked spinach matrix is shown in Figure 16. The calibration range was 5 – 250 ng/g and seven levels were used to generate the curve, 5, 10, 25, 100, and 250 ng/g. The curve was generated by plotting the ratio of the analyte peak area, carbaryl, to the internal standard (IS) peak area with the ratio of the analytes concentration to IS concentration. The $R^2 = 0.998$.

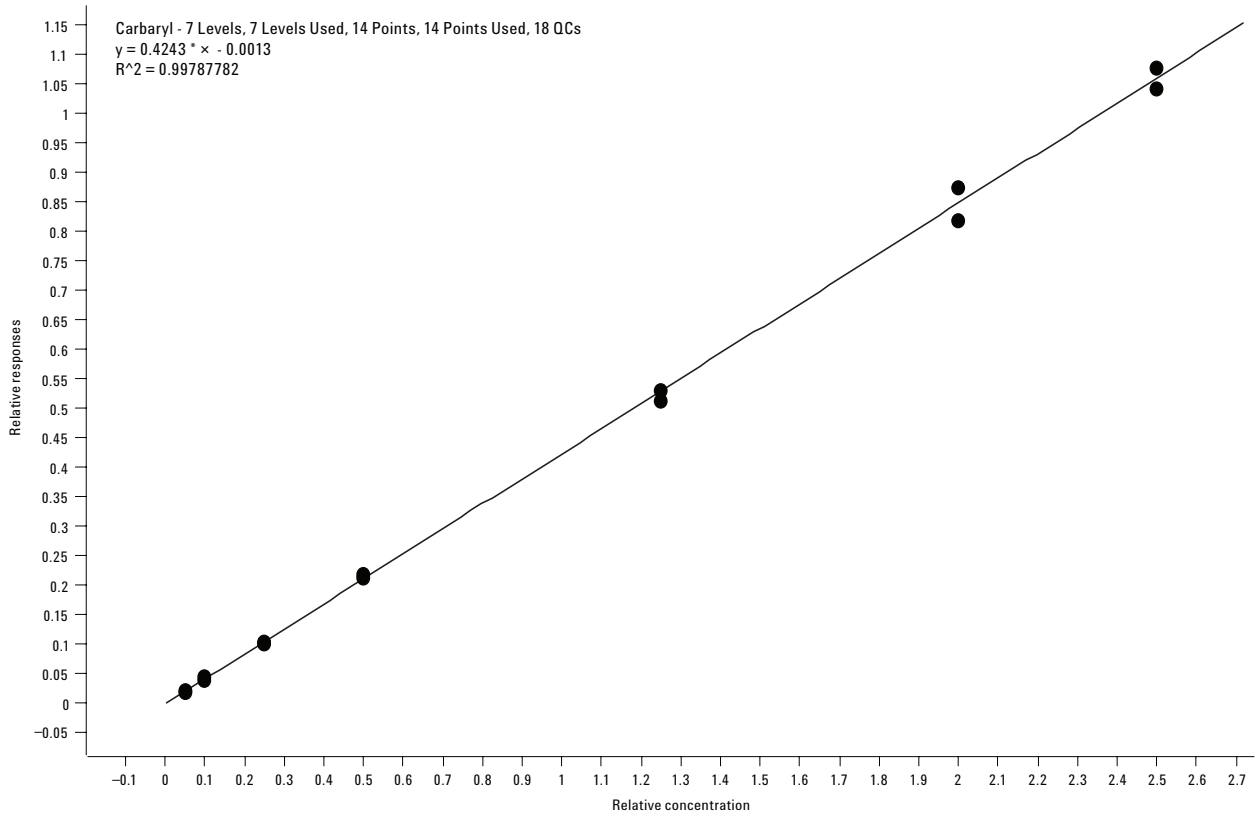


Figure 16. Carbaryl calibration curve.

Conclusions

The **Agilent Pesticide Application Kit** for LC/QQQ provides the user with fast method development for hundreds of pesticides with multiple transitions and the ability to develop those methods customized to his or her specific analytical needs.

This application note demonstrates the use of the Agilent Application Kit for Pesticides using several Agilent technologies for screening large numbers of compounds. The following technologies are used:

- ***600 compound pesticide MRM database*** and the **Agilent MassHunter Data Acquisition and Analysis software.** The combination gives users the ability to generate acquisition and analysis methods quickly. The methods can be easily customized and rapidly modified to meet the needs of future analyses.
- **Dynamic MRM** which maximizes the detection capability of the QQQ when hundreds of residues are being analyzed.
- **Agilent 1200 Series SL RRLC** interfaced to the **Agilent 6400 series triple quadrupoles** for fast and high resolution LC/MS/MS analysis. Use of the Agilent 6460 QQQ with Agilent's Jet Stream Electrospray Ion Source ensures lowest levels of detection of the pesticides. However, any of the Agilent 6400 series LC/QQQ will provide excellent results.
- Easy to use **SampliQ QuEChERS sample preparation kits** included in the Application Kit provide a fast and reproducible method to extract pesticide residues from complex food matrixes in a few simple steps.
- **Ready to use methods** with retention times for Dynamic MRM using the Agilent 1200 Series SL LC system. See all * Appendix methods.[4]

Use of these technologies allows methods to be quickly developed and enables screening of complex matrices containing hundreds of potential residues at femtomole concentrations.

This kit is compatible with all Agilent 1200 Series LC and 6400 series QQQ MS systems and will enable the user to quickly get started running multi-residue pesticides. For the most demanding analyses, the Agilent 1290 Infinity LC with the 6460 QQQ should be considered. Additional methods for this system should be available in the near future.

References

1. Application Note 5990-3595EN, New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Samples.
2. Technical Note 5990-4255EN Pesticide Dynamic Multi-reaction monitoring Database.
3. Technical Note 5990-3494EN Agilent Jet Stream Thermal GradienFocusing Technology.
4. Agilent Publication 5990-4262EN Pesticide analysis with DRMRM database quick start guide.

For More Information

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

Appendix I

LC/MS/MS Conditions for Test mix Positive and Negative Ion Samples

Agilent 1200 Series SL LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm 1.8 µm Agilent p/n 959764-902
Column temperature:	35
Injection volume:	5
Autosampler temperature:	Ambient
Needle wash:	5 s with methanol
Mobile phase:	A = 5 mM acetic acid in water B = 100% acetonitrile
Flow Rate:	0.3 mL/min
Gradient:	5% B at t = 0 to 95% B at t = 12 min
Stop Time:	12 min
Post:	Time 3 min

Jet Stream Conditions

Gas temperature:	250 °C
Gas flow:	7 L/min
Nebulizer:	40 psi
Sheath gas temperature:	325 °C
Sheath gas flow:	1 L/min
Capillary + ion:	3500 V
Nozzle voltage:	0 V
Capillary – ion:	2500 V
Nozzle voltage:	1500 V

MS/MS Scans for positive ions

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window	Polarity
Aminocarb	❑	209	Unit	137	Unit	120	20	3.128	1	Positive
Imazapyr	❑	262	Unit	217	Unit	160	15	3.959	1	Positive
Thiabendazole	❑	202	Unit	131	Unit	120	30	4.072	1	Positive
Dimethoate	❑	230	Unit	171	Unit	80	10	5.064	1	Positive
Imazalil	❑	297	Unit	159	Unit	160	20	5.918	1	Positive
Metoxuron	❑	229.1	Unit	72.1	Unit	93	14	5.992	1	Positive
Carbofuran	❑	222	Unit	123	Unit	120	15	7.019	1	Positive
Atrazine	❑	216	Unit	132	Unit	120	20	7.437	1	Positive
Metosulam	❑	418	Unit	175	Unit	144	26	7.472	1	Positive
Metazachlor	❑	278.1	Unit	134.1	Unit	75	18	8.038	1	Positive
Molinate	❑	188.1	Unit	55.1	Unit	78	22	9.113	1	Positive
Malathion	❑	331	Unit	99	Unit	80	10	9.615	1	Positive
Pyraclostrobin	❑	388	Unit	163	Unit	120	20	10.679	1	Positive
Diazinon	❑	305	Unit	153	Unit	160	20	10.776	1	Positive

MS/MS Scans for negative ions

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window	Polarity
Bentazon	❑	239.1	Unit	132	Unit	80	32	6.572	1	Negative
2,4,5-T	❑	252.9	Unit	194.8	Unit	76	9	8.047	1	Negative
Silvex	❑	266.9	Unit	194.9	Unit	90	5	8.805	1	Negative
Acifluorfen	❑	360	Unit	315.9	Unit	78	5	9.650	1	Negative
Dinoseb	❑	239.1	Unit	207	Unit	154	21	10.503	1	Negative
Hexaflumuron	❑	459	Unit	438.9	Unit	102	5	10.877	1	Negative

Appendix II

LC/MS/MS Conditions for 100-Pesticide Methods

Agilent 1200 Series LC Parameters

Column:		Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 50 mm, 1.8 µm Agilent p/n 959741-902
Column temperature:		35 °C
Injection volume:		1.0 µL
Autosampler temperature:		6 °C
Needle wash:		Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:		A = 0.1% formic acid in water B = 0.1% formic acid in 95:5 acetonitrile:water
Flow rate:		0.6 mL/min
Gradient	Time	%B
	0	10
	10	70B
	15	90B
Stop time	20	10B
Post time	5	

Note that example transitions, fragmentor voltages, and collision energies for this method are shown in Figure 7.

Jet Stream Conditions

Drying gas temperature:	325 °C
Drying gas flow (nitrogen):	6 L/min
Nebulizer gas pressure (nitrogen):	35 psig
Capillary voltage:	4000 V
Sheath gas temperature:	400 °C
Sheath gas flow:	12 L/min
Nozzle voltage:	Off

Agilent 6460A QQQ settings

MS1 and MS2 resolution:	Unit
Time Filtering:	Peak width = 0.03 min
Dynamic MRM transitions:	200
Constant cycle time:	373 ms
Delta EMV:	400 V

Appendix III

LC/MS/MS Conditions for 300-Pesticide Methods using the Agilent 1200 Series SL

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	35 °C
Injection volume:	1.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.5 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
18	No change	600	95
20	No change	600	95
20.01	No change	600	6
Stop time	20 min	10%B	
Post time	5 min		

Jet Stream Conditions

Drying gas temperature:	325 °C
Drying gas flow (nitrogen):	6 L/min
Nebulizer gas pressure (nitrogen):	35 psig
Capillary voltage:	4000 V
Sheath gas temperature:	400 °C
Sheath gas flow:	12 L/min
Nozzle voltage:	Off

Ten representative MS/MS Transitions from 300-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time
Promecarb	❑	208.1	Unit	151	Unit	80	5	11.635
Promecarb	❑	208.1	Unit	109	Unit	80	10	11.635
Flurtamone	❑	334.1	Unit	303	Unit	120	20	11.644
Flurtamone	❑	334.1	Unit	247	Unit	120	30	11.644
Isoxaflutole	❑	377.1	Unit	360.1	Unit	100	5	11.669
Isoxaflutole	❑	360.1	Unit	251	Unit	120	10	11.669
Dimethenamide	❑	276.1	Unit	244	Unit	120	10	11.683
Dimethenamide	❑	276.1	Unit	168	Unit	120	15	11.683
Diethofencarb	❑	268.2	Unit	226	Unit	80	5	11.706
Diethofencarb	❑	268.2	Unit	152	Unit	80	20	11.706

Appendix IV

LC/MS/MS Conditions for 300-Pesticide Methods using the Agilent 1290 Infinity LC

Agilent 1290 LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 150 mm, 1.8 µm RRHD 1200 Series bar columns Agilent p/n 959759-902
Column temperature:	60 °C
Injection volume:	35 µL (stacked injection, 5 µL sample + 30 µL H_2O
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25 + 0.01% formic acid), 10 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = MeOH w/5 mM ammonium formate + 0.01% formic acid
LC flow rate:	0.5 mL/min
LC gradient:	6% B (T = 0) to 98% B (T = 15 min), hold 3 min

MS Parameters

Sheath gas flow:	11 L/min
Sheath gas heater:	375 °C
Charging Electrode:	300 V (pos ion mode)
Capillary voltage:	–4 kV (pos ion mode)
Nebulizer pressure:	35 psig
Drying gas temperature:	325 °C
Drying gas flow:	8 L/min

Ten representative MS/MS Scan Segments from 300-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time
Chloridazon	❑	222	Unit	104	Unit	120	25	5.841
Chloridazon	❑	222	Unit	92	Unit	120	30	5.841
Aminocarb	❑	209.1	Unit	152.1	Unit	120	10	5.841
Aminocarb	❑	209.1	Unit	137	Unit	120	20	5.841
Fluroxypyr	❑	255	Unit	209	Unit	80	10	5.845
Fluroxypyr	❑	255	Unit	181	Unit	80	15	5.845
Acetamiprid	❑	223.1	Unit	126	Unit	80	15	5.858
Acetamiprid	❑	223.1	Unit	56	Unit	80	15	5.858
Vamidothion	❑	288	Unit	146	Unit	80	10	5.996
Vamidothion	❑	288	Unit	118	Unit	80	20	5.996

Appendix V

LC/MS/MS Conditions for Pesticides in Spinach using QuECHERS Extraction

Agilent 1200 Series HPLC conditions

Column:		Agilent ZORBAX Eclipse Plus Phenyl-hexyl, 150 mm × 3 mm, 3.5 µm Agilent p/n 959963-312	
Column temperature:		30 °C	
Injection volume:		10 µL	
Mobile phase:		A = 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H_2O B = 5 mM ammonium acetate, pH 5.0 in ACN	
Needle wash:		1:1:1:1 ACN/MeOH/IPA/H_2O w/0.2% FA	
Gradient:	Time (min)		Flow rate
	(min)	% B	(mL/min)
	0	20	0.3
	0.5	20	0.3
	8.0	100	0.3
	10.0	100	0.3
	10.1	20	0.5
	12.0	100	0.5
Stop time:	13.0 min		
Post run:	4 min		
Total cycle time:	17 min		

Agilent 6410 MS conditions

Positive mode	
Gas temperature:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 psi
Capillary:	4000 V

Appendix VI

LC/MS/MS Conditions for 165-Pesticide Methods using the Agilent 1200 Series Infinity SL

Agilent 1200 Series Infinity SL LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm 1.8 µm Agilent p/n 959764-902
Column temperature:	35 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H2O 75:25) 5 s
Mobile phase:	A = H2O w/5mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in methanol

Gradient — Pump Time Table

Time (min)	Solv ratio B (%)
0.00	10
1.00	10
18.00	100
20.00	100
20.10	10
25.00	10

Jet Stream Conditions

Spray Chamber Conditions

Gas temperature:	200 °C
Dry gas :	6 L/min
Nebulizer:	35 psi
Sheath gas temperature:	250 °C
Sheath gas flow:	12 L/min
Positive cap voltage:	4000 V
Nozzle voltage:	300 V

Ten Representative MS/MS Transitions from 167-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window
Ethiofencarb-sulfon	❑	275	Unit	201	Unit	80	0	6.89	1
Ethiofencarb-sulfon	❑	275	Unit	107	Unit	80	10	6.89	1
Clothianidin	❑	250	Unit	169	Unit	90	5	7.064	1
Clothianidin	❑	250	Unit	132	Unit	90	15	7.064	1
Imidacloprid	❑	256.1	Unit	209	Unit	80	15	7.071	1
Imidacloprid	❑	256.1	Unit	175.1	Unit	80	20	7.071	1
Ethiofencarb-sulfoxid	❑	242	Unit	185	Unit	80	15	7.153	1
Ethiofencarb-sulfoxid	❑	242	Unit	107	Unit	80	5	7.153	1
Monalide	❑	257.1	Unit	200.1	Unit	105	4	7.165	1
Monalide	❑	257.1	Unit	137.1	Unit	105	8	7.165	1

Appendix VII

LC/MS/MS Conditions for 224-Pesticide Methods using the Agilent 1200 Series SL

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	55 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.3 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
14	No change	600	95
17	No change	600	95

Stop time 17 min
Post time 3 min

Jet Stream Conditions

Drying gas temperature:	225 °C
Drying gas flow (nitrogen):	10 L/min
Nebulizer gas pressure (nitrogen):	25 psig
Capillary voltage:	4500 V
Sheath gas temperature:	350 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	500 V

Ten representative MS/MS Transitions from 224-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window
Buprofezin	❑	306.2	Unit	201.1	Unit	115	4	14.321	1
Buprofezin	❑	306.2	Unit	57.2	Unit	115	16	14.321	1
Sulprofos	❑	323	Unit	247.1	Unit	130	5	14.327	1
Sulprofos	❑	323	Unit	219	Unit	130	12	14.327	1
Eprinomectin B1a	❑	914.6	Unit	468.3	Unit	150	5	14.372	1
Eprinomectin B1a	❑	914.6	Unit	330.3	Unit	150	10	14.372	1
Chlorfluazuron	❑	540	Unit	383	Unit	115	16	14.402	1
Chlorfluazuron	❑	540	Unit	158	Unit	115	16	14.402	1
Fenpyroximat	❑	422.2	Unit	366.2	Unit	130	15	14.428	1
Fenpyroximat	❑	422.2	Unit	135	Unit	130	40	14.428	1

Appendix VIII

LC/MS/MS Conditions for 224-Pesticide Methods using the Agilent 1290 Infinity LC

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	55 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.6 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
7	No change	600	95
10	No change	600	95

Stop time 10 min
Post time 3 min

Jet Stream Conditions

Drying gas temperature:	225 °C
Drying gas flow (nitrogen):	10 L/min
Nebulizer gas pressure (nitrogen):	25 psig
Capillary voltage:	4500 V
Sheath gas temperature:	350 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	500 V

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Printed in the USA
October 13, 2009
5990-4253EN

Agilent Technologies

Analysis of Pesticide Residues in Apple by GC/MS using Agilent SampliQ QuEChERS Kits for Pre-injection Cleanup

Application Note

Food Safety

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Abstract

The QuEChERS method, which stands for Quick, Easy, Cheap, Effective, Rugged and Safe was developed in 2003 by scientists at the USDA. This method was created to easily clean up and prepare food samples for multi-class, multi-residue pesticide analysis. This application note describes the use of the original, non-buffered QuEChERS method to prepare apple samples for residue analysis by gas chromatography/mass spectrometry (GC/MS). Fifteen pesticides of different classes were studied. The experiments were done using Agilent QuEChERS extraction kits for 10-g samples and dispersive kits for 1-mL sample volumes. The analysis was done by GC/MS using selective ion monitoring (SIM) mode. The limit of quantitation for all the pesticides studied was 10 ng/g in apple using this method. At 200 ng/g, the recoveries ranged from 89% to 102%, and at 10 ng/g, the recoveries ranged from 72% to103%. The relative standard deviations associated with these recoveries were less than 11% in all cases.

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Introduction

Pesticides, which include herbicides, fungicides and other pest-control chemicals, have long been a part of agriculture. While their use can be widespread and beneficial, pesticides can also be harmful to both humans and animals. Because of this, pesticide handling is monitored by several agencies, including the United States Environmental Protection Agency (EPA) [1]. Of concern to the general population is the maximum residue levels (MRL) for pesticide in food items.

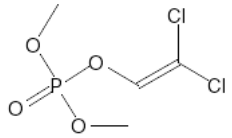
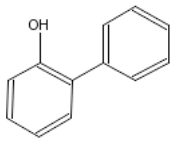
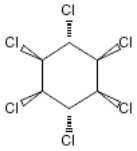
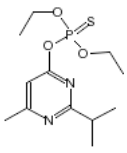
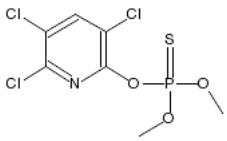
In 2003, scientists at the USDA developed a method for the quick and easy cleanup of food samples for pesticide analysis.[2] This method was given the acronym QuEChERS, which stands for Quick, Easy, Cheap, Effective, Rugged and Safe. The method has since been modified for other analyses. The method for this analysis incorporates a simple acetonitrile/water extraction facilitated by the addition of $MgSO_4$, which salts out water from the sample and includes a liquid/liquid extraction with these two solvents. The extraction step is followed by a dispersive solid phase extraction that combines both a primary secondary amine (PSA) and anhydrous $MgSO_4$ to remove fatty acids and reduce the remaining water in the extract respectively. See the Agilent SampliQ QuEChERs Kit brochure (publication 5990-3562EN) or www.agilent.com/chem/quechers for more information about QuEChERS and suggestions for analyses of different fruits and vegetables.

Experimental

Reagents and Chemicals

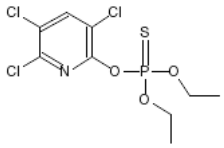
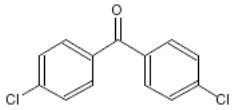
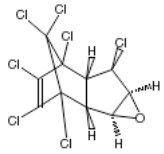
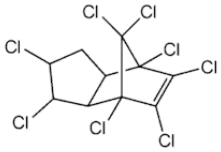
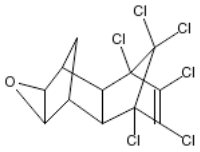
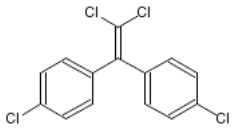
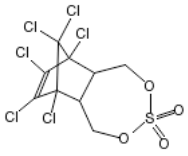
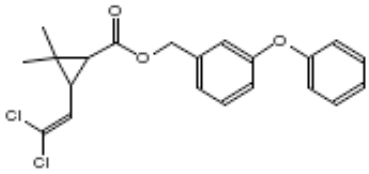
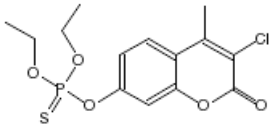
Water (EMD Chemicals, Gibbstown, NJ) and acetonitrile (Burdick and Jackson, Muskegan, MI) were HPLC grade. The pesticides were all analytical grade. Dichlorvos (98.9%), and diazinone (99.5%) were purchased from Ultra Scientific (Kingstown, RI). Coumaphos was purchased from Honeywell Riedel De Haen (Seelze, Germany). All other pesticides were purchased from Chem Service (West Chester, PA). See Table 1 for the chemical and regulatory information for the pesticides used in this study. [3-5] The internal standard, triphenyl phosphate (TPP) was purchased from Sigma-Aldrich (St. Louis, MO).

Table 1. Pesticide Chemical and Regulatory Information.

Analyte	Structure	Category	Log P	pKa	MRLs in apple
Dichlorvos		Organophosphate	1.9	NA	10
σ-phenylphenol		Phenol	3.18	9.4	20
Lindane		Organochlorine	3.69	NA	10
Diazinone		Organophosphate	3.69	2.6	100
Chlorpyrifosmethyl		Organophosphate	4.00	NA	500

(Continued)

Table 1. Pesticide Chemical and Regulatory Information.

Analyte	Structure	Category	Log P	pKa	MRLs in apple
Chlorpyrifos		Organophosphate	4.55	NA	100
dichlorobenzophenone		Organochlorine	4.44	NA	500
Heptachlor-epoxide		Organochlorine	5.83	NA	10
Chlordane		Cyclodiene organochlorine	2.78	NA	20
Dieldrin		Chlorinated hydrocarbon	3.7	NA	10
DDE		organochlorine	6.55	NA	50
Endosulfan sulfate		Organochlorine	3.13	NA	50
Permethrins		Pyrethroid	6.1	NA	50
Coumaphos		Organophosphate	3.86	NA	100

Solutions

Individual stock solutions of the pesticides (2 mg/mL) were prepared in methanol and stored at -20 °C. From these, the High-QC solution which was 20 µg/mL for all the pesticides was prepared in 50:50 acetonitrile/water. The internal standard was 20 µg/mL triphenyl phosphate in acetonitrile.

The High-QC solution was used to prepare all other spiking solutions. A mid range spiking solution (Mid-QC) with a concentration of 5 µg/mL was prepared in 50:50 acetonitrile/water. A low range spiking solution (Low-QC) with a concentration of 1 µg/mL was prepared in 50:50 acetonitrile/water.

Calibration Curve

A 2.5 µg/mL standard working solution was prepared using the High-QC solution. A six-point calibration curve (10, 20, 50, 100, 250 and 400 ng/mL) was created by adding the appropriate volume of this 2.5 µg/mL solution to the matrix blank extract. Internal standard solution was added to have a final concentration of 100 ng/mL.

Sample Preparation

Certified organic, pesticide-free red delicious apples were purchased at a local grocery. Approximately 3 pounds of apples were diced into approximately 1-cm cubes. The seeds were discarded, but the skin was included. The cubes were placed in a plastic bag and frozen at -20 °C overnight. For the first 5 hours in the freezer, the samples were massaged to prevent them from freezing together. When ready to perform the extraction, the amount of sample required was removed from the freezer. A coffee grinder (Mr. Coffee 2.3-oz coffee grinder, Shelton, CT) was used to comminute the sample. If necessary, dry ice may be added to aid this. The sample was checked to ensure that there were no large pieces or lumps remaining prior to extraction. [6].

QuEChERS Cleanup

Step 1, Extraction

The SampliQ Original QuEChERS Method (non-buffered) Extraction Kit, for use with 10g samples (p/n 5982-5550) was used for the extraction step. A 10-g (±0.05g) amount of the homogenized apple sample was placed in a 50-mL centrifuge tube. 100 µL of the appropriate spiking solution was added. The sample was vortexed (VWR vortex mixer model K-550-G, West Chester, PA) for 1 min, then 10 mL of acetonitrile were added. The sample was shaken vigorously for 1 min, then 4 g of $MgSO_4$ and 1 g of NaCl from the extraction salt packet (p/n 5982-5550) were added. The sample was vortexed for 1 min. A 100-µL amount of internal standard solution was added, then the sample was centrifuged (Eppendorf 5810R 15 amp, Westbury, NY) for 5 min at 5000 rpm. See Figure 1.

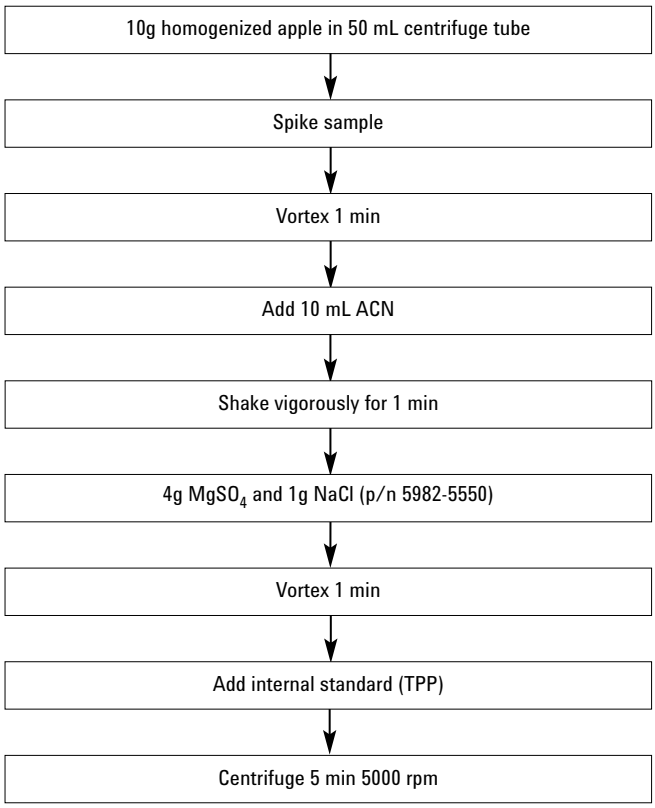


Figure 1. Extraction using SampliQ QuEChERS kit.

Step 2 Dispersive SPE cleanup

The SampliQ QuEChERS Dispersive Kit for General Fruits and Vegetables was used for dispersive SPE cleanup (p/n 5982-5022). This kit removes polar organic acids, some sugars and lipids. One milliliter of the resultant solution was transferred to a 2-mL centrifuge tube containing 50 mg of PSA and 1 50 mg of $MgSO_4$. This was vortexed for 30 sec, then centrifuged for 5 min (VWR micro-centrifuge model 235 B, West Chester, PA). A 0.5-mL amount of the resulting extract was transferred to a sample vial to be analyzed by GC/MS. See Figure 2.

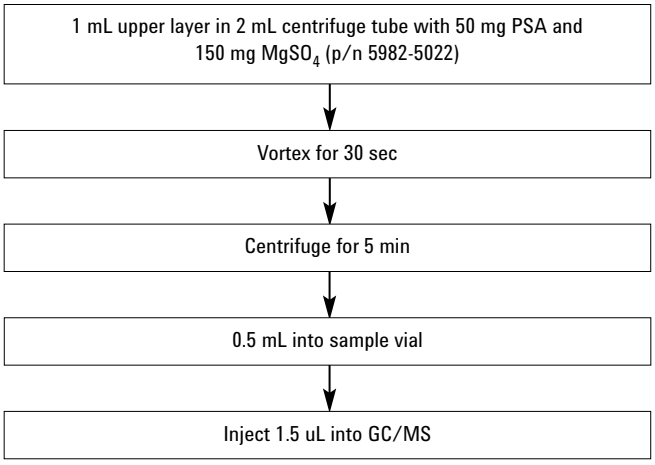


Figure 2. Dispersive SPE using SampliQ QuEChERS kit.

Instrument Conditions

Samples were analyzed using an Agilent 7890A GC system with an Agilent 5975C Series GC/MSD (Agilent Technologies Inc., Santa Clara, CA). An Agilent GC/MS method for pesticide analysis was used with some minor modifications. (7) See Tables 2 and 3 for instrument conditions.

Table 2. GCMS instrument conditions

GC conditions	
Injection source	Manual
Inlet	Splitless
Column	Agilent J&W HP-5ms Ultra Inert, 30 m × 0.250 mm, 0.25 µm film (PN: 190915-433UI)
Carrier Gas	Helium in constant flow mode
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 (7 min)
Injection volume	1 µL
MS Conditions	
Tune File	Atune.u
Mode:	SIM
Source temperature	230 °C
Quad temperature	150 °C
Transfer line temperature	280 °C
Solvent Delay	4.00 minutes
Multiplier Voltage	Autotune voltage

Table 3. Selective Ion Monitoring (SIM) mode conditions.

Peak	Analyte	RT (min)	SIM target	SIM qualifier	Collection window (min)
1	Dichlorvos	6.9	185.00	109.50	4.0–9.0
2	σ-phenylphenol	10.4	170.10	169.10	9.0–14.0
3	Lindane	15.7	180.90	182.90	14.0-16.0
4	Diazinone	16.6	137.10	179.10	16.0–18.0
5	Methyl-chlorpyrifos	18.9	285.90	287.90	18.0–21.0
6	Chlorpyrifos	21.5	196.90	–	21.0–22.0
7	Dichlorobenzophenone	21.5	139.00	–	21.0–22.0
8	Heptachlor-epoxide	23.0	352.90	354.90	22.0–23.6
9	γ-chlordane	24.0	372.90	374.90	23.6–24.3
10	α-chlordane	24.6	372.90	374.90	24.3–25.0
11	Dieldrin	25.4	79.10	–	25.0–27.0
12	DDE	25.5	246.00	317.90	25.0–27.0
13	Endosulfan Sulfate	27.9	271.80	273.80	27.0–28.0
I.S.	TPP	28.6	325.1	326.1	28.0–29.5
14	Permethrin	32.1	183.10	–	29.5–38.0
15	Coumaphos	32.2	96.90	109.00	29.5–38.0

Results and Discussion

As shown in Figure 3b, the apple matrix blank sample had only a few peaks spread across the experimental collection times for the pesticides using the chosen GC/MS method. In the spiked sample (3a), all compounds except coumaphos were free of interferences and gave good linearity as shown in Table 4.

The peak corresponding to coumaphos was difficult to integrate in some samples due to an irregular baseline, which is a possible reason for poor linearity. The QuEChERS method of sample preparation was proven to be quick, easy and effective for this type of analysis. When using the QuEChERS method, samples may still have some impurities that can show up in the chromatograms. In order to achieve the best sensitivity for the analytes of interest, SIM mode was used. Sensitivity for the pesticides was greatly increased by selecting ions corresponding to the analytes of interest to be monitored during different segments of the experiment. In most cases, the highest abundance ion for each analyte was chosen to give the best sensitivity. However, in some cases where selectivity was compromised by this choice, another less abundant ion was used for quantitation. For most of the analytes, a second qualifier ion was also used. The selected ions for each compound and the time segments during which they were monitored are given in Table 3.

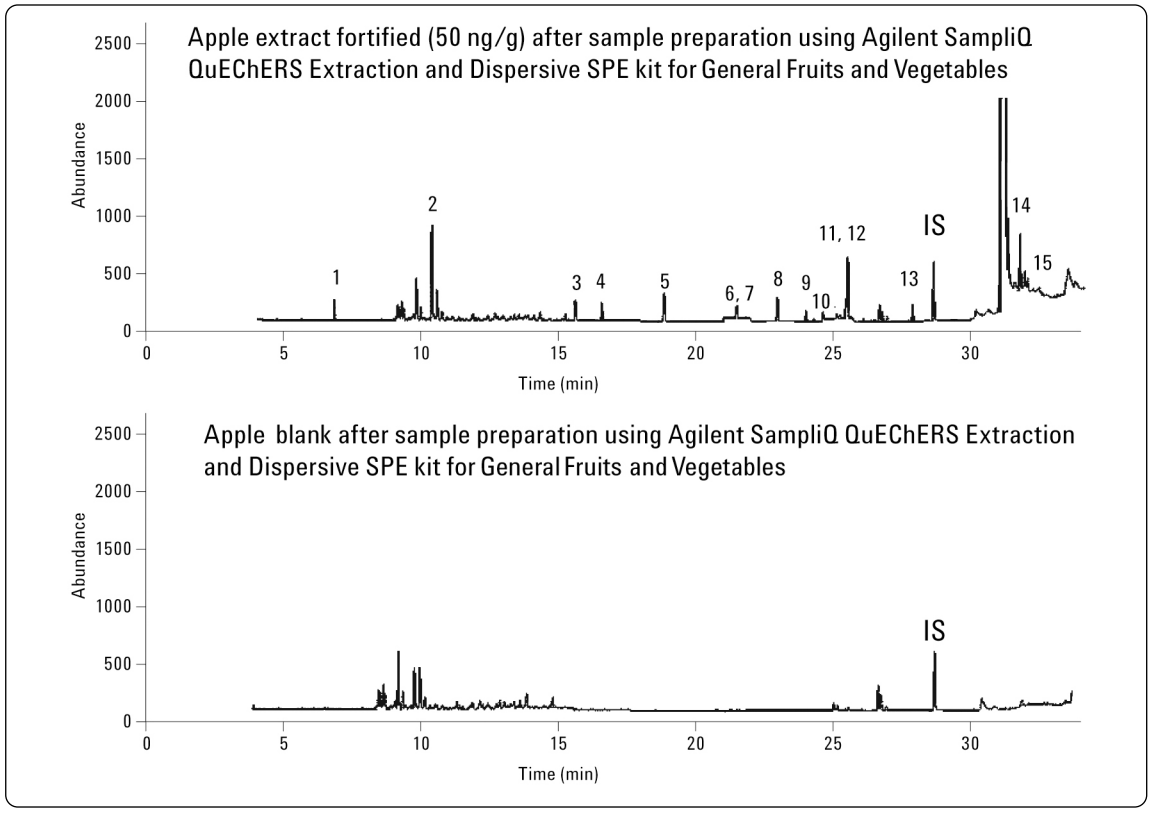


Figure 3. GC/MS chromatograms of apple extract. Peak identifications in Table 3.

Table 4. Regression data for pesticides

Pesticide	Regression Equation	R^2
Dichlorvos	y = 0.1243x - 0.01141	0.9965
σ-phenylphenol	y = 0.6885x - 0.03763	0.9965
Lindane	y = 0.1719x - 0.02280	0.9967
Diazinone	y = 0.1811x - 0.02608	0.9945
Methyl-chlorpyrifos	y = 0.3242x - 0.05026	0.9943
Chlorpyrifos	y = 0.1459x - 0.02455	0.9916
Dichlorobenzophenone	y = 0.1573x - 0.01840	0.9937
Heptachlor-epoxide	y = 0.1995x - 0.02828	0.9906
γ-chlordane	y = 0.07058x - 0.005587	0.9917
α-chlordane	y = 0.05601x - 0.001840	0.9927
Dieldrin	y = 0.2091x - 0.02544	0.9923
DDE	y = 0.4609x - 0.05950	0.9901
Endosulfan Sulfate	y = 0.1262x - 0.01675	0.9897
Permethrin	y = 0.1327x + 0.03232	0.9889
Coumaphos	y = 0.06985x + 0.01864	0.9543

Table 5 shows the recovery and reproducibility for each pesticide in the apple matrix spiked at three different concentrations (200 ng/g, 50 ng/g and 10 ng/g).

Table 5. Recovery and reproducibility of pesticides in apple using the original QuEChERS method (n=4).

Pesticide	High-QC 200ng/g		Mid-QC 50ng/g		Low-QC 10ng/g	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Dichlorvos	99.4	2.8	96.7	10.8	102.8	5.0
σ-phenylphenol	89.5	6.3	79.6	6.8	92.0	6.1
Lindane	92.6	4.2	88.5	9.7	97.9	2.0
Diazinone	102.1	4.4	98.8	5.5	90.5	9.1
Methyl-chlorpyrifos	98.5	3.1	90.0	4.3	88.7	7.1
Chlorpyrifos	100.2	1.2	95.6	4.0	93.5	6.5
Dichlorobenzophenone	99.4	0.6	89.1	6.4	90.3	5.0
Heptachlor-epoxide	95.4	3.9	85.6	5.4	87.0	3.2
γ-chlordane	95.9	2.0	90.0	6.8	92.3	3.5
α-chlordane	93.5	2.6	85.8	6.9	95.5	4.7
Dieldrin	99.9	1.8	93.6	5.3	99.4	4.2
DDE	92.7	1.9	87.1	5.7	94.5	4.2
Endosulfan Sulfate	99.5	2.3	90.8	2.8	97.8	2.3
Permethrin	97.6	2.1	93.0	3.4	100.7	4.8
Coumaphos	96.6	3.0	79.6	3.5	72.5	4.5

Conclusions

The results show that Agilent SampliQ QuEChERS kits offer an effective method of purification for pesticides in an apple matrix. The impurities remaining after the extraction and dispersive steps were minimal. When used in conjunction with the power of GC/MS in the SIM mode, this method of sample preparation offers a quick, easy and complete solution to quantitate pesticides in fruit matrices.

Acknowledgements

The authors would like to acknowledge Limian Zhao and David Schultz of Agilent Technologies for help with the GC/MS software and in acquiring the pesticide solutions. Also, thank you to Bill Davis and Abby Folk of Agilent Technologies for help with the GC/MS hardware configuration.

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Printed in the USA
October 23, 2009
5990-4468EN

Agilent Technologies

Determination of pesticides in baby food by UHPLC/MS/MS using the Agilent 1290 Infinity LC system and the Agilent 6460 triple quadrupole LC/MS

Application Note

Food

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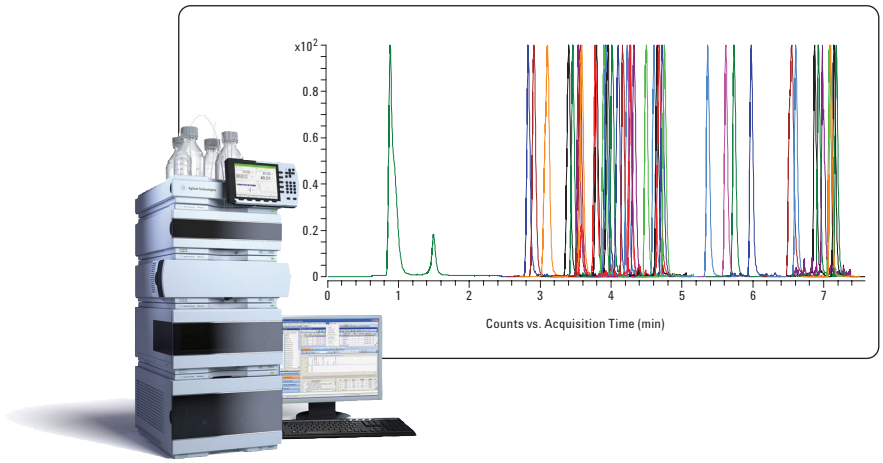

Abstract

The qualitative and quantitative analysis of pesticides at trace levels in baby food matrices using UHPLC and triple quadrupole MS is demonstrated. Sample preparation is performed using an Agilent SampliQ QuEChERS kit for extraction and dispersive SPE. The extracts are analyzed by LC/MS/MS on an Agilent 1290 Infinity LC system coupled to an Agilent 6460 triple quadrupole LC/MS using Dynamic MRM. The method and extraction performance were evaluated in terms of repeatability, linearity and sensitivity. Moreover the influence of the additional dispersive SPE cleanup was investigated. Detection limits were between 500 ng/kg and 10 ng/kg (ppt), which is much lower than the maximum residue level (MRL) of 10 µg/kg (ppb) imposed by the European Union.

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Introduction

Due to diversity of pesticides used in food protection and the globalization of the food industry, the monitoring of programs that cover a large number of pesticides is important. The application of UHPLC systems combined with the new generation triple quadrupole mass spectrometers facilitate the analysis of pesticides in challenging matrices such as food samples. As a result of the high sensitivity and the high scan rate capabilities of the Agilent 6460A triple quadrupole mass spectrometer, the simultaneous qualitative and quantitative multiresidue analysis of a large set of pesticides at trace levels can be performed.

The high sensitivity is essential for the analysis of these compounds in derived products, where the concentrations will be a fraction of the concentration in the raw material. In this respect, baby food is a challenging matrix. This application notes describes the quantitative analysis of 40 pesticides in baby food at levels below the maximum residue level (MRL) (10 µg/kg fruit or vegetable) specified in EC Regulation 396/2005 which was implemented in September 2008.[1] A QuEChERS extraction and dispersive SPE method was applied to isolate the pesticides from the baby food matrix. An Agilent 1290 Infinity LC was used to perform the separation on a Rapid Resolution High Definition (RRHD) ZORBAX Eclipse Plus column. The total analysis time was 10 min (including 1.5 min re-equilibration) and detection limits ranged from 10 to 500 ng/kg using Dynamic MRM and two transitions (quantifier and qualifier) per compound. Three different baby food compositions were analyzed. Extraction performance criteria such as repeatability, recovery (accuracy) and sensitivity were investigated.

Experimental

Instrumentation

An Agilent 1290 Infinity LC system and an Agilent 6460A triple quadrupole LC/MS with Agilent jet stream technology were used. The 1290 Infinity LC system was configured as follows:

Part number	Description
G4220A	Agilent 1290 Infinity Binary Pump with integrated vacuum degasser
G4226A	Agilent 1290 Infinity Autosampler
G1316C	Agilent 1290 Infinity Thermostatted Column Compartment
G4212A	Agilent 1290 Infinity Diode Array Detector

Method parameters:		
Column	Agilent ZORBAX Eclipse Plus RRHD C18, 150 mm L × 2.1 mm id, 1.8 µm d_p	
Mobile phase	A = 0.05% (w/v) ammonium formate + 0.01% (v/v) formic acid in water B = Methanol	
Flow rate	0.5 mL/min	
Gradient	Min	% B
	0 to 5	10 to 65
	5 to 6.5	65 to 95
	6.5 to 8.5	95
	8.5 to 10	10
Temperature	45 °C	
Injection	2 µL, with needle wash (flushport, 5 s, water/methanol 1/1)	
Detection	MS/MS	
Ionization	Electrospray, positive ionization	
Jet Stream parameters		
Drying gas temperature	250 °C	
Drying gas flow	10 L/min	
Nebulizer pressure	30 psig	
Sheath gas temperature	340 °C	
Sheath gas flow	11 L/min	
Capillary voltage	4500 V	
Nozzle voltage	500 V	
Acquisition		
Dynamic MRM	See Table 1	
Delta EMV	50	
Cycle time	200 ms	

Compound		Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor (V)	Collision energy (V)	Retention time (min)	Retention time window (min)
Cyromazine	Q	167.1	85.1	100	25	1.20	1.5
Cyromazine	q	167.1	125.1	100	25	1.20	1.5
Flonicamid	Q	230.1	203.1	80	15	2.85	0.8
Flonicamid	q	230.1	174.1	80	15	2.85	0.8
Thiamethoxam	Q	292.2	211.0	85	4	2.92	0.8
Thiamethoxam	q	292.2	181.0	85	16	2.92	0.8
Monocrotofos	Q	224.1	127.0	85	10	3.11	0.8
Monocrotofos	q	224.1	193.0	85	5	3.11	0.8
Dicrotofos	Q	238.1	112.1	90	5	3.41	0.8
Dicrotofos	q	238.1	127.0	90	15	3.41	0.8
Ethiofencarb-sulfone	Q	258.1	107.1	80	10	3.47	0.8
Ethiofencarb-sulfone	q	258.1	201.1	80	10	3.47	0.8
Imidacloprid	Q	256.1	175.1	90	20	3.55	0.8
Imidacloprid	q	256.1	209.0	90	15	3.55	0.8
Clothianidin	Q	250.0	169.1	90	7	3.58	0.8
Clothianidin	q	250.0	132.1	90	15	3.58	0.8
Ethiofencarb-sulfoxide	Q	242.1	107.1	80	15	3.60	0.8
Ethiofencarb-sulfoxide	q	242.1	185.1	80	15	3.60	0.8
Methiocarb-sulfoxide	Q	242.0	185.1	80	10	3.79	0.8
Methiocarb-sulfoxide	q	242.0	170.0	90	15	3.79	0.8
Thiofanox-sulfone	Q	251.1	57.1	100	15	3.80	0.8
Thiofanox-sulfone	q	251.1	76.1	100	15	3.80	0.8
Trichlorfon	Q	256.9	109.0	100	15	3.92	0.8
Trichlorfon	q	256.9	221.0	100	15	3.92	0.8
Vamidothion	Q	288.1	146.1	80	10	3.94	0.8
Vamidothion	q	288.1	118.1	80	20	3.94	0.8
Acetamiprid	Q	223.1	126.0	100	15	3.94	0.8
Acetamiprid	q	223.1	56.0	100	15	3.94	0.8
Carbofuran-3-OH	Q	238.1	163.1	85	5	3.96	0.8
Carbofuran-3-OH	q	238.1	181.1	85	5	3.96	0.8
Fenthion-oxon-sulfoxide	Q	279.0	104.1	125	30	4.03	0.8
Fenthion-oxon-sulfoxide	q	279.0	121.1	125	30	4.03	0.8
Carbendazim	Q	192.1	160.1	100	15	4.11	0.8
Carbendazim	q	192.1	132.1	100	25	4.11	0.8
Fenthion-oxon-sulfone	Q	295.0	217.1	125	25	4.18	0.8
Fenthion-oxon-sulfone	q	295.0	104.1	125	25	4.18	0.8
Cymoxanil	Q	199.2	128.0	65	5	4.24	0.8
Cymoxanil	q	199.2	111.0	100	20	4.24	0.8
Oxycarboxin	Q	268.1	175.0	100	10	4.27	0.8
Oxycarboxin	q	268.1	146.9	100	25	4.27	0.8
Chlothiamid	Q	205.9	189.0	85	20	4.29	0.8
Chlothiamid	q	205.9	172.0	85	20	4.29	0.8

Table 1
Dynamic MRM data acquisition parameters for the compounds under investigation. Q = quantifier, q = qualifier.

Compound		Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor (V)	Collision energy (V)	Retention time (min)	Retention time window (min)
Thiacloprid	Q	253.1	126.0	100	20	4.34	0.8
Thiacloprid	q	253.1	186.0	100	10	4.34	0.8
Florasulam	Q	360.0	129.1	100	20	4.51	0.8
Florasulam	q	360.0	191.9	100	10	4.51	0.8
Tricyclazole	Q	190.1	163.2	100	20	4.62	0.8
Tricyclazole	q	190.1	136.2	100	25	4.62	0.8
Butocarboxim	Q	213.1	75.1	110	15	4.66	0.8
Butocarboxim	q	213.1	156.1	110	5	4.66	0.8
Thiabendazole	Q	202.0	175.0	120	25	4.69	0.8
Thiabendazole	q	202.0	131.0	120	35	4.69	0.8
Aldicarb	Q	208.0	116.0	70	0	4.73	0.8
Aldicarb	q	208.0	89.1	70	5	4.73	0.8
DMSA	Q	201.0	92.1	85	15	4.76	0.8
DMSA	q	201.0	137.1	85	10	4.76	0.8
Propoxur	Q	210.1	111.1	50	10	5.36	0.8
Propoxur	q	210.1	93.0	50	20	5.36	0.8
Carbaryl	Q	202.1	145.1	50	2	5.62	0.8
Carbaryl	q	202.1	127.0	50	20	5.62	0.8
Monolinuron	Q	215.2	126.0	100	20	5.75	0.8
Monolinuron	q	215.2	148.1	100	20	5.75	0.8
Fluazifop	Q	328.1	282.1	120	20	5.99	0.8
Fluazifop	q	328.1	254.1	120	20	5.99	0.8
Spiroxamine	Q	298.4	144.2	100	10	6.54	0.8
Spiroxamine	q	298.4	100.2	100	10	6.54	0.8
Pyrimethanil	Q	200.1	107.1	100	25	6.61	0.8
Pyrimethanil	q	200.1	82.0	100	30	6.61	0.8
Fenhexamid	Q	302.1	97.0	120	10	6.88	0.8
Fenhexamid	q	302.1	142.1	100	5	6.88	0.8
Fenbuconazole	Q	337.2	125.0	120	15	6.94	0.8
Fenbuconazole	q	337.2	194.1	120	15	6.94	0.8
Iprodion	Q	330.0	244.9	110	10	6.98	0.8
Iprodion	q	330.0	287.9	110	5	6.98	0.8
Kresoxim-methyl	Q	314.2	116.0	70	10	7.08	0.8
Kresoxim-methyl	q	314.2	222.0	70	10	7.08	0.8
Penconazole	Q	284.1	69.9	85	15	7.11	0.8
Penconazole	q	284.1	158.8	85	30	7.11	0.8
TPP	Q	327.1	77.0	180	40	7.14	0.8
TPP	q	327.1	151.9	180	40	7.14	0.8
Pyraclostrobin	Q	388.2	194.1	100	10	7.18	0.8
Pyraclostrobin	q	388.2	296.2	100	10	7.18	0.8

Table 1
Dynamic MRM data acquisition parameters for the compounds under investigation. Q = quantifier, q = qualifier. (continued)

Solutions and Samples

Stock solutions of the pesticides were prepared in acetonitrile. These solutions were diluted to the appropriate concentration (range 0.05 ppb to 1 ppm) in 1% v/v acetic acid in acetonitrile. An internal standard solution of triphenylphosphate (TPP, 20 µg/mL) was prepared in the same solvent.

Sample Preparation

Three baby food products were obtained from a local supermarket. According to the labels, the samples were composed of the following ingredients:

- Sample 1: carrots (40%), potatoes (18%), tomatoes (18%), beans (13%), beef (10%)
- Sample 2: water (37%), potatoes (30%), spinach (17%), chicken (10%)
- Sample 3: carrots (54%), potato (23%), water (16%), rice (7%)

The sample preparation was performed using Agilent SampliQ QuEChERS AOAC kits for extraction and dispersive SPE cleanup. The procedure is described below.

Extraction

1. Weigh 15 g of sample into a 50-mL centrifuge tube.
2. Add 100 µL TPP solution.
3. Add spiking solution, if necessary.
4. Vortex for 30 s.
5. Add 15 mL of 1% v/v acetic acid in acetonitrile and the SampliQ AOAC extraction salt (p/n 5982-5755).
6. Cap tubes and shake vigorously by hand for 1 min.
7. Centrifuge at 4,000 rpm for 5 min.
8. Filter 1 mL of sample through a syringe filter (0.2 µm pore size, regenerated cellulose, p/n 5061-3366) and analyze directly (no SPE) or (additional clean-up).

Dispersive SPE

1. Transfer 8 mL from the centrifuged extract into a 15-mL SampliQ AOAC dispersive SPE tube for fatty samples (p/n 5982-5158).
2. Vortex for 30 s.
3. Centrifuge at 13,000 rpm for 2 min.
4. Filter 1 mL through a syringe filter (0.2 µm pore size, regenerated cellulose, p/n 5061-3366) and analyze.

Results and discussion

State-of-the-art LC/MS/MS equipment enables fast multiresidue analysis of pesticides at low levels in complex matrices. The Agilent 1290 Infinity LC provides the necessary power to perform analysis of the 40 selected pesticides within the 10-min total analysis time (run time and equilibration time). A 15 cm column was preferred above a 10 or 5 cm column because of the higher resolving power. This is useful to minimize ion suppression or response enhancement due to matrix effects. Methanol was chosen as an organic modifier because of the significantly improved sensitivity compared to acetonitrile for this analysis.

During the analysis, a total of 82 transitions (2 per solute + 2 for IS) had to be performed. The dynamic MRM function allows MRM transition lists to be built based on a retention time window specified for each analyte. Consequently, the pesticides are only monitored during that elution window in the analytical run. This approach leads to equivalent or better results in terms of sensitivity and quantification (data points) compared to the traditional time segment based methods [2]. With the Dynamic MRM enabled, the maximum number of concurrent MRMs was 32. Using an MRM cycle time of 200 ms, the minimal and maximal transition dwell times were 2.75 and 96.50 ms (values given by MassHunter acquisition software), respectively. The resulting number of data points across the peaks was above 20 for all compounds which is largely sufficient for quantitation purposes.

The performance of the LC/MS/MS method was tested by the analysis of standard solutions. The chromatogram (overlaid MRMs of quantification ions) for a 10 ppb solution is shown in Figure 1. Figures of merit are summarized in Table 2. The injection precision was tested at two concentration levels (1 and 10 ppb). The standard solutions were each injected five consecutive times. The linearity of the method was evaluated between 0.05 and 20 ppb at eight levels (0.05, 0.10, 0.20, 0.50, 1,2,10, and 20 ppb). Each solution was injected once. The lowest level is below the detection limit for some compounds. For these analytes, the calibration curve was started at the limit of detection.

The sensitivity was excellent and all compounds could be analyzed at the sub-ppb level. An example of the ion traces (quantification ion transition and qualifier ion transition) and the corresponding calibration curves for fluazifop

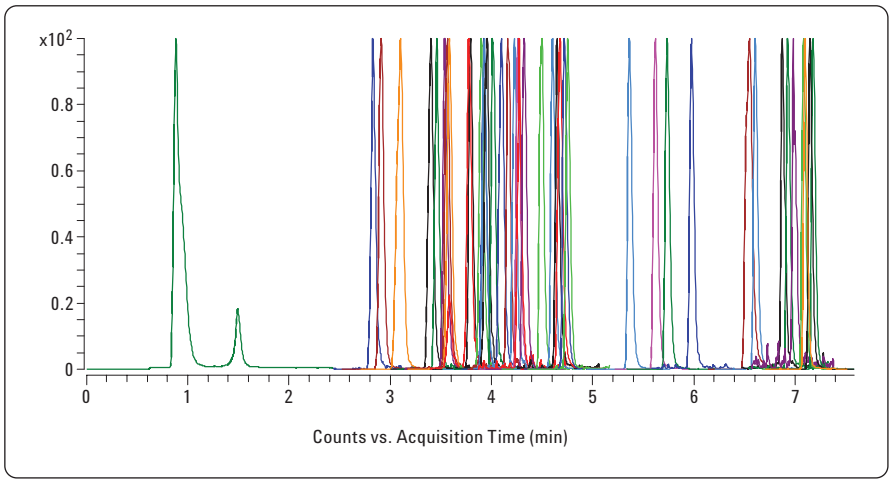

Figure 1
MRM of a 10 ppb standard solution (only quantifier transitions are shown).

Compound	Repeatability of injection (% RSD) 1 ppb	10 ppb	Linearity (R^2)	Detection limit (ppb) Q	q
Acetamiprid	2.20	1.62	0.9999	0.02	0.02
Aldicarb	4.82	2.03	0.9999	0.01	0.02
Butocarboxim	19.93	2.36	0.9996**	0.20	0.50
Carbaryl	1.70	1.73	0.9996	0.01	0.01
Carbendazim	2.93	1.28	0.9997	0.01	0.05
Carbofuran-3-OH	14.68	2.50	0.9996*	0.10	0.10
Chlothiamid	20.64	7.28	0.9979*	0.20	1.00
Clothianidin	7.69	2.14	0.9999*	0.10	0.20
Cymoxanil	7.30	3.88	0.9998*	0.10	0.50
Cyromazine	2.02	1.08	0.9993	<0.50[1]	0.50
Dicrotofos	3.69	1.01	0.9994	0.01	0.02
DMSA	5.13	2.36	0.9996	0.05	0.20
Ethiofencarb-sulfone	2.69	2.25	0.9998	0.05	0.20
Ethiofencarb-sulfoxide	6.24	2.02	0.9991*	0.10	0.10
Fenbuconazole	11.29	1.24	0.9994**	0.20	1.00
Fenhexamid	5.19	4.93	0.9988**	0.20	1.00
Fenthion-oxon-sulfone	13.96	7.10	0.9988	0.05	0.05
Fenthion-oxon-sulfoxide	13.13	2.90	0.9986	0.05	0.10
Flonicamid	10.87	2.55	0.9980	0.05	0.20
Florasulam	9.51	3.25	0.9999	0.05	0.20
Fluazifop	5.77	3.28	0.9998**	0.20	0.50
Imidacloprid	3.31	1.15	0.9998	0.05	0.05
Iprodione	24.53	4.28	0.9984***	0.50	5.00
Kresoxim-methyl	4.46	1.16	0.9999	0.01	0.05
Methiocarb-sulfoxide	4.02	2.85	0.9991*	0.10	0.20
Monocrotofos	1.71	1.45	0.9996	0.01	0.02
Monolinuron	0.67	0.27	0.9999	0.05	0.05
Oxycarboxin	5.92	1.93	0.9991	0.05	0.05
Penconazole	2.02	1.60	0.9997	0.01	0.02
Propoxur	0.70	0.94	0.9998	0.01	0.01
Pyraclostrobin	1.23	0.93	0.9996	0.01	0.02
Pyrimethanil	5.55	0.60	0.9997	0.02	0.05
Spiroxamine	0.91	0.87	0.9997	<0.01	<0.01
Thiabendazole	2.99	0.96	0.9999	0.02	0.02
Thiacloprid	1.57	1.10	0.9995	0.02	0.05
Thiamethoxam	1.38	1.89	0.9998	0.01	0.05
Thiofanox-sulfone	5.13	2.14	0.9998	0.05	0.10
Trichlorfon	6.34	4.31	0.9988	0.05	0.20
Tricyclazole	1.66	0.85	0.9999	0.02	0.02
Vamidothion	4.56	1.16	0.9997	0.01	0.01

* Detection limit is 0.10 ppb, calibration starts at 0.10 ppb.

** Detection limit is 0.20 ppb, calibration starts at 0.20 ppb.

*** Detection limit is 0.50 ppb, calibration starts at 0.50 ppb.

1 High due to interference of a system peak.

Table 2
Method performance results.

(a compound with relatively low sensitivity) and for propoxur (a compound with relatively good sensitivity) are shown in Figures 2 and 3, respectively. Most of the compounds have detection limits below 0.05 ppb. The sensitivity for spiroxamine is below the lowest level injected (0.01 ppb) which is significantly better compared to the other pesticides. No accurate detection limit could be determined for cyromazine due to a system peak that interfered at low levels.

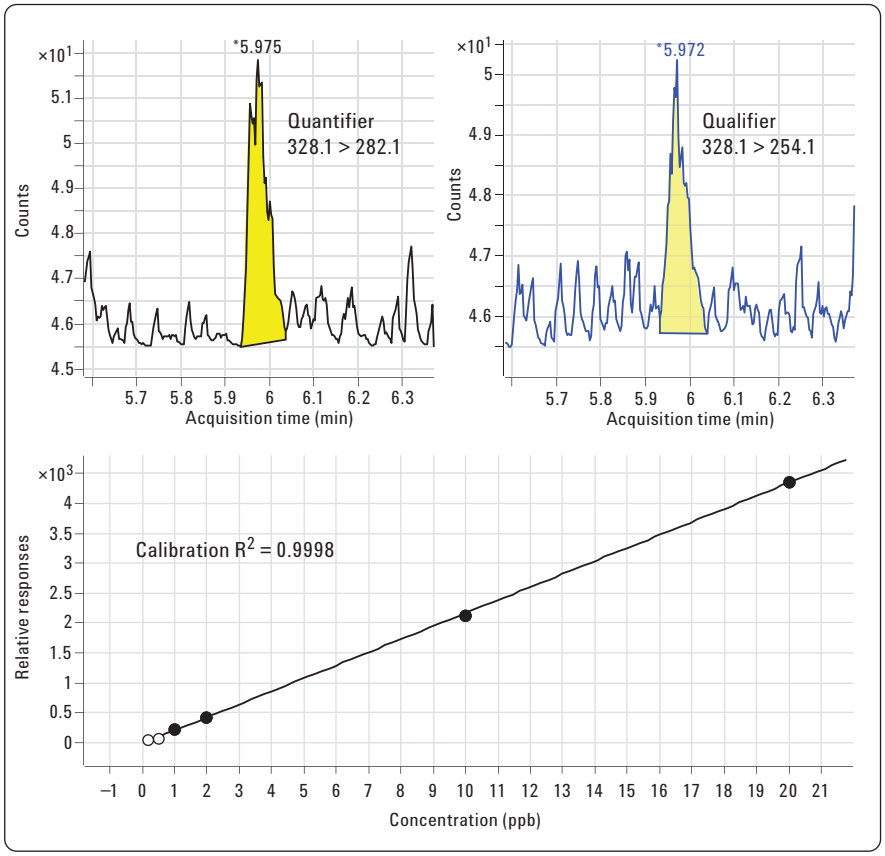

Figure 2
Ion traces for two transitions at the LOD (0.5 ppb standard solution) and calibration curve for fluazifop.

The QuEChERS sample preparation procedure was applied to three baby food samples. Extracts were analyzed with and without additional dispersive SPE cleanup. There were no target compounds detected above the LOD in non-spiked samples. The resulting chromatogram, shown as an overlay of quantification transitions for a sample spiked at 1-ppb level with all 40 pesticides, is depicted in Figure 4. The signals for the quantifier and qualifier transitions for fluazifop and propoxur in the spiked sample at 1-ppb level are shown in Figure 5. From these traces it is clear that excellent selectivity and sensitivity are obtained. The relative response of the quantification transition and qualifier transition are clearly within the limits for positive identification.

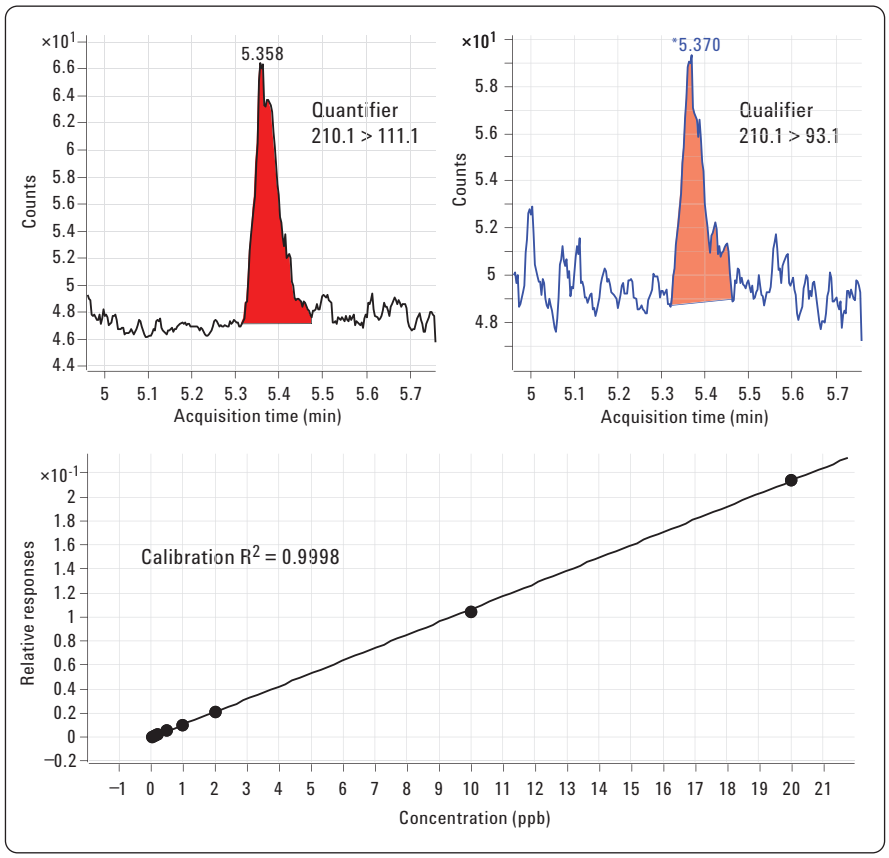

Figure 3
Ion traces for two transitions at the LOD (0.01 ppb standard solution) and calibration curve for propoxur.

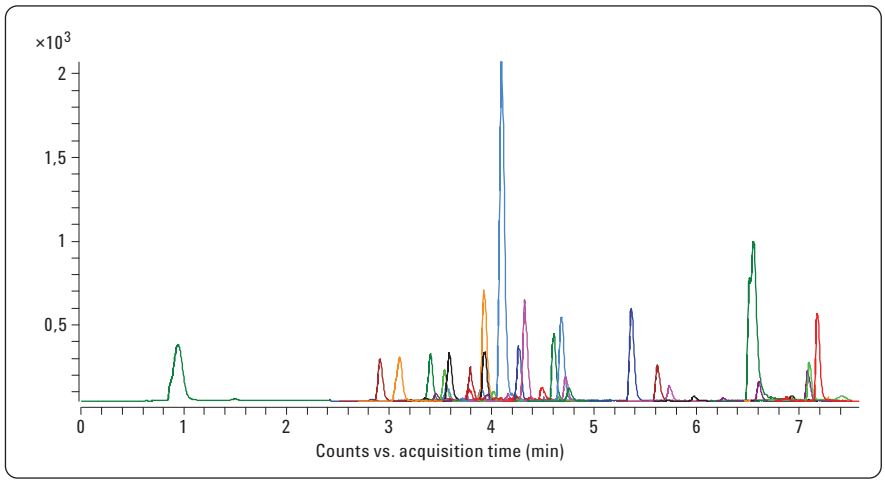

Figure 4
MRM of an extract of sample 2 spiked with 1 ppb (only quantifier transitions are shown). No dispersive SPE performed on the sample. The transition for the internal standard is not shown.

The performance criteria of the sample preparation and analysis method are summarized in Table 3. The extraction repeatability is calculated on sample 1, spiked at the 10-ppb level and repeated (extraction + analysis) five times. Most RSDs are below 10%, with the exception of iprodione and fluazifop, where higher values are obtained after SPE. The average recovery (response spike sample / response calibration sample) for the three different samples was between 70% and 110% at 1 and 10 ppb spike level in most cases. No significant differences were observed between the different matrices. The recovery is satisfactory even at the 1-ppb level and in most cases there is no significant difference between extracts that have been subjected to the additional SPE procedure and those that have not. For cyromazine, better values are obtained after SPE. For fluazifop, on the other hand, very low recoveries (and high RSD) are obtained when additional dispersive SPE is used. In this case, analysis without additional SPE is recommended.

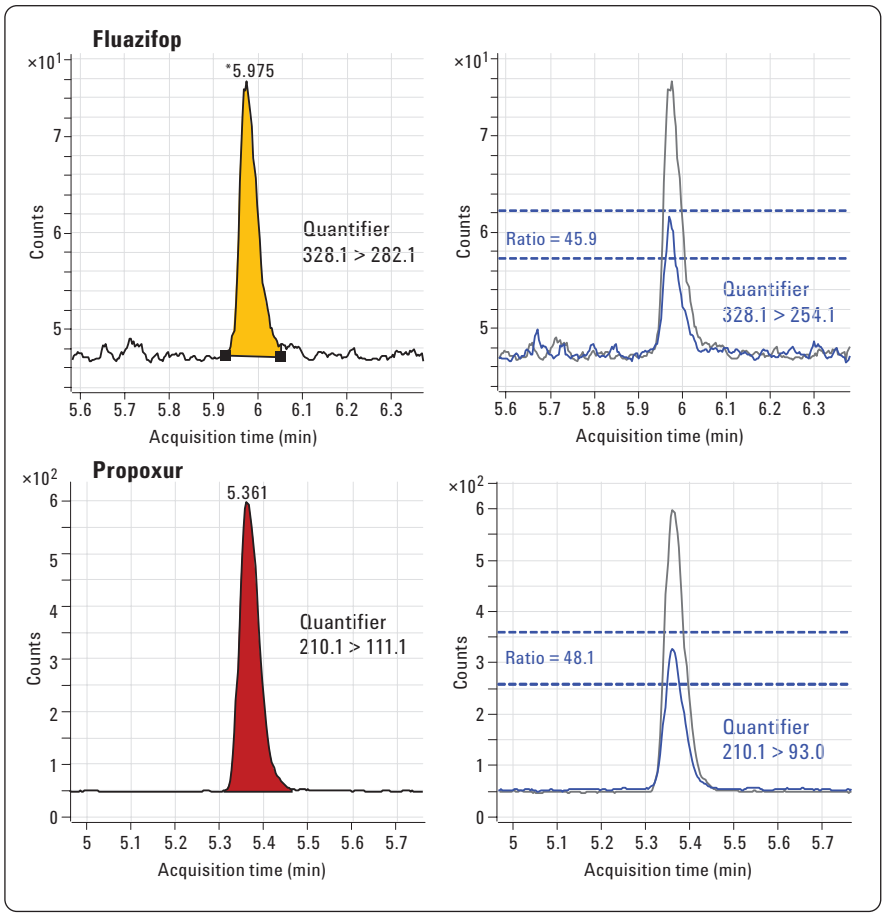

Figure 5
Ion traces for 2 transitions for fluazifop and propoxur in an extract of sample 2 spiked with 1 ppb. No dispersive SPE performed on the sample. The uncertainty was set at 20% (dotted lines).

	Repeatability of extraction (% RSD)1		Average recovery (%)2				Lowest level detected in extract (ppb)3
			1 ppb		10 ppb		
Compound	No IS SPE	No IS No SPE	SPE	No SPE	SPE	No SPE	
Acetamiprid	1.50	1.55	107.0	83.4	99.3	92.2	0.10
Aldicarb	1.74	1.69	91.9	82.8	92.4	81.8	0.10
Butocarboxim	4.41	4.17	95.4	79.6	91.8	86.9	1.00
Carbaryl	1.84	1.30	92.3	75.4	93.2	80.1	0.10
Carbendazim	1.87	1.23	88.6	79.4	90.5	78.1	0.10
Carbofuran-3-OH	6.13	4.62	85.8	114.4	100.5	98.2	1.00
Chlothiamid	4.59	9.54	87.5	105.1	91.8	70.1	1.00
Clothianidin	3.01	1.70	87.0	117.7	103.7	103.2	1.00
Cymoxanil	5.27	5.11	101.5	72.1	99.0	98.1	1.00
Cyromazine	1.70	0.54	108.7	87.1	73.0	57.8	<10.00[4]
Dicrotofos	2.73	1.98	102.9	83.0	93.5	83.5	0.10
DMSA	2.13	1.73	96.3	109.9	103.0	108.1	0.10
Ethiofencarb-sulfone	1.52	2.96	92.1	85.6	91.8	84.2	1.00
Ethiofencarb-sulfoxide	2.33	0.74	90.8	88.9	92.1	83.2	0.10
Fenbuconazole	3.77	6.28	107.5	80.6	90.8	99.8	1.00
Fenhexamid	5.22	7.29	74.0	100.8	91.5	83.5	1.00
Fenthion-oxon-sulfone	6.08	4.49	91.4	78.4	89.8	89.8	1.00
Fenthion-oxon-sulfoxide	2.63	0.90	122.8	96.5	100.8	90.0	0.10
Flonicamid	2.77	3.02	94.4	86.1	94.3	91.8	0.10
Florasulam	5.98	3.76	72.2	103.3	73.8	115.9	0.10
Fluazifop	20.71	1.45	14.4	117.7	18.8	92.0	1.00
Imidacloprid	2.98	2.35	115.2	114.9	111.5	117.6	0.10
Iprodion	14.30	4.37	87.0	90.8	89.8	91.0	1.00
Kresoxim-methyl	4.16	3.82	74.0	80.6	71.8	80.7	0.10
Methiocarb-sulfoxide	3.06	1.63	94.5	98.5	93.1	87.1	0.10
Monocrotofos	1.54	0.72	90.2	81.6	90.9	83.1	0.10
Monolinuron	1.66	0.71	90.0	80.9	92.9	84.3	0.10
Oxycarboxin	2.04	1.74	89.8	107.4	101.0	105.6	0.10
Penconazole	4.25	2.77	73.4	78.2	76.9	83.6	0.10
Propoxur	1.61	0.25	94.7	83.0	95.6	84.9	0.10
Pyraclostrobin	3.62	4.59	86.1	89.3	84.8	90.9	0.10
Pyrimethanil	1.99	2.22	85.5	84.4	86.8	78.3	0.10
Spiroxamine	3.91	1.50	79.6	91.7	78.4	85.8	0.10
Thiabendazole	1.29	1.52	92.7	74.5	91.1	78.7	0.10
Thiacloprid	2.51	1.74	96.7	90.7	94.3	86.4	0.10
Thiamethoxam	2.09	1.12	104.8	108.5	112.4	108.7	0.10
Thiofanox-sulfone	2.70	1.48	99.0	93.3	91.5	84.1	0.10
Trichlorfon	6.94	1.93	86.9	86.5	99.7	92.4	1.00
Tricyclazole	0.90	1.58	91.3	72.0	90.9	75.6	0.10
Vamidothion	1.75	2.63	92.4	79.2	90.0	81.8	0.10

1 Sample 1, spiked with 10 ppb, extracted 5 times. 1 injection per extract.

2 Average of samples 1 to 3, spiked at 1 ppb and at 10 ppb and extracted once. 1 injection per extract.

3 Samples were spiked at 0.1, 1, and 10 ppb level. Lowest detected level is reported.

4 High due to interference of a system peak.

Table 3
Extraction performance.

Conclusion

The multiresidue LC/MS/MS method enabled the analysis of 40 pesticides at low levels in baby food. Sample preparation was performed using an Agilent SampliQ QuEChERS AOAC kit. The total analysis time using the Agilent 1290 Infinity LC system and the Agilent 6460A triple quadrupole LC/MS was 10 min. All compounds could be detected at 0.5 µg/kg or lower in the samples, which is 20 times lower than the MRL for these compounds in baby food according to EU regulation. The extraction repeatability and recovery were good. No difference on extraction and analytical performance due to differences in sample matrix were observed. The optional dispersive SPE cleanup procedure can be applied but for some solutes larger standard deviation and lower recoveries were observed after SPE.

References

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European Parliament and Council Regulation No. 396/2005 on Maximum Residue Levels of Pesticides In or On Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC, 23 February 2005.

2.
P. Stone, T. Glauner, K. Kuhlmann, T. Schlabach, K. Miller, "New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantitation in Complex Analyses," Agilent Technologies, Technical Overview, 5990-3595EN, June 2009.

www.agilent.com/chem/1290

December 1, 2009
Publication Number 5990-5028EN

Agilent Technologies

PBDE Analysis Using an Agilent J&W DB-5ms Ultra Inert GC Column

Application Brief

Environmental

Authors

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Introduction

Polybrominated diphenyl ether (PBDE) analyses are important tools for understanding food supply and environmental quality worldwide. In this application, trace-level PBDE analysis is demonstrated using electron impact GC–MS. For these challenging separations, knowing that each GC column has been thoroughly tested for column inertness gives the analyst higher confidence in the accuracy of the results.

Agilent 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 extremely active probes, including 1-propionic acid, 4-picoline and trimethyl phosphate, are used to verify each column's inertness performance. Capillary GC column activity as a potential source of result uncertainty has been virtually eliminated with the Ultra Inert series of columns.

PBDE Analyses

PBDE-209 is a particularly challenging analyte because of its long retention and tendency to degrade with high-temperature exposure. High-temperature thermal stability is an issue for this class of compounds, but is more pronounced for BDE-209, as it is highly brominated and well retained. One key to successful BDE analysis is to limit the time that these compounds are exposed to high temperatures. A 15-m long column, as opposed to a typical 30-m long column was used in this instance to limit residence time for BDE-209. Fortunately, the BDEs resolve well, with symmetrical peak shapes, when using Agilent J&W DB-5ms ultra inert phase, enabling successful separation on the shorter column. Figure 1 shows a total ion chromatogram of the eight BDEs investigated in this study.

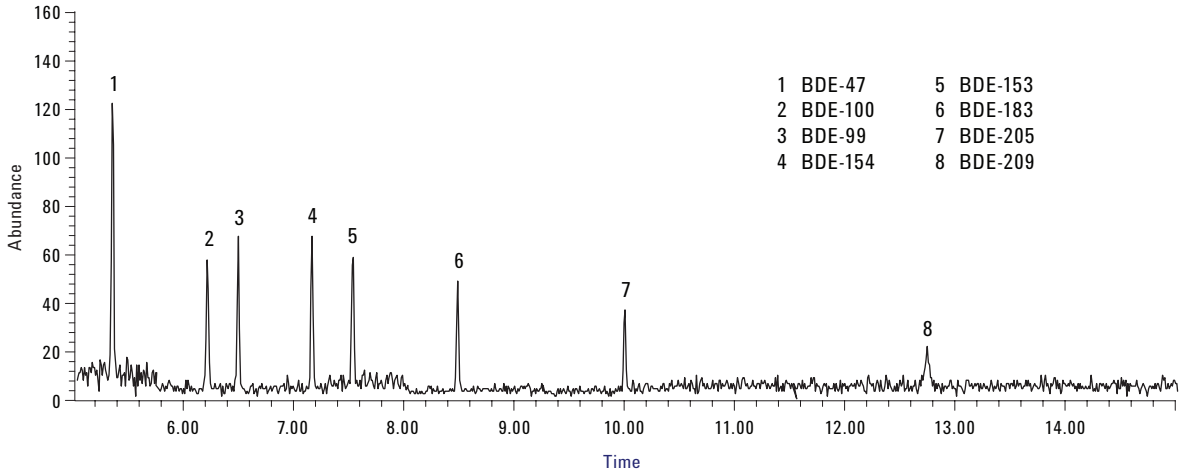

Figure 1. Total ion chromatogram (SIM mode) of a 0.005 ng (BDEs -47, -100, -99, -154, -153, -183 and -205) and 0.025 ng (BDE-209) on-column loading on an Agilent J&W DB-5ms Ultra Inert 15 m x 0.25 mm, 0.25 µm capillary GC column (p/n 122-5512UI).

Table 1 Chromatographic Conditions

GC	Agilent 6890N/5973B MSD
Sampler	Agilent 7683B, 5.0 µL syringe (Agilent p/n 5188-5246), 1.0 µL splitless injection, 5 ng each component on column
Carrier	Helium 72 cm/s, constant flow
Inlet	Pulsed splitless; 325 °C, 20 psi until 1.5 min, purge flow 50 mL/min at 2.0 min
Inlet liner	Deactivated dual taper direct connect (Agilent p/n G1544-80700)
Column	Agilent J&W DB-5ms Ultra Inert 15 m × 0.25 mm, 0.25 µm (Agilent p/n 122-5512UI)
Oven	150 to 325 °C (17 °C/min), hold 5 min
Detection	MSD source at 300 °C, quadrupole at 150 °C, transfer line at 300 °C, scan range 200–1000 amu

This application successfully demonstrates the use of a 15 m Agilent J&W DB-5ms Ultra Inert capillary GC column for trace-level BDEs in a 15-min analysis. Linearity was excellent for all eight BDEs studied, yielding 0.997 or greater R^2 values down to a 0.005 ng (0.025 ng for BDE-209) on-column loading of each component. One of the reasons for the 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.

The Agilent 6890/5975B GC/MSD (SIM mode) equipped with an inert electron impact source had excellent sensitivity with even the most challenging BDE in this set, BDE-209. The signal-to-noise ratio for a 0.025 ng on-column loading of BDE-209 was greater than three to one with this system. This result shows clearly the power of using an Agilent J&W DB-5ms Ultra Inert capillary GC column for trace-level BDE analysis. Lower limits of quantification are expected when using one of Agilent's latest GC/MS offerings, such as the 6890N/5975C GC/MSD Triple-Axis Detector coupled with an Agilent J&W DB-5ms Ultra Inert GC capillary column.

Access the full application note at:
http://www.chem.agilent.com/Library/applications/5989-9571EN.pdf

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Printed in the USA
February 11, 2011
5990-5651EN

Agilent Technologies

PAH Analysis Using an Agilent J&W DB-5ms Ultra Inert Capillary GC Column

Application Brief

Environmental

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Introduction

Trace- and ultra trace-level polycyclic aromatic hydrocarbon (PAH) analyses are important tools for accessing environmental quality and foodstuff purity worldwide. In this application, trace-level PAH analyses are demonstrated using electron impact single quadrupole scanning mass spectrometry. In these challenging separations, knowing that the GC column has been thoroughly investigated for column inertness gives the analyst higher confidence in the accuracy of the results.

Agilent has implemented a new testing procedure to more effectively evaluate GC column inertness performance. This new testing procedure employs 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. This is a rigorous approach that establishes consistent baseline inertness profiles for each column in the Agilent J&W Ultra Inert GC column series.

PAH Analysis

In this application, a 16-component PAH standard mixture was evaluated over a concentration range of 0.05 µg/mL to 5 µg/mL on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm, 0.25 µm capillary GC column (p/n 122-5532UI). Excellent sensitivity was observed for each of these components across the range studied. Good resolution was obtained in a 15-min analysis for each of the PAHs, with the exception of indeno [1,2,3-c,d]pyrene and dibenz[a,h]anthrancene, which were only partially resolved. Figure 1 shows the total ion chromatogram for a standard injection at the 0.5 µg/mL level; GC conditions are listed in Figure 1.

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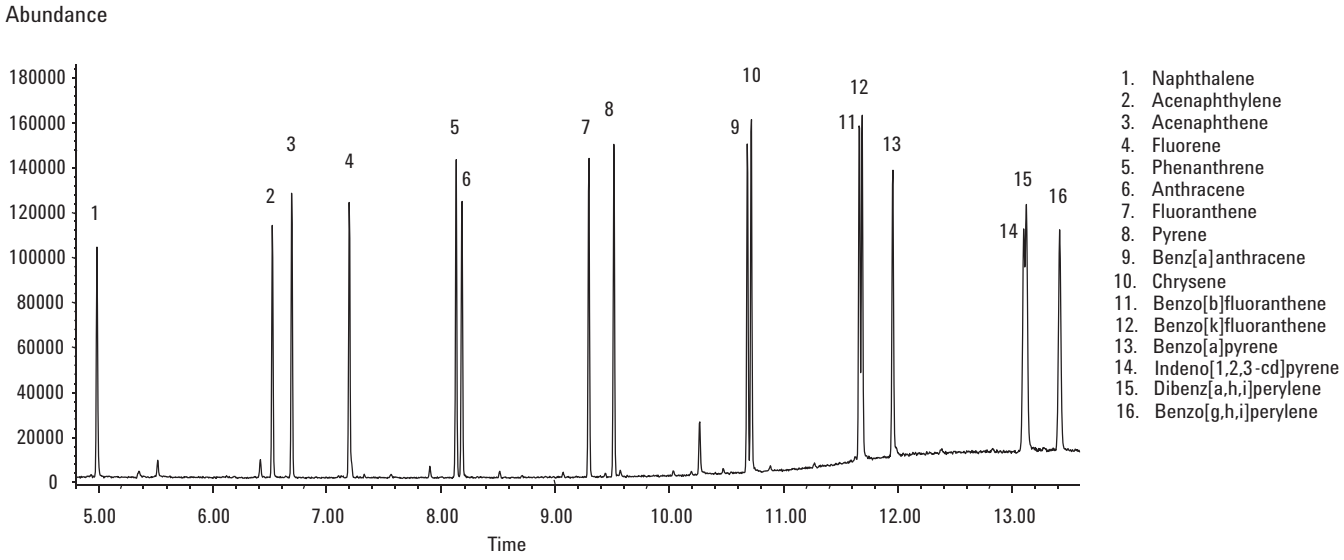

Figure 1. Total ion chromatogram (scanning mode) of a 1 µL injection of the 0.5 µg/mL standard solution on an Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm, 0.25 µm capillary GC column (p/n 122-5532UI). This injection represents an on-column loading of 0.5 ng per component.

Table1. Chromatographic Conditions

GC	Agilent 6890N/5973B MSD
Sampler	Agilent 7683B, 5.0 µL syringe (Agilent p/n 5188-5246), 1.0 µL splitless injection, 5 ng each component on column
Carrier	Helium 45 cm/s, constant flow
Inlet	Pulsed splitless; 300 °C, 40 psi until 0.2 min, purge flow 30 mL/min at 0.75 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	55 °C (1 min) to 320 °C (25 °C/min), hold 3 min
Detection	MSD source at 300 °C, quadrupole at 180 °C, transfer line at 280 °C, scan range 45 to 450 AMU

This application successfully demonstrates the use of an Agilent J&W DB-5ms Ultra Inert capillary GC column for trace-level PAHs in a 15 minute analysis. Linearity was excellent for all 16 PAHs studied, yielding 0.995 or greater R^2 values down to a 0.05 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 excellent choices for trace-level applications.

This study was done exclusively using scanning mode on an Agilent 6890N/5975B GC/MSD equipped with an inert electron impact source. The signal-to-noise ratio for a 0.05 ng on-column loading of benzo[a]pyrene was greater than 9 to 1 with this system. This result clearly shows the power of using an Agilent J&W DB-5ms Ultra Inert column for trace-level PAH analysis.

Access the full application note at:
http://www.chem.agilent.com/Library/applications/5989-9181EN.pdf

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Printed in the USA
February 11, 2011
5990-5652EN

Agilent Technologies

Analysis of Low-level Polycyclic Aromatic Hydrocarbons (PAHs) in Rubber and Plastic Articles Using Agilent J&W DB-EUPAH GC column

Application Note

Gas Chromatography/Mass Spectrometry

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Abstract

Agilent J&W DB-EUPAH GC columns are designed for the analysis of EU-regulated priority PAHs. This application demonstrates a GC/MS method for the determination of 20 PAHs including 16 EPA-regulated priority PAHs and four commonly monitored PAHs including benzo(j)fluoranthene, benzo[e]pyrene, 2-methylnaphthalene and 1-methylnaphthalene in rubber and plastics using this type of column. To ensure the accuracy of results, the quantitation was performed with internal standardization using five isotopically-labeled PAHs including naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12 along with p-terphenyl-d14 as the surrogate standard. All 26 compounds were separated well with the DB-EUPAH column. The resulting good linearity and sample recovery demonstrate the high selectivity of the described method in this application note for trace-level detection and confirmation of the targeted PAHs in complex sample matrices.

Agilent Technologies

Introduction

Polycyclic aromatic hydrocarbons (PAHs), containing two to eight aromatic rings [1], are identified as some of the most persistent organic pollutants (POPs). Due to their well known carcinogenic and mutagenic properties, some PAHs are classified as priority pollutants by both the U.S. EPA and the European Commission. The U.S. EPA designated 16 PAH compounds as priority pollutants, including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene. The 16 EPA priority PAHs are often targeted for measurement in environmental samples.

PAHs may be present in oil, coal, rubber, and plastics. Some rubber products such as tires are produced using extender oils that may unintentionally contain various levels of PAHs. These extender oils along with the PAHs are incorporated into the rubber matrix and remain locked in the final products. In 2005, the European Commission adopted a Directive [2] restricting the marketing and use of certain PAHs in extender oils for tire production. All tires produced after 1 January 2010 are required to comply with the new Directive.

In the Directive, extender oils may not be used for the production of tires if they contain more than 1 mg/kg benzo(a)pyrene (BaP), or more than 10 mg/kg of the sum of the eight PAHs of concern including benzo(a)pyrene (BaP), benzo(e)pyren (BeP), benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbFA), benzo(j)fluoranthene (BjFA), benzo(k)fluoranthene (BkFA) and dibenzo(a,h)anthracene (DBAhA). BeP and BjFA were not included in 16 EPA-regulated priority PAHs.

Five percent phenyl methylpolysiloxane stationary phase was the most commonly-cited GC column for the analysis of the 16 EPA-regulated PAHs, [3,4]. The resolution of the Benzo(b,k,j)fluoranthenes isomers were not easily obtained using this GC column.

In this application note, a DB-EUPAH column was chosen to provide the necessary separation for all 20 PAHs of interest in rubber and plastic articles. This includes the resolution of all the critical isomers such as the benzo(b,k.j)fluoranthenes. With its exceptional thermal stability, low column bleed at elevated temperatures, and consistent column inertness, the Agilent J&W DB-EUPAH column delivers fast, reliable results while meeting demanding regulatory requirements.

Experimental

The experiments were performed on one Agilent 7890 gas chromatograph equipped with an Agilent 5975C series GC/MSD, and an Agilent 7683 Automatic Liquid Sampler (ALS). The instrument conditions are listed in Table 1.

Chemicals and Standards

All standards in the experiment were purchased from Sigma-Aldrich (St. Louis, MO, USA). The CAS numbers and nominal molecular mass are provided in Table 2.

Surrogate Spiking Solution

p-Terphenyl-d14 was used as a surrogate standard in this experiment. The surrogate spiking solution was prepared from aliquots of pure compound diluted with toluene to a concentration of 10 µg/mL, and served as a stock solution. Surrogate solution was added to all samples and all quality control samples prior to extraction.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC Conditions	
Column:	Agilent J&W DB-EUPAH, 20 m × 0.18 mm × 0.14 µm (Agilent p/n 121-9627）
Inlet temperature:	290 °C
Carrier gas:	Helium, constant flow mode, 52 cm/s
Injection mode:	Splitless, purge flow 50 mL/min at 0.75 min
Injection volume:	1 µL
Oven:	120 °C (1 min), 8 °C/min to 200 °C (0.5 min), 11 °C/min to 270 °C, 2 °C/min to 300 °C,
Post run:	320 °C (4 min)
MS Conditions	
Solvent delay:	2.8 min
MS temp:	250 °C (Source); 180 °C (Quad)
Transfer line:	290 °C
MS:	EI, SIM/Scan
Scan mode:	Mass range (50-450 amu)
For other parameters, see Table 2	
Miscellaneous Parts	
Septa:	Long-lifetime septa (Agilent p/n 5183-4761)
Liner:	Splitless deactivated dual taper direct connect liner (Agilent p/n G1544-80700).
Syringe	5 µL syringe (Agilent p/n 5181-1273)

Table 2. Polycyclic Aromatic Hydrocarbons, CAS Number, Nominal Molecular Mass and Corresponding Ions

Compound	CAS No.	Nominal molecular mass	Corresponding Ions
Naphthalene-d8*	1146-65-2	136	**136**, 108, 68
Acenaphthene-d10*	15067-26-2	164	**164**, 160
Phenanthrene-d10*	1719-06-8	188	**188**
Chrysene-d12*	1719-03-5	240	**240**, 236, 120
p-Terphenyl-d14**	1718-51-0	244	**244**, 122 ,212
Perylene-d12*	1520-96-3	264	**264**, 265, 260
Naphthalene	91-20-3	128	**128**, 127, 129
2-methylnaphthalene	91-57-6	142	**142**, 141, 115
1-methylnaphthalene	90-12-0	142	**142**, 141, 115
Acenaphthylene	208-96-8	152	**152**, 153, 151
Acenaphthene	83-32-9	154	**153**, 154, 152
Fluorene	86-73-7	166	**166**, 165, 167
Phenanthrene	85-01-8	178	**178**, 176, 179
Anthracene	120-12-7	178	**178**, 176, 179
Fluoranthene	206-44-0	202	**202**, 200, 101
Pyrene	129-00-0	202	**202**, 200, 101
Benzo(a)anthracene	56-55-3	228	**228**, 226, 229
Chrysene	218-01-9	228	**228**, 226, 229
Benzo(b)fluoranthene	505-99-2	252	**252**, 253, 126
Benzo(j)fluoranthene	205-82-3	252	**252**, 253, 126
Benzo(k)fluoranthene	207-08-9	252	**252**, 253, 126
Benzo(e)pyrene	192-97-2	252	**252**, 253, 126
Benzo(a)pyrene	50-32-8	252	**252**, 253, 126
Indeno(1,2,3-cd)pyrene	193-39-5	276	**276**, 138, 277
Dibenzo(a,h)anthracen	53-70-3	278	**278**, 139, 279
Benzo(g,h,i)perylene	191-24-2	276	**276**, 138, 277

1. * Internal standard
2. ** surrogate standard
3. Suggested quantitative ions are in bold.

Internal Standard Solution

The internal standard solution included naphthalene-d8, acenaphthlene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12. The internal standard solution was made from aliquots of pure compounds, diluted with toluene to a concentration of 10 µg/mL, and served as a stock solution.

Calibration Solution

Calibrations solutions were prepared in five different concentrations ranging from 5 to 500 ppb by diluting commercially available certified solutions containing analytes of interest. Each standard solution contained 500 µg/L of internal standards (ISTDs).

Sample Preparation

According to ZLS standard ZEK 01.2-08 to prepare samples, rubber and plastic articles were cut into pieces no larger than 2–3 mm in size. Five hundred milligrams of cut pieces were extracted by 20 mL of toluene and mixed with internal standards for 1 hour in the ultrasonic bath at a temperature of 60 °C. After cooling to room temperature, an aliquot was taken from the extract for analysis.

One rubber sample and one plastic sample spiked with PAHs of interest at the 100 ppb level respectively were treated according to the procedure described above.

Results and Discussion

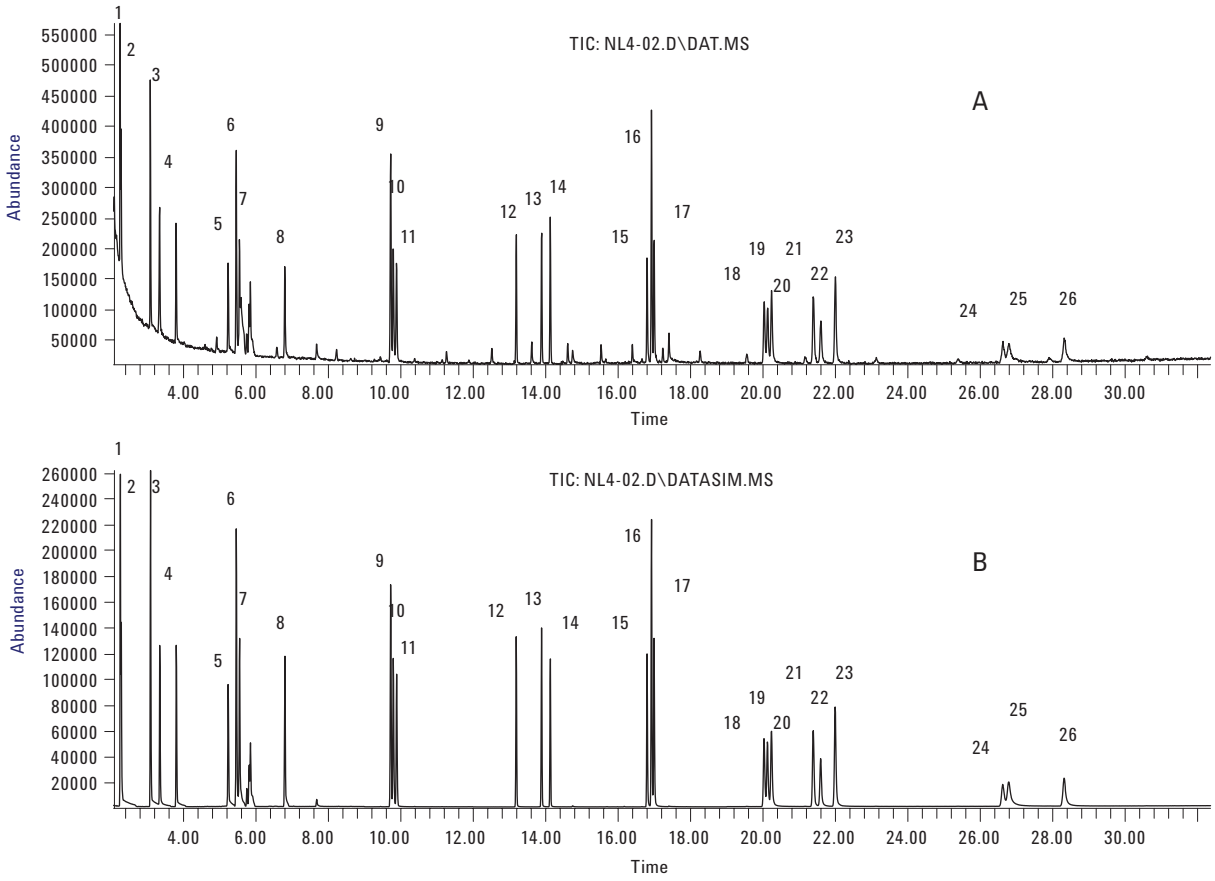
Figure 1 shows the GC/MS total ion chromatograms for the compounds of interest. Due to the presence of isomers, some compounds listed in Table 2 with the same quantitation ions could be eluting quite closely. Therefore good resolution is very important for these isomers to achieve accurate quantitative results. As shown in Figure 1, all 20 targeted PAH compounds were well-resolved with the DB-EUPAH column.

Figure 2 exhibits the baseline resolution of some critical PAH pairs of interest, including phenanthrene and anthracene (*m/z* 178), fluoranthene and pyrene (*m/z* 202), benzo[a]anthracene and chrysene (*m/z* 228), with excellent peak shapes using the DB-EUPAH column.

Benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(j)fluoranthene, benzo(e)pyrene and benzo(a)pyrene are isomers with the same quantitation ion (*m/z* 252). According to 2005/ 69/ EC Directive, the content of benzo(a)pyrene was restricted to be less than 1 mg/kg. Figure 2 shows that benzo(a)pyrene can be separated well, and the resolution of benzo(a)pyrene and benzo(e)pyrene is 2.82 with the DB-EUPAH column. Benzo(b, k,j)fluoranthenes are difficult-to-chromatograph isomers. Benzo(b)fluoranthene (BbFA) and benzo(j)fluoranthene (BjFA) often show coelution on the commonly used 5% phenyl methylpolysiloxane stationary phase GC column. The resolution of benzo(b,j,k)fluoranthene is 1.41

and 1.63 respectively, meeting the requirement of analysis. The three benzo(b,k,j)fluoranthene isomers could be baseline separated, however, with the compromise of a extra 10-min run time. Therefore, it is a trade-off between speed and resolution. The described method in this application note was chosen to provide a reasonable sample run time when meeting the regulatory requirements.

Calibration curves were constructed from the data obtained by the 1-µL injections of standards at 5, 50, 100, 250, 500 ppb. Each standard solution contained 500 ppb of internal standards (ISTDs). All the PAHs have excellent linearity with calibration coefficients greater than 0.998 as shown in Table 3.



1. Naphthalene-d8
2. Naphthalene
3. 2-methylnaphthalene
4. 1-methylnaphthalene
5. Acenaphthylene
6. Acenaphthene-d10
7. Acenaphthene
8. Fluorene
9. Phenanthrene-d10
10. Phenanthrene
11. Anthracene
12. Fluoranthene
13. Pyrene
14. p-Terphenyl-d14
15. Benzo(a)anthracene
16. Chrysene-d12
17. Chrysene
18. Benzo(b)fluoranthene
19. Benzo(k)fluoranthene
20. Benzo(j)fluoranthene
21. Benzo(e)pyrene
22. Benzo(a)pyrene
23. Perylene-d12
24. Indeno(1,2,3-cd)pyrene
25. Dibenzo(a,h)anthracen
26. Benzo(g,h,i)perylene

Figure 1. Twenty PAHs at 250 ppb each with ISTDs at 500 ppb each and surrogate at 250 ppb, using synchronous SIM/SCAN mode, A: SCAN mode B: SIM mode.

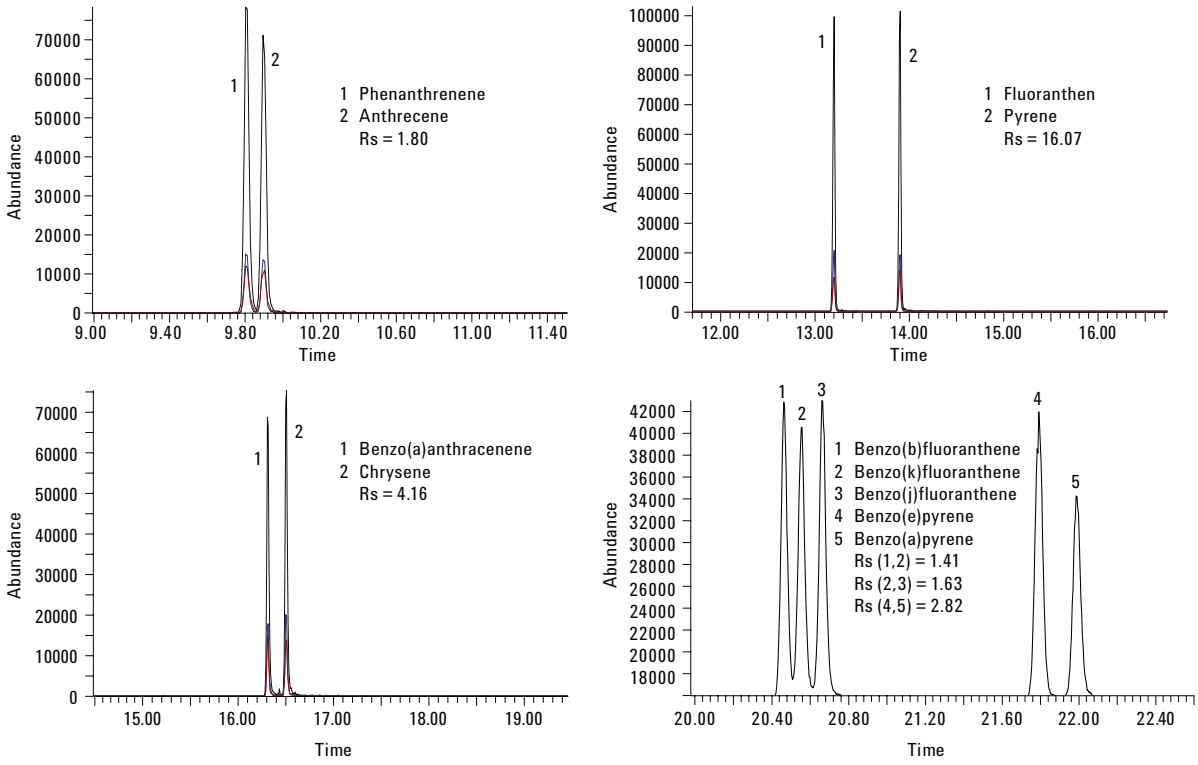


Figure 2. Resolution of Critical isomer Pairs with the DB-EUPAH column, 20 m × 0.18 mm × 0.14 µm (Agilent p/n 121-9627).

The GC/MS TIC for matrix spiked extract is illustrated in Figure 3. The spiked samples were treated according to the procedure described in the sample preparation. The recovery data for the spiked samples are listed in Table 4. All data were based on three replicates of matrix spikes with the 20 targeted PAHs at the 100 ppb level. Good recoveries were achieved for all the compounds, ranging from 73.5% to 119.4%, satisfying both the US-EPA and EU regulatory requirements.

Conclusion

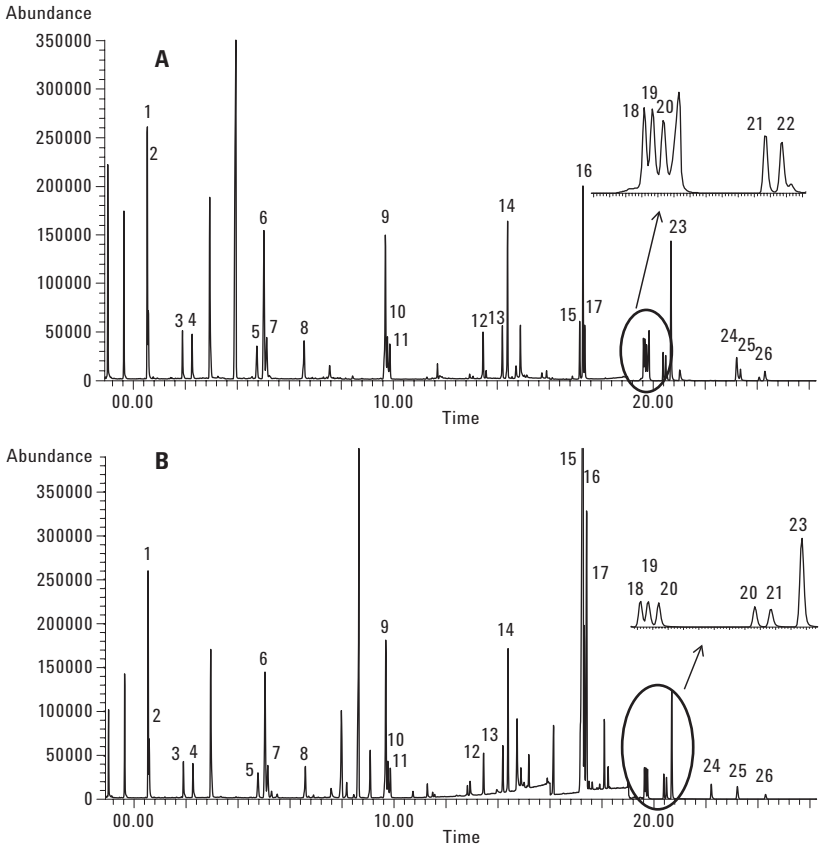
This application demonstrates a highly sensitive and selective GC/MS method for PAH analysis in rubber and plastic products using an Agilent J&W DB-EUPAH GC column. The DB-EUPAH column can effectively separate the 20 PAHs of interest, resolving all the critical, difficult-to-separate pairs. The system allows for trace-level detection of the PAHs in rubber and plastic articles. Good linearity and recoveries were achieved for all targeted compounds. The Agilent J&W DB-EUPAH column delivers fast, reliable results while meeting the requirements of both EPA and EU regulatory methods.

Table 3. The Regression Equations and Correlation Coefficient of PAHs

Compound	Range of linearity (ng)	Correlation coefficient (R^2)
Naphthalene	0.005-0.5	0.9997
2-Methylnaphthalene	0.005-0.5	0.9999
1-Methylnaphthalene	0.005-0.5	0.9998
Acenaphthylene	0.005-0.5	0.9996
Acenaphthene	0.005-0.5	0.9999
Fluorene	0.005-0.5	0.9994
Phenanthrene	0.005-0.5	0.9999
Anthracene	0.005-0.5	0.9992
Fluoranthene	0.005-0.5	0.9990
Pyrene	0.005-0.5	0.9996
Benzo[a]anthracene	0.005-0.5	0.9985
Chrysene	0.005-0.5	0.9998
Benzo[b]fluoranthene	0.005-0.5	0.9998
Benzo[j]fluoranthene	0.005-0.5	0.9983
Benzo[k]fluoranthene	0.005-0.5	0.9990
Benzo[e]pyrene	0.005-0.5	0.9992
Benzo[a]pyrene	0.005-0.5	0.9997
Indeno[1,2,3-c,d]pyrene	0.005-0.5	0.9989
Dibenzo[a,h]anthracene	0.005-0.5	0.9989
Benzo[g,h,i]perylene	0.005-0.5	0.9993

Table 4. Recoveries of PAHs in Substantial Plastic Sample and Rubble Sample

Compounds	Spiked (ppb)	Recovery (%) plastic sample	Recovery (%) rubber sample
Naphthalene	100	101.99	106.7
2-Methylnaphthalene	100	93.3	95.7
1-Methylnaphthalene	100	91.22	92.6
Acenaphthylene	100	111.32	120.8
Acenaphthene	100	98.45	109.0
Fluorene	100	107.66	114.6
Phenanthrene	100	92.54	109.1
Anthracene	100	106	110.8
Fluoranthene	100	110.52	119.4
Pyrene	100	111.62	104.0
Benzo[a]anthracene	100	102.61	118.5
Chrysene	100	107.67	95.9
Benzo[b]fluoranthene	100	103.18	114.6
Benzo[j]fluoranthene	100	103.02	118.9
Benzo[k]fluoranthene	100	91.13	88.8
Benzo[e]pyrene	100	90.8	92.3
Benzo[a]pyrene	100	106.58	119.2
Indenol(1,2,3-cd)pyrene	100	75.36	79.9
Dibenzo(a,h)anthracen	100	80.47	89.9
Benzo(g,h,i)perylene	100	76.45	73.5



1. Naphthalene-d8
2. Naphthalene
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5. Acenaphthylene
6. Acenaphthene-d10
7. Acenaphthene
8. Fluorene
9. Phenanthrene-d10
10. Phenanthrene
11. Anthracene
12. Fluoranthene
13. Pyrene
14. p-Terphenyl-d14
15. Benzo(a)anthracene
16. Chrysene-d12
17. Chrysene
18. Benzo(b)fluoranthene
19. Benzo(k)fluoranthene
20. Benzo(j)fluoranthene
21. Benzo(e)pyrene
22. Benzo(a)pyrene
23. Perylene-d12
24. Indeno(1,2,3-cd)pyrene
25. Dibenzo(a,h)anthracen
26. Benzo(g,h,i)perylene

Figure 3. TIC of matrix spiked extract using Agilent GC/MS system and Agilent J&W DB-EUPAH 20 m × 0.18 mm, 0.14 µm column (Agilent p/n 121-9627) A: plastic, B: rubber.

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Printed in the USA
August 12, 2010
5990-6155EN

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EU's Scientific Committee on Food PAH 151 Analysis Using a ZORBAX Eclipse PAH Column

Application Brief

Food

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Introduction

The 16 Environmental Protection Agency (EPA) priority pollutant polycyclic aromatic hydrocarbons (PAHs) are the dominant analytes in PAH methods. In fact, Agilent ZORBAX Eclipse PAH columns' ruggedness, longevity, batch-to-batch reproducibility and unvarying selectivity among three particle sizes were demonstrated using the EPA priority pollutants PAH mixture.[1] But for food and other environmental analyses, both subsets and additional PAHs must be separated. Different PAH separations including a fast food screening method and a complex environmental standard (24 PAHs) were previously shown.[2] A method for The European Union's (EU's) Scientific Committee on Food (SCF) 15 priority PAHs with benzo[c]fluorene, deemed relevant by the Joint FAO/WHO Experts Committee on Food Additives (JECFA), is presented here.

Experimental

This complex PAH separation was developed on an Agilent ZORBAX Eclipse PAH 2.1mm x 50 mm, 1.8 µm column, p/n 959741-918 and an Agilent Rapid Resolution 1200 Series LC (RRLC) system. It could be scaled up to a more traditional 4.6 mm x 150 mm, 5 or 3.5 µm column if desired, but the shorter, highly efficient, high throughput column was chosen to speed up method development. The "15+1" mixture was made by dissolving individual neat compounds in toluene or methylene chloride then combining them. Dilutions were made with ethanol.

Figure 1 shows the rapid analysis of 18 PAHs associated with food safety including the 15 classified as priority from the SCF (peaks #4–18), benzo[c]fluorene and two additional PAHs, benzo[c]phenanthrene and triphenylene that elute near benzo[c]fluorene. The short 50 mm column length and highly efficient 1.8 µm Eclipse PAH particles resolve the 18 components in 10 minutes, including re-equilibration.

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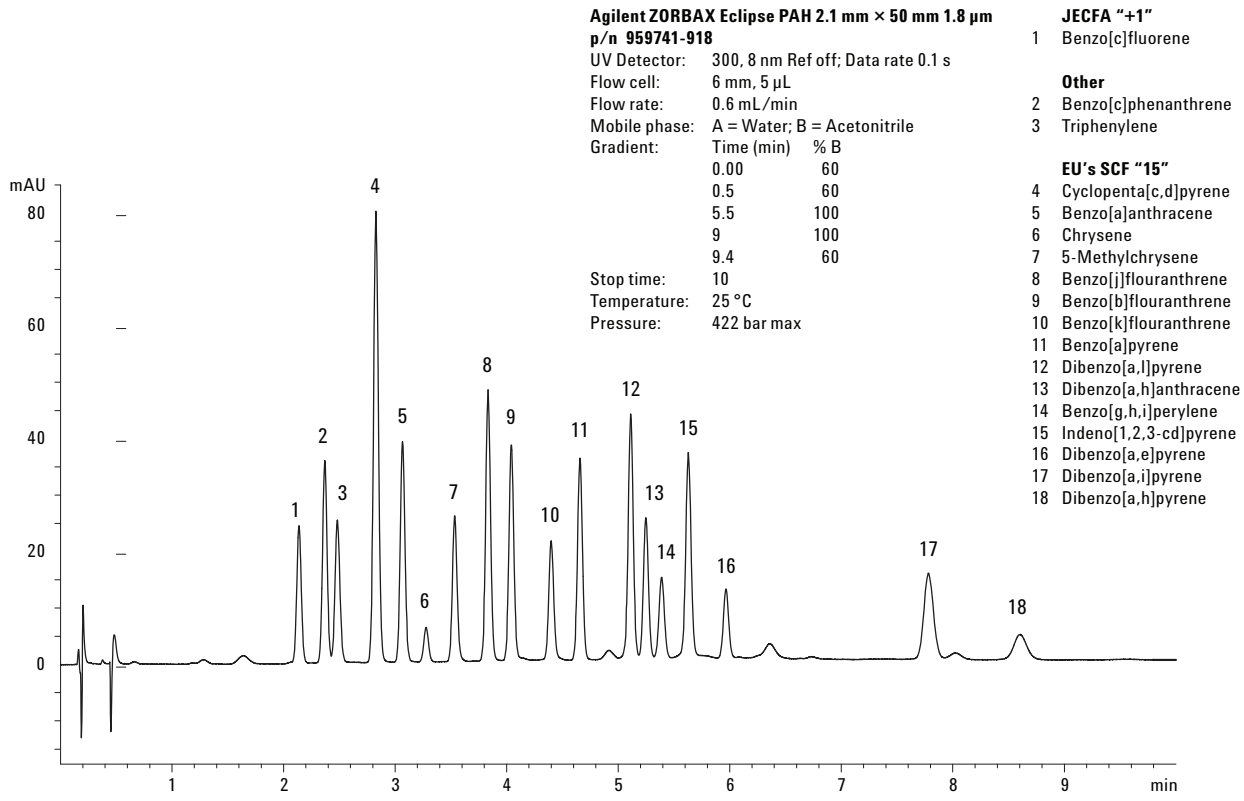

Figure 1. EU's SCF and JECFA "15+1" with two additional PAHs separated by the Agilent ZORBAX Eclipse PAH 2.1 mm x 50 mm, 1.8 µm column.

Conclusion

Many PAH methods are established for the EPA priority PAHs, but fewer have been established for the EU's SCF and JECFA "15+1" mix designed for the food industry. Agilent ZORBAX Eclipse PAH columns' proven robustness and selectivity make them ideal for the broad variety of PAH samples in matrices such as air, water, soil and food, including the "15+1" PAH mix. The 1.8 µm Eclipse PAH high throughput column produced a rapid separation of 18 PAHs associated with the food safety industry in 10 minutes, including re-equilibration. With choices in column lengths, diameters and particle sizes, the Eclipse PAH column gives the analyst valuable analysis options for difficult PAH sample matrices.

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Printed in the USA
February 11, 2011
5990-5650EN

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GC-µECD Analysis and Confirmation of Contract Laboratory Protocol Pesticides in Olive Oil

Application Note

Food Safety and Environmental Markets

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Abstract

An olive oil sample obtained from a local grocery store is analyzed for 20 contract laboratory protocol (CLP) pesticides. A QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) with dispersive solid phase extraction (dSPE) procedure cleaned the sample prior to analysis. A dual µECD and dual capillary GC column approach accomplished simultaneous primary and confirmatory analysis. The primary column, an Agilent J&W DB-35ms 30 m × 0.25 mm × 0.25 µm and a confirmatory column, an Agilent J&W DB-XLB 30 m × 0.25 mm × 0.50 µm effectively resolved all 20 CLP pesticides. An unpurged two-way capillary flow technology splitter divided the flow from a single injection port to the two analytical GC columns. Endosulfan sulfate and endosulfan 1 were present in the olive oil sample.

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Introduction

Lower cholesterol and reduced cancer risk are among the reported beneficial health effects obtained from the antioxidants and monounsaturated fats found in olive oil. The potential health benefits have bolstered the popularity of using olive oil as a cooking oil, salad dressing, or direct replacement in baking for other traditional food oils. This rise in olive oil's popularity has led to higher demand and production levels.

Higher olive oil production levels increase the potential for olive crop destruction by pests. Therefore the industry is more reliant on the use of pesticides, leading to increased regulation of pesticide use [1]. For example, food safety concerns have led to tighter government regulation of pesticide residues found in foods such as olive oil. Production of olive oil has a tendency to concentrate pesticide residues in the oil because it takes four kilograms of olives to make one kilogram of olive oil [2]. Olive oil producers need inexpensive, robust analytical methods for necessary monitoring and testing.

In previous studies of pesticides residue analysis in olive oil, the extraction procedures varied from liquid-liquid followed by solid phase extraction (SPE), gel permeation chromatography (GPC), and more recently to the use of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) with dispersive solid phase extraction (dSPE) [3-4]. QuEChERS and dSPE are convenient ways to clean up sample matrixes sufficiently to remove chromatographic interferences, while retaining sensitivity for analytes of interest. In this olive oil example, the high boiling lipid portion of the sample is removed along with its potential for carryover and interference with peaks of interest.

A dual column, dual µECD system with an Agilent J&W DB-35ms 30 m × 0.25 mm × 0.25 µm primary analysis column and an Agilent J&W DB-XLB 30 m × 0.25 mm × 0.50 µm confirmatory column was used to separate the CLP pesticides in olive oil [5-6]. Continuous improvements and stringent process control with respect to column activity make this column pair a particularly good choice for analysis of active analytes such as pesticides.

The GC was also fitted with an unpurged two-way splitter capillary flow technology (CFT) device. This device allows the operator to disassemble and service the inlet, the inlet transfer line or either of the analytical columns individually. System maintenance and troubleshooting are faster with reusable connections to the CFT.

Experimental

Standard Preparation

CLP pesticide (PPM-808C-1) and surrogate standards (ISM-320-1) were purchased from Ultra Scientific, 250 Smith St, N. Kingstown, RI. The CLP pesticide standard solution was prepared by diluting the standard to 8000 ng/mL with 2,2,4-Trimethylpentane (Ultra-Resi grade from VWR International, West Chester, PA). The 2000 ng/ml stock surrogate solution was made by diluting the surrogate standard with 2,2,4-Trimethylpentane. Working solutions of 4, 8, 80 and 800 ng/mL for the CLP pesticides and 1, 2, 20, and 200 ng/mL of the surrogates were used for spiking and calibration samples. 2,2,4-Trimethylpentane was used as a solvent blank and syringe wash solvent.

Sample Preparation

A sample of extra virgin olive oil was purchased at a local grocery store. The sample extraction method used the QuEChERS method followed by dSPE. Figure 1 illustrates the sample preparation procedure graphically in a flow chart.

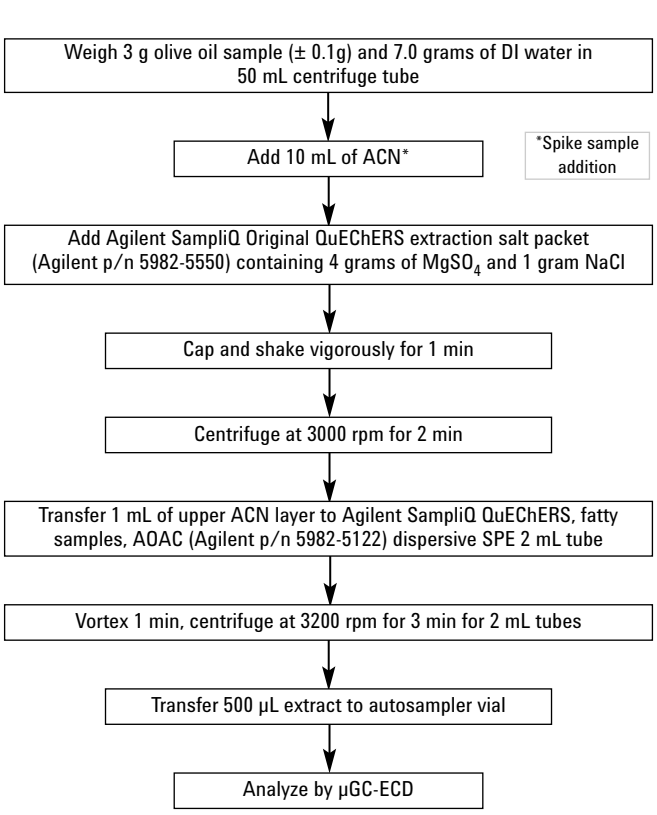

Figure 1: Flow chart of the Agilent SampliQ QuEChERS original extraction procedure for olive oil sample.

A 3.0-g olive oil aliquot and 7.0-g aliquot of deionized water were weighed into a centrifuge tube. The sample, spiked sample and blank each received 10-mL aliquots of acetonitrile (HPLC grade from VWR International). An Agilent SampliQ QuEChERS extraction salt packet (Agilent p/n 5982-5550) containing 4 g of $MgSO_4$ and 1 g NaCl was added to each centrifuge tube. The capped tubes were shaken for 1 min by hand and then 1 min on a mechanical shaker. The samples were centrifuged at 3000 rpm for 2 min.

A 1-mL aliquot of the upper layer was transferred into an Agilent SampliQ QuEChERS, fatty samples, AOAC (Agilent p/n 5982-5122) dispersive SPE 2 mL tube. The dSPE tube was vortexed for 1 min and then centrifuged at 3200 rpm for 3 min to complete the sample extraction. The liquid from the dSPE tube was transferred to a GC vial and run on the GC-µECD using the chromatographic conditions listed in Table 1 and Table 2.

Table 1. Chromatographic Conditions

GC/Dual µECD:	7890A equipped with dual µECD detection and a 7873B auto sampler
CFT Device:	2-way unpurged splitter capillary flow technology (Agilent p/n G3181B)
Column 1:	DB-35 ms 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-3832)
Column 2:	DB-XLB 30 m × 0.25 mm × 0.50 µm (Agilent p/n 122-1236)
Carrier Gas:	Hydrogen 56 cm/sec
Oven:	110 °C (1.4 min), 21 °C/min to 285 °C (1 min), 30 °C/min to 300 °C (2 min)
Injection:	1µL, 250 °C splitless, purge 50 mL/min at 0.3 min, gas saver 50 mL/min on at 2 min
Dual µ-ECD:	350 °C, N_2 makeup; constant column + makeup = 30 mL/min

Table 2 Flow Path Supplies

Vials:	Amber screw top glass vials (Agilent p/n 5183-2072)
Vial Caps:	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 5183-4729)
Septum:	Advanced green (Agilent p/n 5183-4759)
Inlet Seal:	Gold plated inlet seal (Agilent p/n 5188-5367)
Inlet liners:	Dual taper direct connect linear (Agilent p/n G1544-80700)
Ferrules:	0.4 mm id short; 85/15 vespel/graphite (Agilent p/n 5181-3323)
CFT fittings:	Internal nut (Agilent p/n G2855-20530)
CFT ferrules:	SilTite ferrules, 0.25 mm id (Agilent p/n 5188-5361)
20x magnifier :	20x Magnifier loop (Agilent p/n 430-1020)

To produce the spiked sample, a 3-mL aliquot of the 80 ng/mL CLP standard solution was added to the olive oil and water mixture, before the SampliQ QuEChERS original extraction salt packet addition. Extractions of water and acetonitrile aliquots in the same manner as the samples and the spiked sample served as reagent blanks.

Discussion of Results

The CLP pesticides and surrogates standards were resolved on the Agilent J&W DB-35ms 30 m × 0.25 mm × 0.25 µm primary analysis column in less than 12 min. Figure 2 show the separation of a 4.0 ng/mL CLP standard solution with the surrogate standards added at a concentration of 1.0 ng/mL. Peak numbers in the chromatogram label the peaks of interest and a compound key is included with the figure. Figure 3 shows a chromatogram of the same 4 ng/mL CLP standard (0.5 ng/mL surrogate standard) injection on the Agilent J&W DB-XLB 30 m × 0.25 mm × 0.50 µm confirmatory analysis column. Although peaks 10 and 11 (α-chlordane and endosulfan 1) are not completely resolved, the separation is suitable for confirming the presence of these analytes when observed on the primary analysis (DB-35ms) column.

The performance of the dual column set yielded acceptable linearity, limits of detection (LOD), and quantitative (LOQ) in accordance with current EU guidelines for these analytes. The linearity of the column set as defined by the R^2 values of the CLP pesticide standard curve ranged from 0.994-0.999. Individual pesticide values are shown in Table 3. The LOD (S/N = 3) and LOQ (S/N = 10) were determined through close examination of an expanded section of chromatograms on each column relative to a known concentration peak in the lowest standard. Figure 4A shows this comparison for the tetrachloro-m-xylene peak on the primary analysis DB-35ms column and Figure 4B shows the same comparison on the DB-XLB confirmation column. The average LOD (S/N = 3) and LOQ (S/N =10) across both columns using the lowest concentration (0.5 ng/mL) of the standard CLP pesticides was 0.3 ng/mL and 1.0 ng/mL respectively. The dual column set of a primary analytical column and a confirmatory column on one instrument allows simultaneous confirmation of the presence of the CLP pesticides. The single injection, dual column approach saves instrument and analyst time and offers the analyst an alternative to GC/MS screening for olive oil samples.

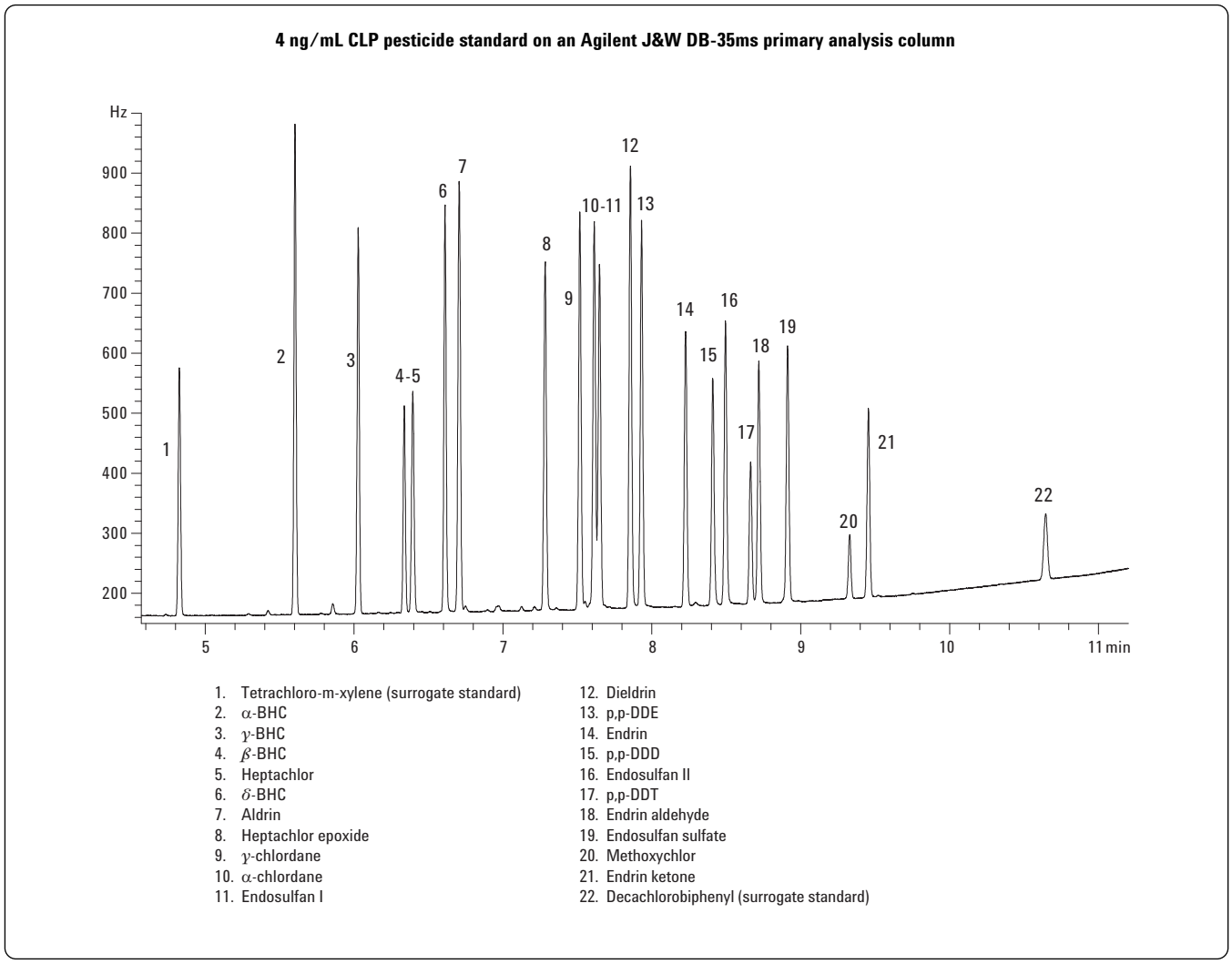

Figure 2. GC-μECD chromatogram of 4 ng/mL standard of CLP pesticides and surrogates standard analyzed on an Agilent J&W DB-35ms, 30 m × 0.25 mm × 0.25 μm column.

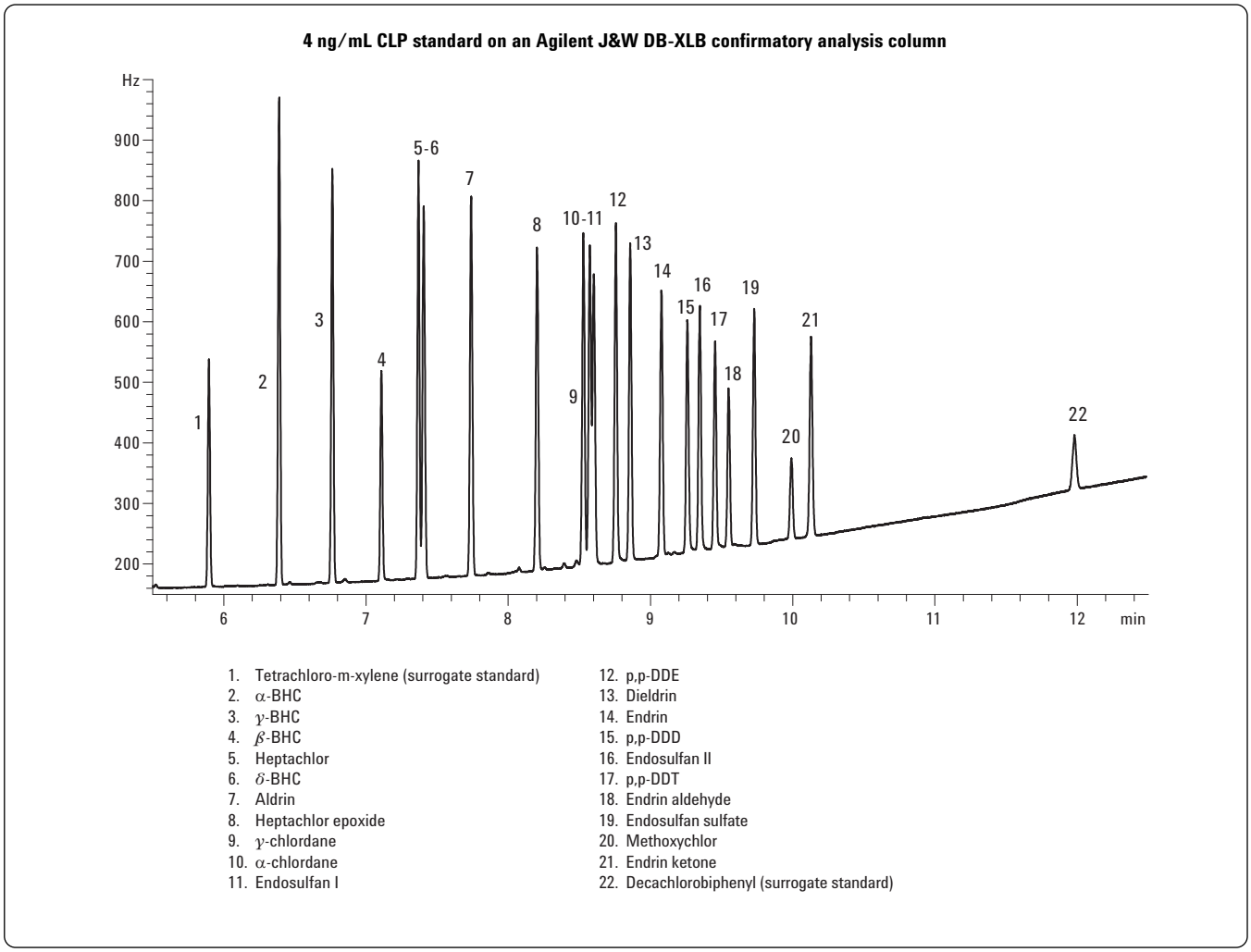

Figure 3. GC-μECD chromatogram of 4 ng/mL standard of CLP pesticides and surrogates standard analyzed on an Agilent J&W DB-XLB, 30 m × 0.25 mm × 0.50 μm column.

Table 3. Calibration Curve - Calibration Standards of 4, 8, 10, 20, and 40 ng/mL Were Prepared in 2,4-Trimethylpentane.

	DB-35 ms	DB-XLB
Compounds	r^2	r^2
α-BHC	0.998	0.998
γ-BHC	0.998	0.998
β-BHC	0.997	0.998
Heptachlor	0.998	0.998
δ-BHC	0.997	0.995
Aldrin	0.997	0.998
Heptachlor epoxide	0.998	0.998
γ-chlordane	0.998	0.997
α-chlordane	0.998	0.996
Endosulfan I	0.997	0.996
p,p-DDE	0.998	0.998
Dieldrin	0.997	0.998
Endrin	0.995	0.997
Endosulfan II	0.999	0.998
Endrin Aldhyde	0.998	0.995
p,p-DDD	0.998	0.998
p,p-DDT	0.999	0.998
Endosulfan sulfate	0.995	0.998
Methoxychlor	0.998	0.996
Endrin Ketone	0.998	0.994

A dual column µECD system resolved the 20 CLP pesticides in the spiked olive oil sample. The primary analytical column DB-35ms separated the 20 CLP pesticides with minor matrix interference in a sample of olive oil spiked with an 80 ng/mL CLP pesticide and surrogate standard. There are several olive oil matrix peaks observed in the spiked samples, including one large peak, that fortunately do not co-elute with the CLP pesticides. Figure 5 clearly shows the separation of all 20 CLP pesticides in an olive oil matrix. In Figure 6, the DB-XLB confirms the presence and separation of the CLP pesticides in the spiked olive oil sample.

Figures 7 and 9 show the overlaid chromatograms on the DB-35ms primary analysis column of the spiked and native olive oil sample with peaks for endosulfan sulfate and endosulfan 1 labeled. Figures 8 and 10 show the overlaid chromatograms on the DB-XLB confirmatory column with peaks for endosulfan sulfate and endosulfan 1 labeled. Chromatogram overlays of the native and spiked olive oil samples identify and confirm endosulfan sulfate and endosulfan 1 as pesticide residues in the olive oil sample. Concentrations from the CLP calibration standard curves were 23.1 ng/mL for endosulfan sulfate and 7.1 ng/mL for endosulfan 1.

The extraction process using the QuEChERS followed by dispersive SPE was effective in retaining the pesticides in the spiked olive oil sample as well as cleaning up the sample matrix for GC-µECD analysis. The fact that endosulfan sulfate and endosulfan 1 were present and detected at low levels in the olive oil sample investigated underscores this point.

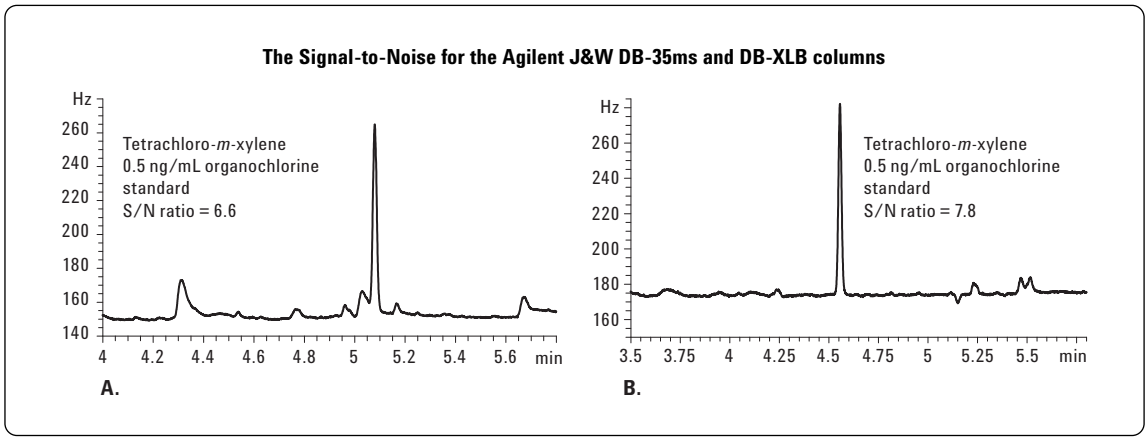

Figure 4. GC-µECD chromatogram of 0.5 ng/mL standard of CLP pesticides and surrogates standard. A.) The S/N on the Agilent J&W DB-35ms, 30 m × 0.25 mm × 0.25 µm column is 6.6. B.) The S/N on an Agilent J&W DB-XLB, 30 m × 0.25 mm × 0.50 µm column is 7.8.

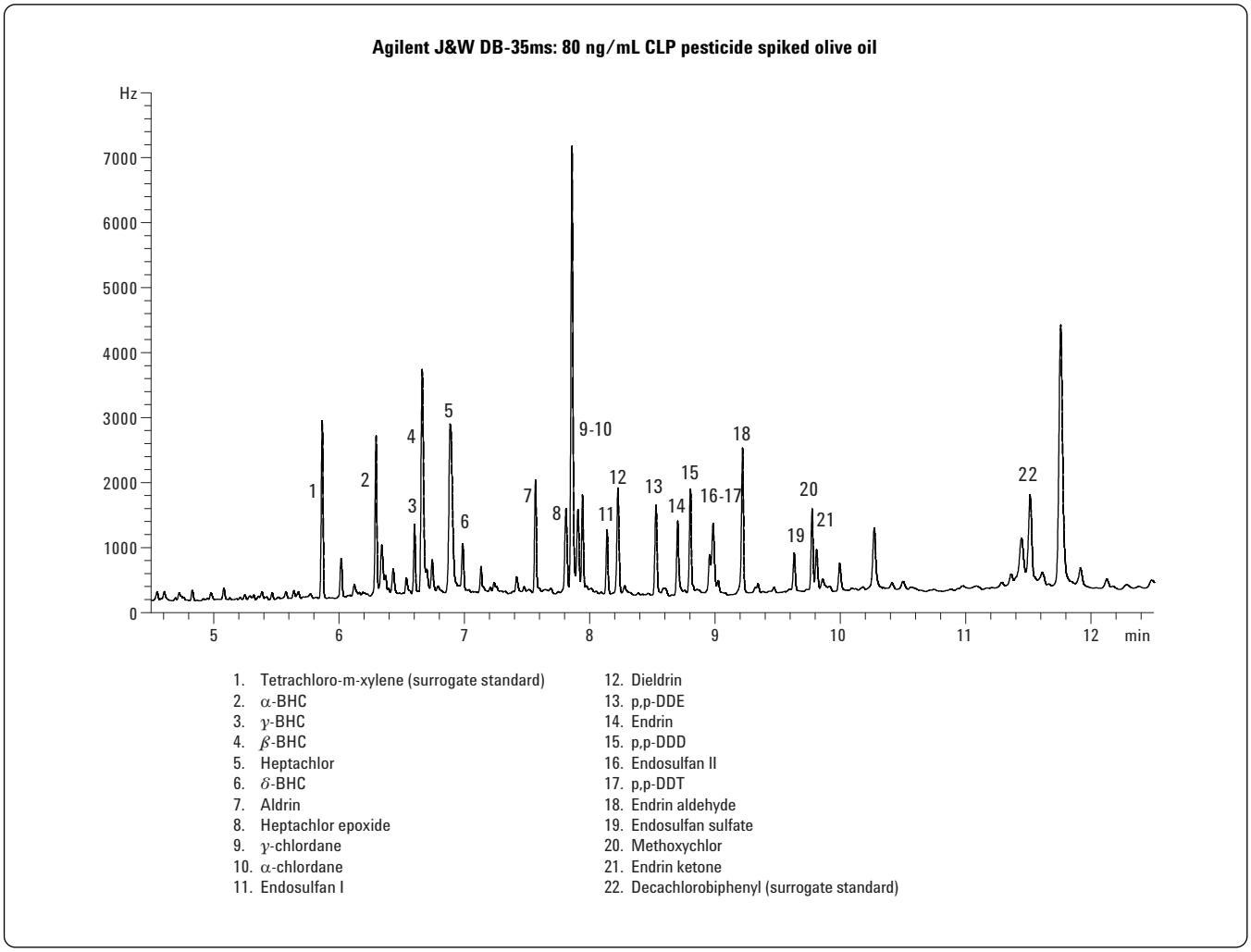

Figure 5. GC-µECD chromatogram of olive oil sample spiked with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-35ms 30 m × 0.25 mm × 0.25 µm column.

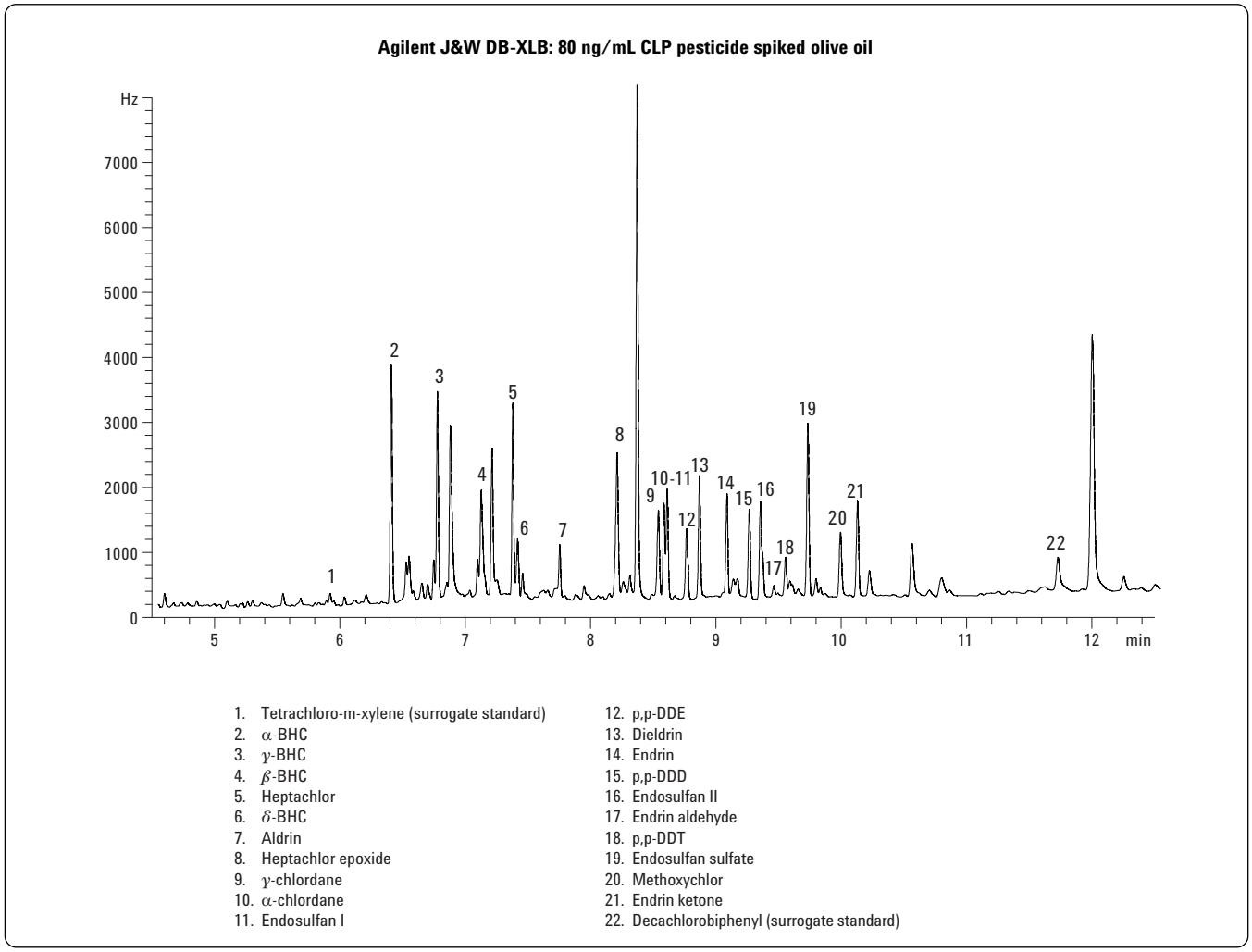

Figure 6. GC-µECD chromatogram of olive oil sample spiked with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-XLB 30 m × 0.25 mm × 0.25 µm column.

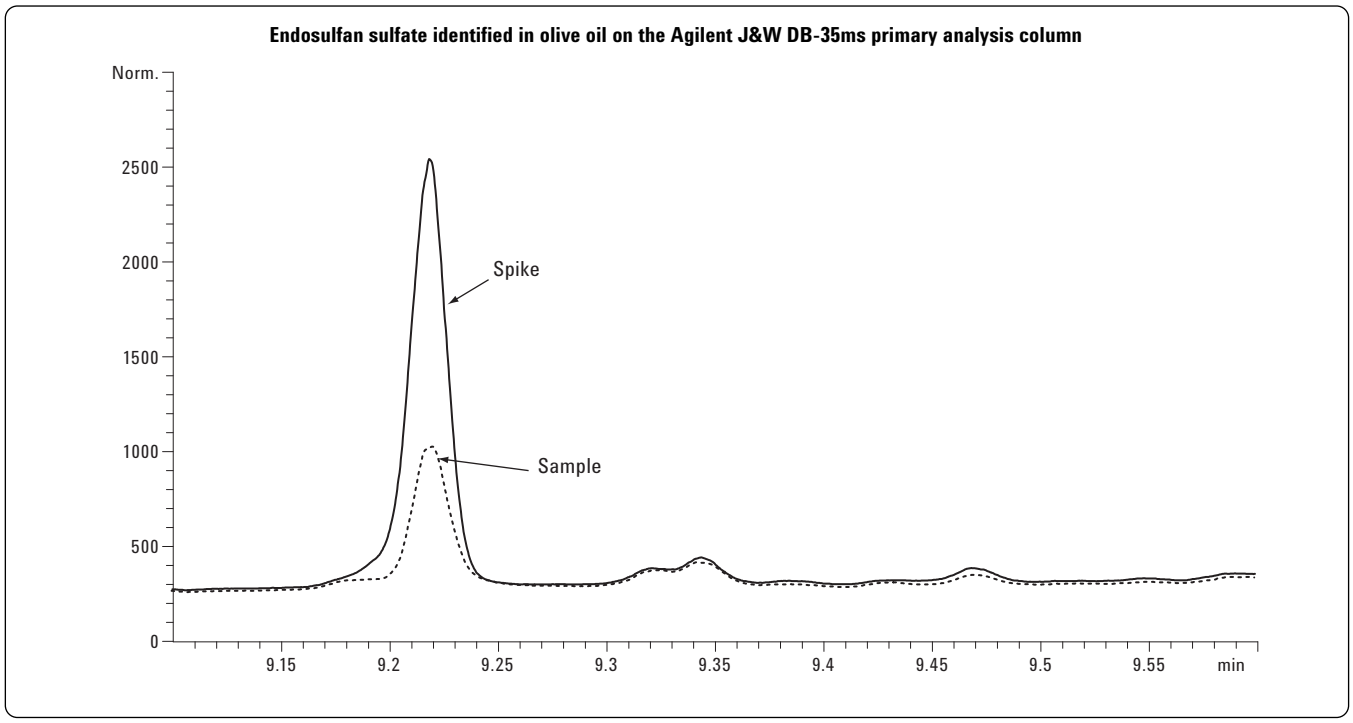

Figure 7. GC-µECD chromatogram of olive oil sample overlaid with olive oil sample spike with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-35ms, 30 m × 0.25 mm × 0.25 µm column. This overlay offers more evidence of the presence of endosulfan sulfates on the Agilent J&W DB-35ms column.

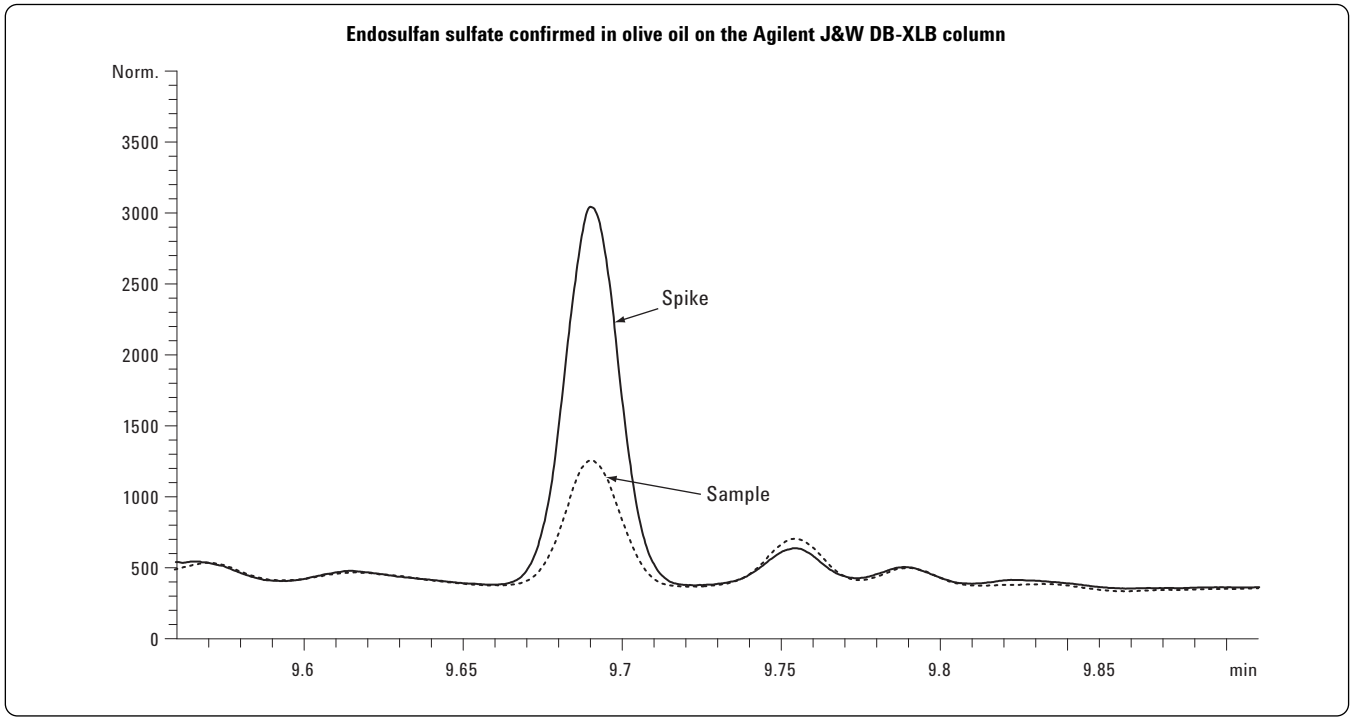

Figure 8. GC-µECD chromatogram of olive oil sample overlaid with olive oil sample spike with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-XLB, 30 m × 0.25 mm × 0.50 µm column. This overlay offers more evidence of endosulfan sulfates presence on the Agilent J&W DB-XLB column.

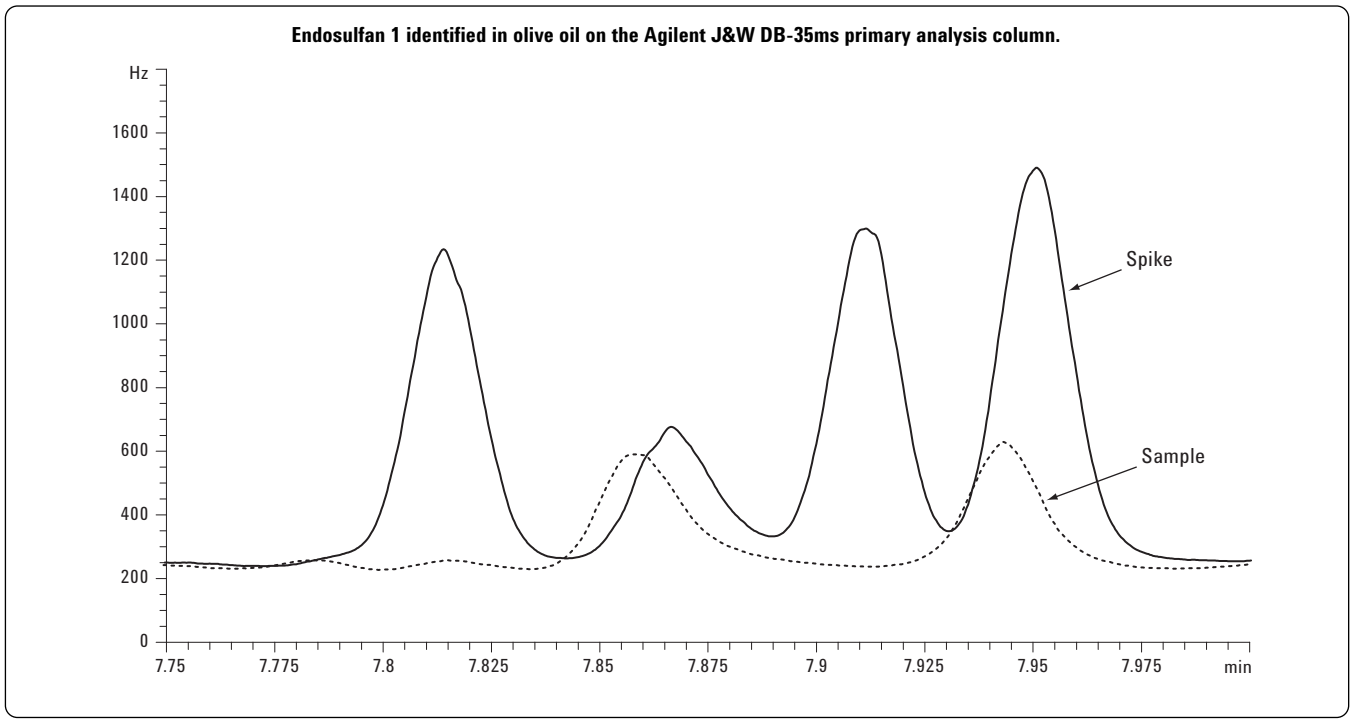

Figure 9. GC-µECD chromatogram of olive oil sample overlaid with olive oil sample spike with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-35ms, 30 m × 0.25 mm × 0.25 µm column. This overlay offers more evidence of endosulfan 1 presence on the Agilent J&W DB-35ms column.

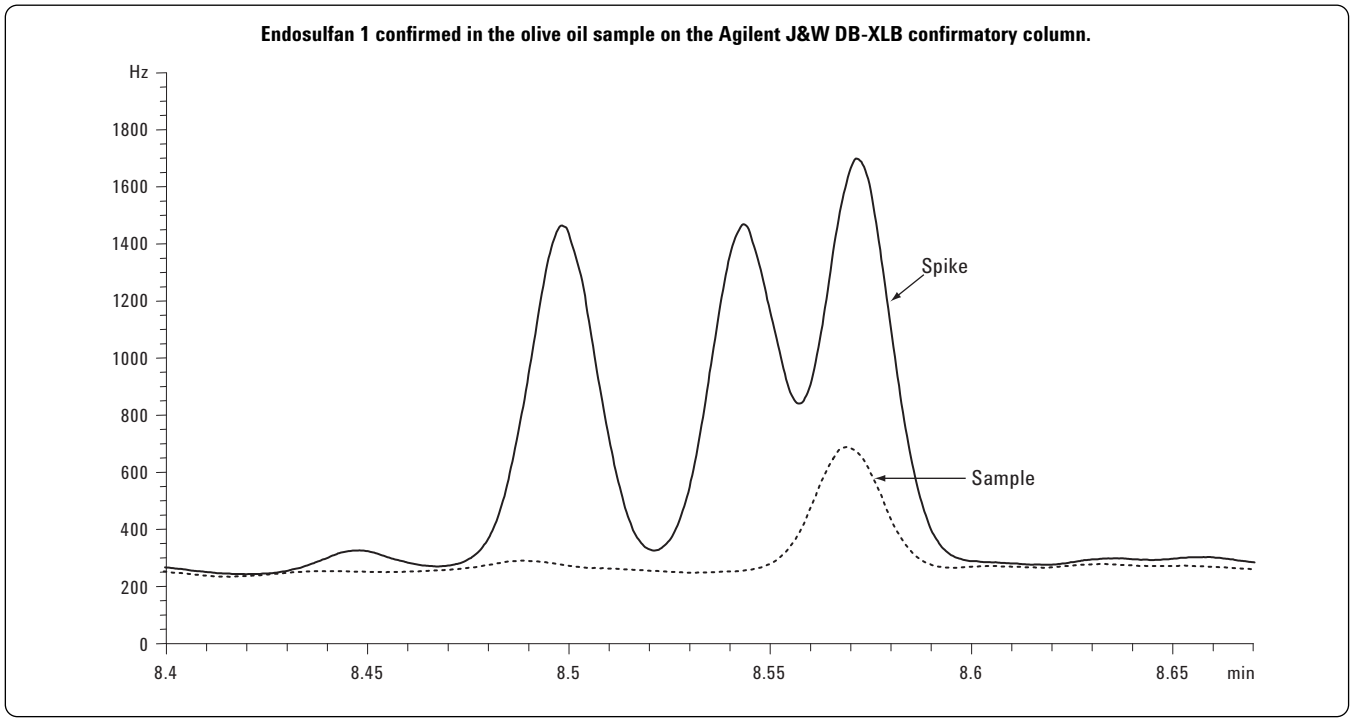

Figure 10. GC-µECD chromatogram of olive oil sample overlaid with olive oil sample spike with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-XLB, 30 m × 0.25 mm × 0.50 µm column. This overlay offers more evidence of endosulfan 1 presence on the Agilent J&W DB-XLB column.

Conclusions

The dual column set of an Agilent J&W DB-35ms primary analytical column and an Agilent J&W DB-XLB confirmatory column on one instrument allows simultaneous identification and confirmation of the presence of the CLP pesticides. The DB-35ms primary analysis and a DB-XLB confirmatory column with dual µ-ECD detection were effective at analyzing 20 CLP pesticides in an olive oil matrix following sample matrix cleanup. The single injection, dual column approach improves productivity by saving instrument and analyst time. Continuous improvements and stringent process control with respect to column activity make the DB-35ms and DB-XLB column pair an excellent choice for analysis of active analytes such as pesticides.

This note successfully shows a robust, inexpensive analytical method to monitor CLP pesticides in olive oil suitable to address food safety concerns. This method demonstrates the feasibility of using a dual column µECD approach for routine olive oil screening as an alternative to GC/MS.

QuEChERS followed by dSPE were effective at providing just enough sample cleanup to avoid matrix interferences while still maintaining low-level analyte detection. The performance of the dual column set DB-35ms and DB-XLB on the GC-µECD had excellent linearity over the range of concentrations studied with R^2 values between 0.994 and 0.999 for the individual pesticides. The limits of detection (LOD) quantitative (LOQ) for this analysis were 0.3 ng/mL and 1.0 ng/mL, demonstrating low level detection capability. QuEChERS followed by dSPE also provided a fast and effective way to prepare the sample.

The primary analysis DB-35ms column identified endosulfan sulfate and endosulfan 1 in the olive oil sample. The DB-XLB confirmatory column confirmed that endosulfan sulfate and endosulfan 1 were present in the olive oil sample. Overlaid chromatograms of pesticide spiked and native olive oil samples on both columns provided additional evidence confirming the presence of these two pesticides through a process of pattern recognition. The evidence proves that these two pesticides were present in this grocery store olive oil.

The fact that a single randomly selected commercial olive oil sample came back positive for two pesticide residues indicates residues are present in these oils. Clearly, there is a need to monitor olive oil for pesticide residues through tests such as the dual column dual GC-µECD analysis described in this note.

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Printed in the USA
March 30, 2010
5990-5553EN

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Determination of Dioxin-Like and Non-Dioxin-Like Polychlorinated Biphenyl Congeners in Foodstuffs and Animal Feed Using the Agilent 7000 Triple Quadrupole GC/MS System

Application Note

Food Safety

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Abstract

Two methods have been developed on the Agilent 7000 Triple Quadrupole GC/MS system for the analysis of polychlorinated biphenyl (PCB) congeners in foodstuffs and animal feed. The methods were shown to give linear response over the required concentration ranges. In addition, quantitative results for dioxin-like PCB (dl-PCB) congeners down to low pg TEQ/g levels and non-dioxin-like PCB (ndl-PCB) congeners at levels below 1 ng/g product were in good agreement with values obtained using a GC-High Resolution mass spectrometer. This application note demonstrates the determination of the 12 dl-PCB comprising eight mono-*ortho* PCB congeners (# 105, 114, 118, 123, 156, 157, 167 and 189) and four non-*ortho* PCB congeners (# 77, 81, 126 and 169) as well as the six ndl-PCB congeners (# 28, 52, 101, 138, 153 and 180) that are also known as “Indicator PCB” congeners.

Agilent Technologies

Introduction

Polychlorinated biphenyls (PCB) are highly toxic Persistent Organic Pollutants (POP) with properties that are detrimental to human health. They have been linked to cancer, endocrine disruption and reproductive disorders. Until their ban in the late 20th Century, PCBs were widely manufactured for use in hundreds of industrial and commercial applications including electrical products and hydraulic equipment and as plasticizers in paints, plastics and, rubber products. PCB congeners that have been released into the environment can bio-accumulate in animal tissues and thereby enter the human food chain.

Current legislation in the United States [1] and the European Union (EU) [2], [5] require the confirmation and quantitation of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) and dioxin-like polychlorinated biphenyl congeners (dl-PCB) in foodstuffs and animal feed by isotope dilution capillary gas chromatography–high resolution mass spectrometry (GC-HRMS). The analysis of dioxins and furans in foodstuffs and animal feed by gas chromatography-triple quadrupole mass spectrometry is shown in a previously published Agilent application note [3].

Maximum levels for PCDD, PCDF and dl-PCB congeners in foodstuffs and animal feed are given in additional EU regulations [4], [6]. dl-PCB congeners have each been assigned a Toxic Equivalency Factor (TEF) that relates the toxicity of each individual dl-PCB congener to 2,3,7,8 Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), which itself is assigned a TEF of 1. The individual concentration of each dl-PCB found in foodstuffs and animal feed samples is multiplied by it's respective TEF and after summation the total concentration is expressed as the Toxic Equivalent (TEQ) in terms of pg TEQ/g fat, pg TEQ/g fresh weight (fish) or ng TEQ/kg in 88% dry feed.

The World Health Organization (WHO) through the International Program on Chemical Safety (IPCS) originally established and then re-evaluated TEF for PCDD, PCDF and dl-PCB. Original TEF values were established by WHO/IPCS expert consultation in 1997 and re-evaluated in 2005. As a result, some TEF values have been changed and it is important to clearly state the set of TEF values used by indicating the year in which they were first expressed (TEF_{WHO98} or TEF_{WHO05}) and the resultant TEQ_{WHO98} and TEQ_{WHO05} values. TEF values assigned to PCDD, PCDF and dl-PCB are shown in Table 1.

The maximum levels (based on TEF_{WHO98} values) for PCDD, PCDF and dl-PCB in certain foodstuffs as prescribed by EU legislation are given in Table 2.

Table 1. WHO Toxic Equivalency Factors (TEF_{WHO98} and TEF_{WHO05}) for PCDD, PCDF and dl-PCB Congeners from Assessments Made in 1998 and 2005 (Changed Values in Italics)

Compound	TEF WHO_{98}	TEF WHO_{05}	Compound	TEF WHO_{98}	TEF WHO_{05}
Chlorinated dibenzo-p-dioxins			**Non-*ortho* substituted PCB**		
2378-TCDD	1	1	PCB-77	0.0001	0.0001
12378-PeCDD	1	1	PCB-81	0.0001	*0.0003*
123478-HxCDD	0.1	0.1	PCB-126	0.1	0.1
123678-HxCDD	0.1	0.1	PCB-169	0.01	*0.03*
123789-HxCDD	0.1	0.1			
1234678-HpCDD	0.01	0.01			
OCDD	0.0001	*0.0003*			
Chlorinated dibenzofurans			**Mono-*ortho* substituted PCB**		
2378-TCDF	0.1	0.1	PCB-105	0.0001	*0.00003*
12378-PeCDF	0.05	*0.03*	PCB-114	0.0005	*0.00003*
23478-PeCDF	0.5	*0.3*	PCB-118	0.0001	*0.00003*
123478-HxCDF	0.1	0.1	PCB-123	0.0001	*0.00003*
123678-HxCDF	0.1	0.1	PCB-156	0.0005	*0.00003*
234678-HxCDF	0.1	0.1	PCB-157	0.0005	*0.00003*
123789-HxCDF	0.1	0.1	PCB-167	0.00001	*0.00003*
1234678-HpCDF	0.01	0.01	PCB-189	0.0001	*0.00003*
1234789-HpCDF	0.01	0.01			
OCDF	0.0001	*0.0003*			

Table 2. Maximum Levels (Upperbound concentrations) for PCDD, PCDF and dl-PCB Congeners in Certain Foodstuffs, as Specified in EU Regulation (EC) No 1881/2006

Foodstuff	Maximum levels Sum of dioxins (WHO-PCDD/F-TEQ)	Maximum levels Sum of dioxins and dl-PCB (WHO-PCDD/F-PCB-TEQ)
Meat and meat products (excluding edible offal) of the following animals :		
- Bovine animals and sheep	3.0 pg/g fat	4.5 pg/g fat
- Poultry	2.0 pg/g fat	4.0 pg/g fat
- Pigs	1.0 pg/g fat	1.5 pg/g fat
- Raw milk and dairy products, including butter fat	3.0 pg/g fat	6.0 pg/g fat
- Hens eggs and egg products	3.0 pg/g fat	6.0 pg/g fat

The action levels for the sum of the 12 dl-PCB congeners in certain foodstuffs and certain feedstuffs are shown in Tables 3 and 4, respectively.

The six ndl-PCB congeners do not have TEF values and their results are expressed simply as the sum of the six individual congeners.

The chemical structures of the 12 dl-PCB congeners and 6 ndl-PCB congeners are shown in Figures 1 and 2, respectively.

This application note describes sensitive and reproducible methods for the screening of dl-PCB congeners and ndl-PCB congeners in foodstuffs and animal feed using the Agilent 7000 Triple Quadrupole GC/MS system that meets the requirements of EU Legislation for a screening method.

Table 3. Action Levels (Upperbound Concentrations) for the Sum of dl-PCB Congeners in Certain Foodstuffs, as Specified in EU Commission recommendation 2006/88

Food	Action level for dioxin-like PCBs (TEQ_{WHO98})
Meat and meat products of	
Runinants (bovine animals, sheep)	1.0 pg/g fat
Poultry and farmed game	1.5 pg/g fat
Pigs	0.5 pg/g fat
Liver and derived products of terrestrial animals	4.0 pg/g fat
Muscle meat of fish and fishery products and products thereof with the exception of eel	3.0 pg/g fresh weight
Muscle meat of eel (Anguilla anguilla) and products thereof	6.0 pg/g fresh weight
Milk and milk products, including butter fat	2.0 pg/g fat
Hens eggs and egg products	2.0 pg/g fat

Table 4. Action Levels (Upperbound Concentrations) for the Sum of dl-PCB Congeners in Certain Feedstuffs, as Specified in EU Regulation 2002/32/EC

Product intended for animal feed	Action threshold relative to a feeding stuff with a moisture content of 12%
Feed materials of plant origin with the exception of vegetable oils and their byproducts	0.35 ng TEQ/kg
Vegetable oils and their byproducts	0.5 ng TEQ/kg
Feed materials of mineral origin	0.35 ng TEQ/kg
Animal fat, including milk fat and egg fat	0.75 ng TEQ/kg
Other land animal products including milk and milk products and eggs and egg products	0.35 ng TEQ/kg
Fish oil	14.0 ng TEQ/kg

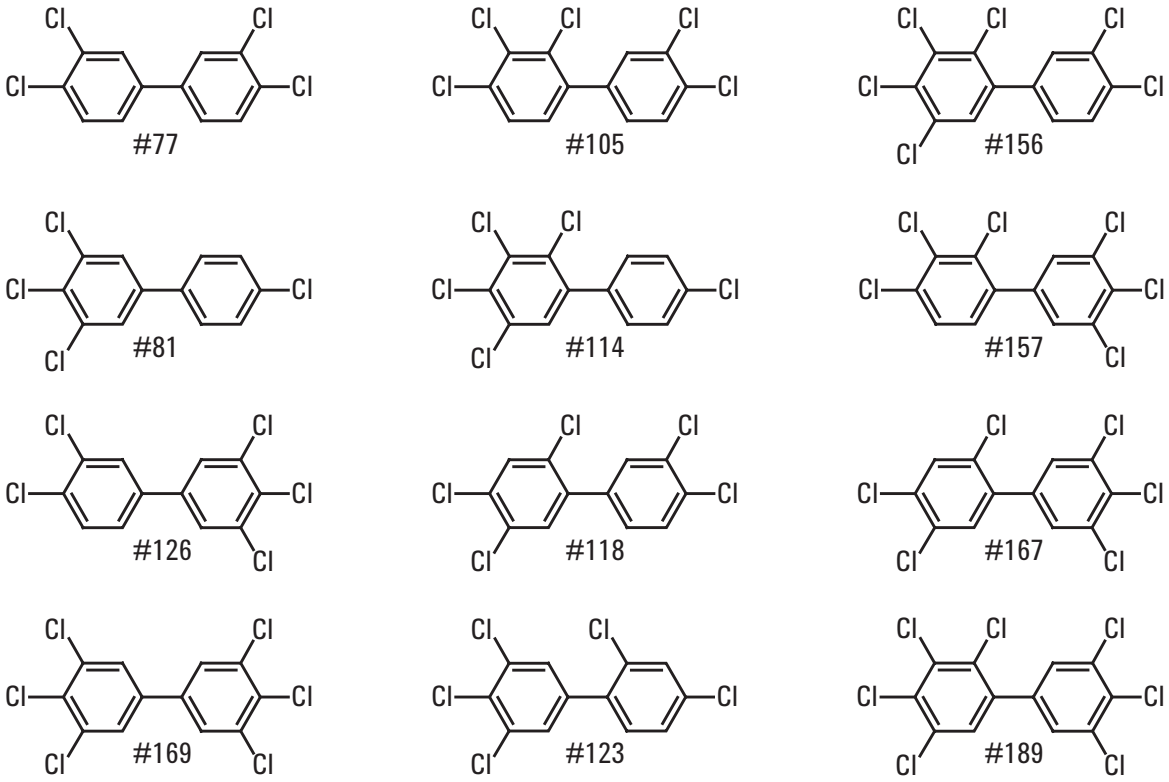

Figure 1. Chemical structures of dl-PCB congeners.

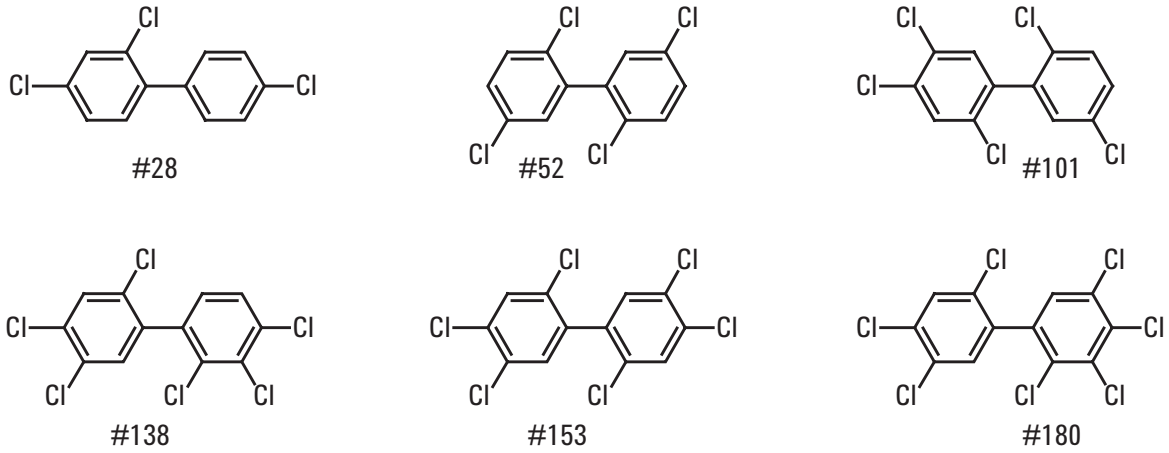

Figure 2. Chemical structures of ndl-PCB congeners.

Experimental

Calibration Standards

Calibration mixtures of native PCB congeners and their ^{13}C-isotope labelled internal standards were obtained from Cambridge Isotope Laboratories and Wellington Laboratories Inc.

Sample Preparation and Analysis

The most frequently used methods for the determination of PCDD, PCDF, dl-PCB congeners and ndl-PCB congeners in foodstuffs and animal feed combine fat extraction (for example, Soxhlet or extraction with organic solvents) with cleanup steps using different column chromatographies such as silica gel coated with sulfuric acid, florisil, alumina, and active carbon. The final extracts are collected as three fractions containing the mono-*ortho* PCB congeners and indicator PCB congeners (1a, Figure 3), non-*ortho* PCB congeners (1b, Figure 3) and PCDD/F (2, Figure 3), by eluting with various solvents. After addition of a syringe spike of ^{13}C- labelled PCB internal standards, the extracts were evaporated under a gentle stream of nitrogen and subsequently reconstituted with toluene and analyzed with GC/MS/MS. The PCDD/F fraction was reconstituted with 20 µL of toluene, the non-*ortho* PCB fraction with 40 µL of toluene and the mono-*ortho*/indicator PCB fraction with 250 µL of toluene.

A flow diagram summarizing the sample preparation steps is shown in Figure 3.

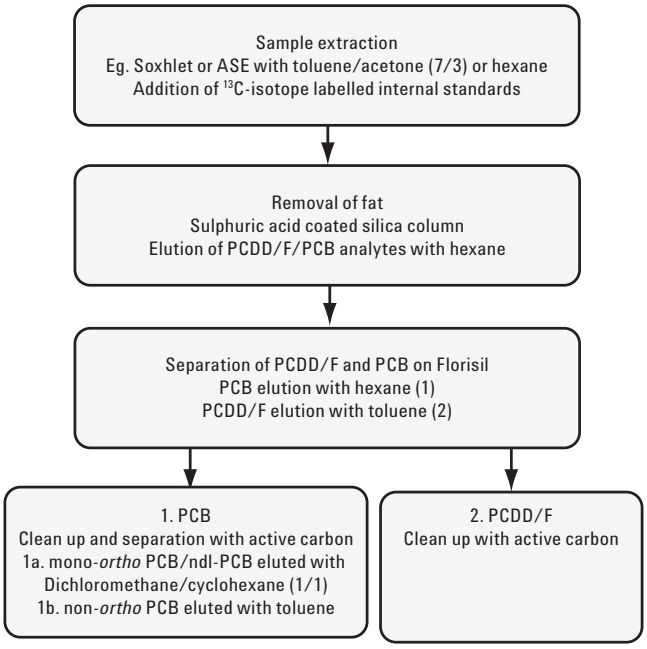

Figure 3. Flow diagram of the sample extraction and cleanup procedures.

The analyses were performed on an Agilent 7890 GC and an Agilent 7000 Triple Quadrupole GC/MS system. The 7890 Series GC was configured with a carbon dioxide cooled Multimode Inlet (MMI) and an HT-8 50 m × 0.22 mm, 0.25 µm capillary column.

The GC instrument conditions for the mono-*ortho* PCB congeners are listed in Table 5 and the GC instrument conditions for the non-*ortho* PCB congeners are given in Table 6. MS parameters, common to both sets of PCB congeners, are shown in Table 7. The 7000 Triple Quadrupole GC/MS was operated in MS/MS-EI (electron ionization) Multiple Reaction Monitoring (MRM) mode. Each analyte and its associated ^{13}C-Internal standard (ISTD) were measured using two different precursor ions and two different product ions.

Table 5. GC Conditions for Mono-ortho and dl-PCB Congeners

Column	HT-8 50 m × 0.22 mm id, 0.25 µm
Injection	2 µL cold splitless using CO_2 cooled Multimode Inlet (MMI)
Injection port liner	4 mm id, unpacked
Inlet temperature program	100 °C (0.02 min), 500 °C/min to 300 °C
Purge flow to split vent	50 mL/min at 1.0 min
Carrier gas	Helium, constant flow 1.2 mL/min
Oven program	80 °C (3.0 min hold), 20 °C/min to 160 °C (0 min), 4 deg °C/min to 300 °C (8 min), (Total run time = 50.0 minutes)
MS transfer line temp	280 °C

Table 6. GC Conditions for Non-ortho-PCB Congeners

Column	HT-8 50 m × 0.22 mm id, 0.25 µm
Injection	2 µL cold splitless using CO_2 cooled Multimode Inlet (MMI)
Injection port liner	4 mm id with glass wool
Inlet temperature program	100 °C (0.02 min), 500 °C/min to 300 °C
Purge flow to split vent	50 mL/min at 1.0 min
Carrier gas	Helium, constant flow 1.2 mL/min
Oven program	120 °C (2.0 min hold), 40 °C/min to 160 °C (0 min), 7 deg °C/min to 300 °C (10 min), (Total run time = 33.0 minutes)
MS transfer line temp	280 °C

Table 7. MS Setpoints for all PCB Congeners

Electron energy	–78 EV
Tune	EI Autotune
EM gain	100
MS1 resolution	Unit
MS2 resolution	Wide
Quant/Qual transitions	Table 8/Table 9
Dwell times	Table 8/Table 9
Collision energies	Table 8/Table 9
Collision cell gas flows	Nitrogen at 1.5 mL/min, Helium at 2.25 mL/min
MS temperatures	Ion source 280 °C, MS1 150 °C, MS2 150 °C

A full list of the analyte retention times, MRM settings and dwell times for the mono-*ortho* and ndl-PCB congeners and the non-*ortho* PCB congeners are given in Tables 8 and 9, respectively.

An Agilent 7693A Automatic Liquid Sampler with the sampler tray cooled to 5 °C was used and 2 µL cold splitless injections were made using a 10-µL syringe.

Table 8. MS/MS Settings for Native Mono-Ortho and ndl-PCB Congeners (ndl-PCB Congeners Shown in Bold Italics) and ^{13}C-Internal Standards

TS	Segment start time (min)	Analyte	RT (min)	Quant pre-cursor	Product	Dwell (ms)	CE (V)	Qual pre-cursor	Product	Dwell (ms)	CE (V)
1	22.0	***^{13}C-PCB 28***	***24.34***	***268.0***	***198.1***	***25***	***26***	***270.0***	***198.1***	***25***	***26***
		PCB 28	***24.35***	***256.0***	***186.0***	***75***	***26***	***258.0***	***186.0***	***75***	***26***
		^{13}C-PCB 52	***25.66***	***302.0***	***232.0***	***25***	***28***	***304.0***	***234.0***	***25***	***28***
		PCB 52	***25.67***	***289.9***	***220.0***	***75***	***28***	***291.9***	***222.0***	***75***	***28***
2	29.0	***^{13}C-PCB 101***	***30.15***	***335.9***	***266.0***	***25***	***28***	***337.9***	***268.0***	***25***	***28***
		PCB 101	***30.16***	***323.9***	***253.9***	***75***	***28***	***325.9***	***255.9***	***75***	***28***
		^{13}C-PCB 123	33.55	335.9	266.0	25	28	337.9	268.0	25	28
		PCB 123	33.56	323.9	253.9	75	28	325.9	255.9	75	28
		^{13}C-PCB 118	33.76	335.9	266.0	25	28	337.9	268.0	25	28
		PCB 118	33.77	323.9	253.9	75	28	325.9	255.9	75	28
		^{13}C-PCB 141	34.00	371.9	301.9	25	28	369.9	299.9	25	28
		^{13}C-PCB 114	34.19	335.9	266.0	25	28	337.9	268.0	25	28
		PCB 114	34.20	323.9	253.9	75	28	325.9	255.9	75	28
		^{13}C-PCB 153	***34.50***	***371.9***	***301.9***	***25***	***28***	***369.9***	***299.9***	***25***	***28***
		PCB 153	***34.51***	***359.8***	***289.9***	***75***	***28***	***357.8***	***287.9***	***75***	***28***
		^{13}C-PCB 105	35.15	335.9	266.0	25	28	337.9	268.0	25	28
		PCB 105	35.16	323.9	253.9	75	28	325.9	255.9	75	28
		^{13}C-PCB 138	***35.88***	***371.9***	***301.9***	***25***	***28***	***369.9***	***299.9***	***25***	***28***
		PCB 138	***35.89***	***359.8***	***289.9***	***75***	***28***	***357.8***	***287.9***	***75***	***28***
		^{13}C-PCB 167	37.64	371.9	301.9	25	28	369.9	299.9	25	28
		PCB 167	37.65	359.8	289.9	75	28	357.8	287.9	75	28
3	38.5	^{13}C-PCB 156	38.78	371.9	301.9	25	28	369.9	299.9	25	28
		PCB 156	38.79	359.8	289.9	75	28	357.8	287.9	75	28
		^{13}C-PCB 157	39.06	371.9	301.9	25	28	369.9	299.9	25	28
		PCB 157	39.07	359.8	289.9	75	28	357.8	287.9	75	28
		^{13}C-PCB 180	***39.17***	***407.8***	***337.9***	***25***	***30***	***405.8***	***335.9***	***25***	***30***
		PCB 180	***39.18***	***393.8***	***323.9***	***75***	***30***	***395.8***	***325.9***	***75***	***30***
		^{13}C-PCB 189	42.43	407.8	337.9	25	30	405.8	335.9	25	30
		PCB 189	42.44	393.8	323.9	75	30	395.8	325.9	75	30

Table 9. MS/MS Settings for Native Non-Ortho PCB Congeners and ^{13}C-Internal Standards

TS	Segment start time (min)	Analyte	RT (min)	Quant pre-cursor	Product	Dwell (ms)	CE (V)	Qual pre-cursor	Product	Dwell (ms)	CE (V)
1	19.0	^{13}C-PCB 81	20.74	301.9	232.0	25	28	303.9	234.0	25	28
		PCB 81	20.75	289.9	220.0	125	28	291.9	222.0	125	28
		^{13}C-PCB 77	21.12	301.9	232.0	25	28	303.9	234.0	25	28
		PCB 77	21.13	289.9	220.0	125	28	291.9	222.0	125	28
2	22.0	^{13}C-PCB 126	23.55	335.9	265.9	25	28	337.9	267.9	25	28
		PCB 126	23.56	323.9	253.9	125	28	325.9	255.9	125	28
3	25.0	^{13}C-PCB 169	26.26	371.9	301.9	25	28	369.9	299.9	25	28
		PCB 169	26.27	359.9	289.9	125	28	357.8	287.9	125	28

Results and Discussion

Chromatography

The multiple reaction monitoring (MRM) chromatograms for the native mono-*ortho* and ndl-PCB congeners, with an analysis time of 50 minutes, are shown in Figure 4. The multiple reaction monitoring (MRM) chromatograms for the native non-*ortho* PCB congeners, with an analysis time of 33 minutes, are shown in Figure 5.

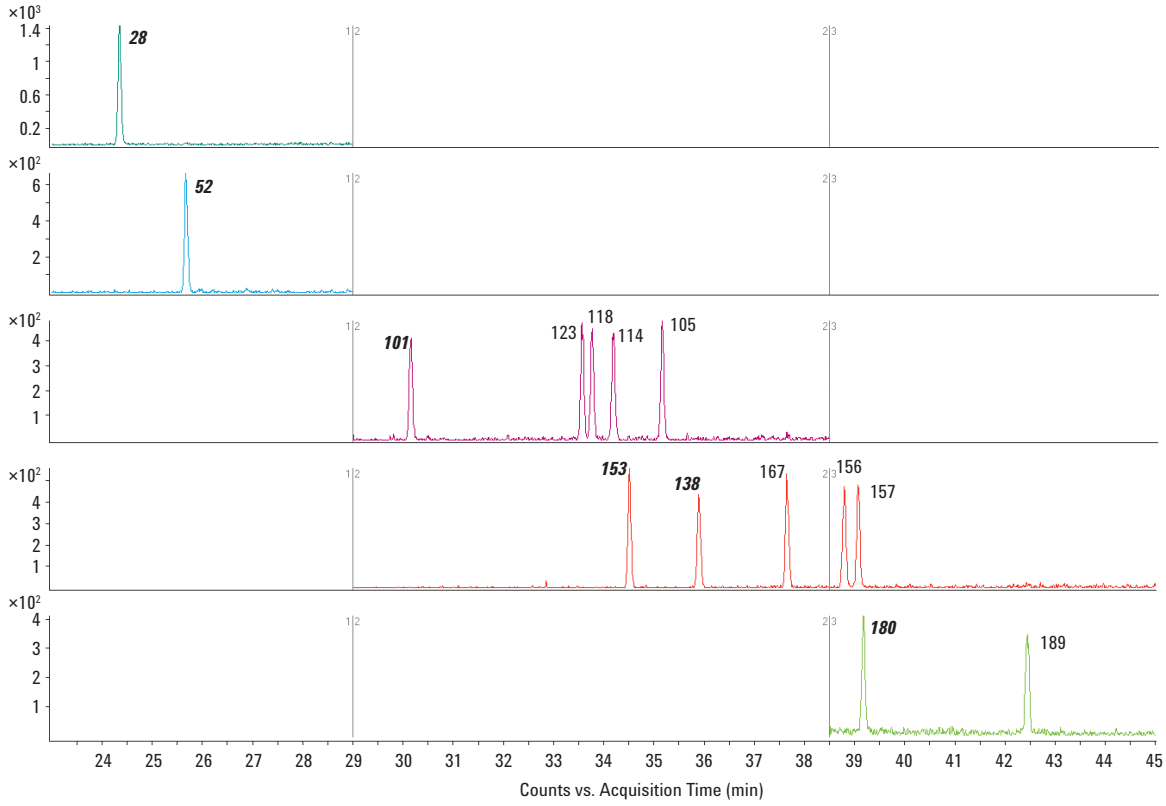

Figure 4. MRM chromatograms of native Mono-ortho and ndl-PCB congeners (ndl-PCB congeners labelled in bold italics).

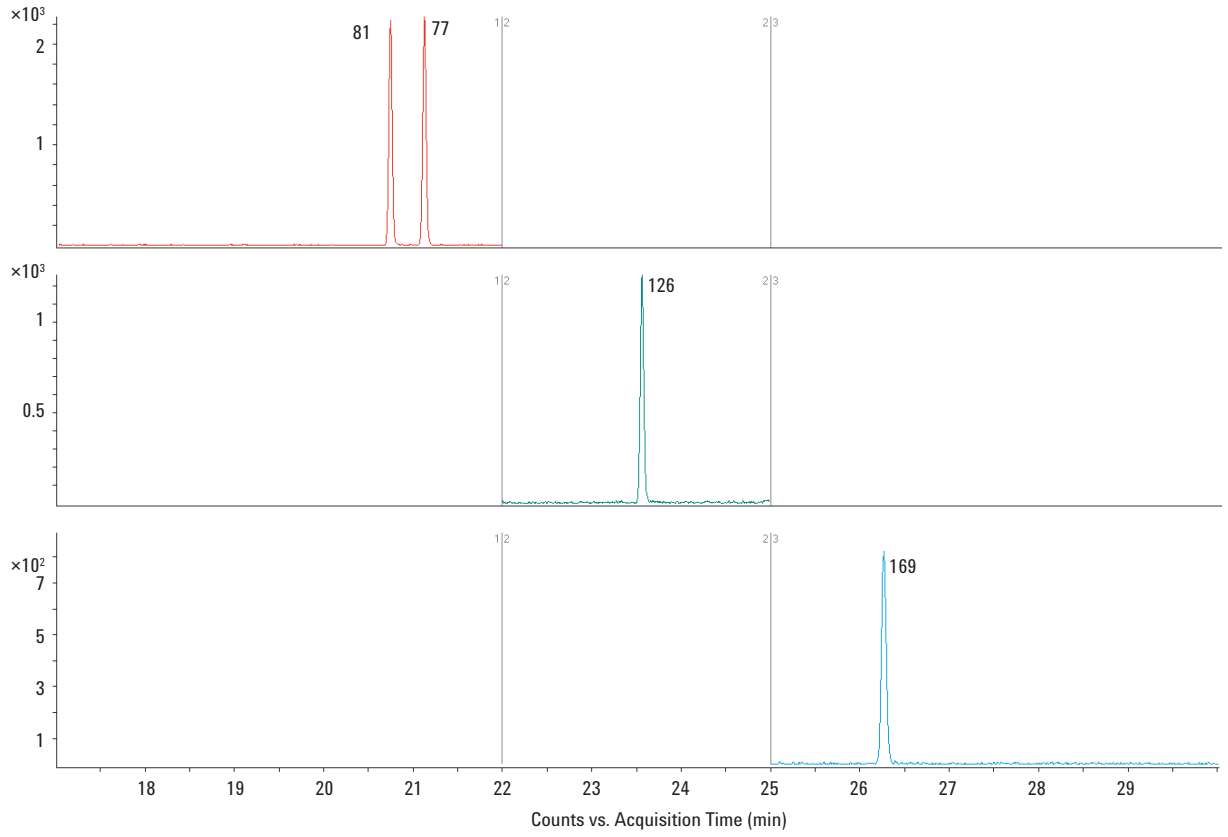

Figure 5. MRM chromatograms of native Non-ortho PCB congeners.

Linearity of Response

All PCB congeners were measured using ^{13}C-labelled internal standard (ISTD) calibration. Seven-point ISTD calibration curves were created using calibration standard solutions at the concentrations given in Tables 10 and 11, respectively.

Table 10. Concentration of Native Mono-Ortho and ndl-PCB Congeners and their ^{13}C-ISTD Calibration Standards

Mono-ortho PCB	Natives pg/µL	^{13}C pg/µL	^{13}C (PCB 180, 153, 138 141 = recovery) pg/µL
M1	0.05	5.00	50.0
M2	0.15	5.00	50.0
M3	0.50	5.00	50.0
M4	1.50	5.00	50.0
M5	5.00	5.00	50.0
M6	15.00	5.00	50.0
M7	50.00	5.00	50.0

Table 11. Concentration of Native Non-Ortho PCB Congeners and their ^{13}C- ISTD Calibration Standards

Non-Ortho PCB	Natives pg/µL	^{13}C pg/µL
C1	0.10	2.50
C2	0.25	2.50
C3	0.50	2.50
C4	1.00	2.50
C5	2.50	2.50
C6	5.00	2.50
C7	10.00	2.50

Excellent linearity was obtained over the required concentration range for all the PCB congeners and example calibration curves for PCB 126 and PCB 169 are shown in Figures 6 and 7, respectively.

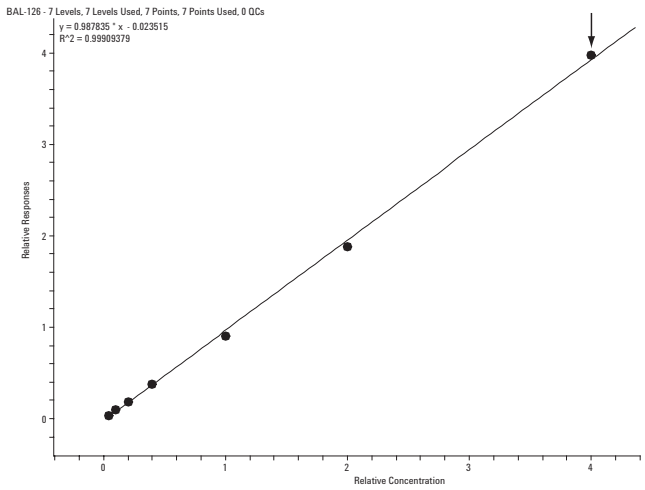

Figure 6. 7-point ISTD calibration curve for PCB 126 with linear fit.

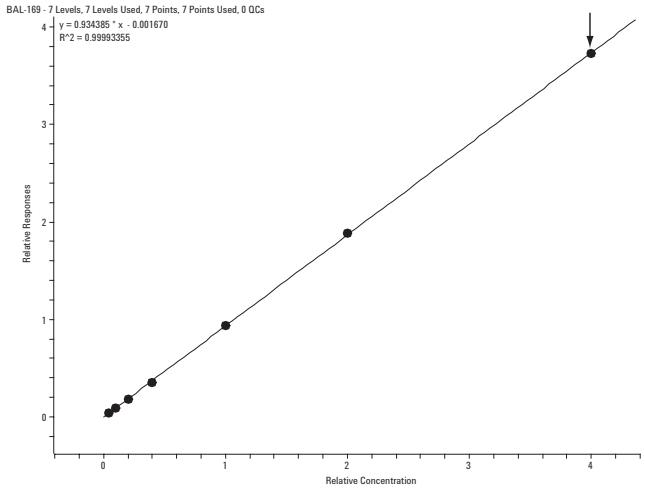

Figure 7. Seven-point ISTD calibration curve for PCB 169 with linear fit.

The linear calibration curve fits for all the PCB congeners are shown in Table 12. All analytes gave linear curve fit coefficients (R^2) greater than 0.998.

Sample Analysis

Eighty samples of four different foodstuffs and animal feed: animal feed (n = 45), cows' milk (n = 11), meat (n = 19) and liver (n = 5) were extracted and analyzed using a GC-High Resolution Mass Spectrometer (GC-HRMS) at a resolution of

Table 12. Linear Correlation Coefficients for Seven-Point ISTD Calibration Curves Over the Range 0.05 pg/µL – 50 pg/µL for Mono-Ortho and ndl-PCB Congeners and 0.1 pg/µL – 10 pg/µL for Non-Ortho PCB Congeners, Injection Volume = 2 µL

Mono-*ortho* PCB	R^2	Non-*ortho* PCB	R^2
PCB 28	0.9999	PCB 81	0.9992
PCB 52	0.9993	PCB 77	0.9991
PCB 101	0.9991	PCB 126	0.9991
PCB 123	0.9997	PCB 169	0.9999
PCB 118	0.9994		
PCB 114	0.9998		
PCB 153	0.9997		
PCB 105	0.9999		
PCB 138	0.9993		
PCB 167	0.9988		
PCB 156	0.9985		
PCB 157	0.9987		
PCB 180	0.9995		
PCB 189	0.9990		

R = 10,000. The same sample vials were then transferred to the Agilent 7000 Triple Quadrupole GC /MS system and reanalyzed.

Figure 8 shows the comparative sample results (total TEQ-dl-PCB, upperbound values) of the two sets of measurements expressed as the percentage difference between the results obtained by the GC-HRMS and GC/MS/MS analyses.

The agreement between the results obtained for the total of the 12 dl-PCB congeners on the GC-HRMS and the GC/MS/MS system for foodstuffs and animal feed samples at levels above 1 pg TEQ/g were within the range of ± 10%.

The comparative results for the 68 foodstuffs and animal feed samples that gave total dl-PCB results less than 1.2 TEQ pg/g are shown in Figure 9.

The agreement between the results obtained for the sum of the 12 dl-PCB congeners on the GC-HRMS and the GC/MS/MS system for foodstuffs and animal feed samples at levels between 0.1 and 1 pg TEQ/g was within the range of ± 15%. Only those animal feed samples with total dl-PCB congener concentrations below 0.1 TEQ pg/g gave some results with percentage differences greater than 15%.

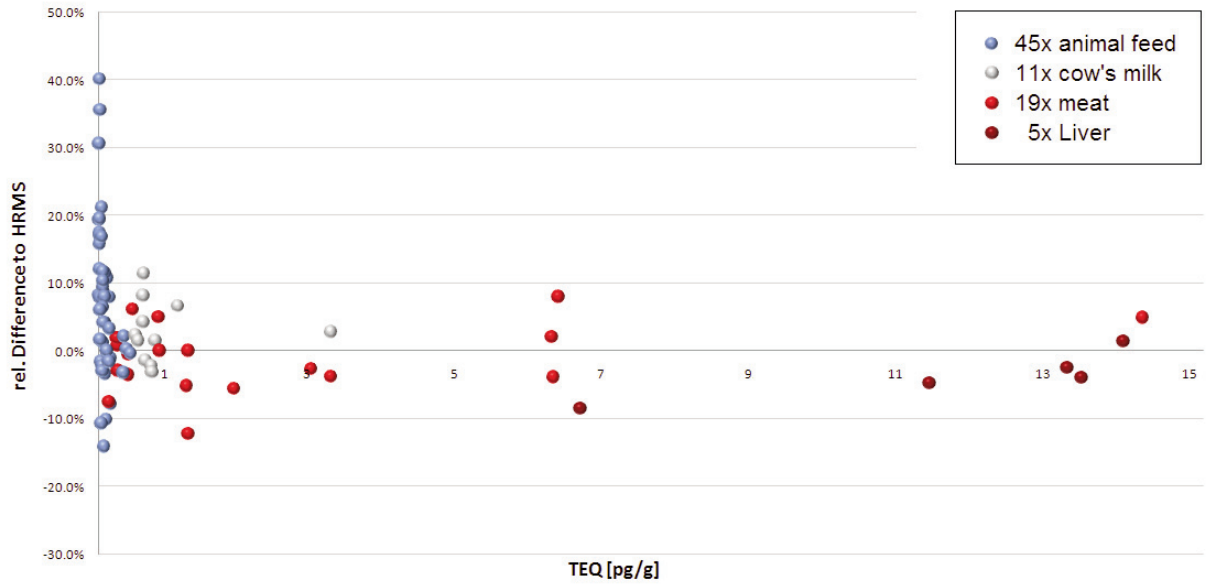

Figure 8. Comparative results for the sum of the 12 dl-PCB congeners (TEQ_{WHO98} upperbound values) for 80 foodstuffs and animal feed samples analyzed by GC-HRMS and GC/MS/MS.

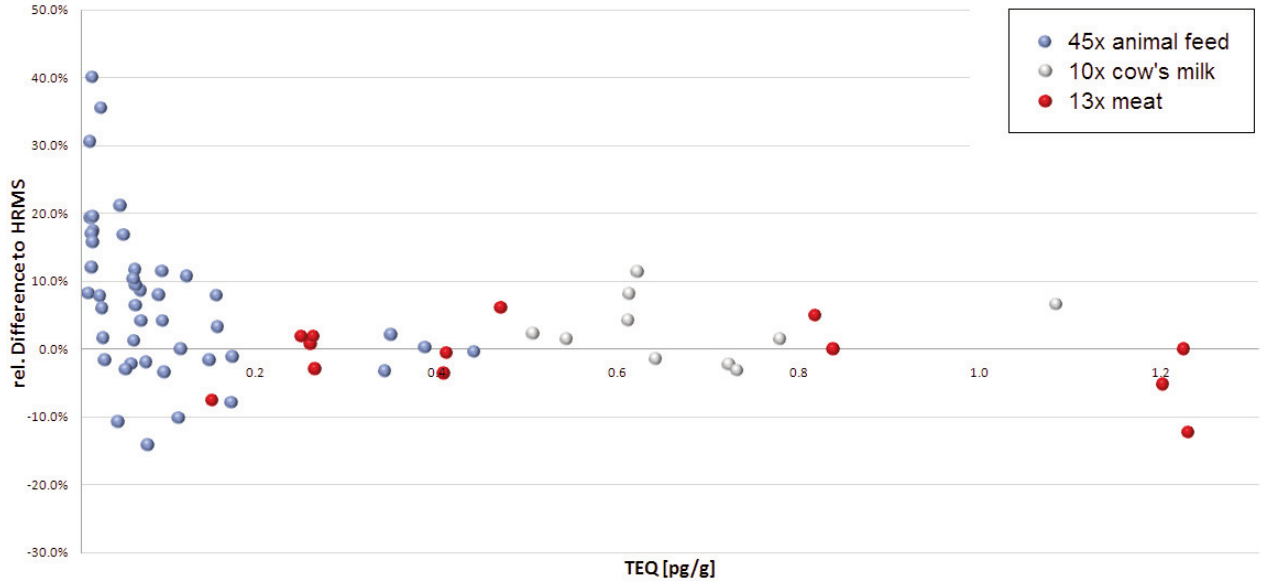

Figure 9. Comparative results for the sum of the 12 dl-PCB congeners (TEQ_{WHO98} upperbound values) for 68 foodstuffs and animal feed samples analyzed by GC-HRMS and GC/MS/MS that gave values less than ~1.2 pg TEQ/g product.

Figure 10 shows the comparative sample results (total ndl-PCB congeners, upperbound values) of the two sets of measurements expressed as the percentage difference between the results obtained by the GC-HRMS and GC/MS/MS analyses.

The agreement between the sum of the results obtained for the six ndl-PCB congeners on the GC-HRMS and the GC/MS/MS for foodstuffs and animal feed samples at levels between 0.5 and 10 ng/g was within the range of ± 10%. Some animal feed samples with total ndl-PCB congener concentrations below 0.5 ng/g gave results with percentage differences greater than + 10%.

Conclusion

The Agilent 7000 Triple Quadrupole GC/MS system provides linear, reproducible and sensitive detection of dl-PCB congeners in foodstuffs and animal feed samples down to low pg TEQ/g values. Comparison of analytical results for foodstuffs and animal feed samples by GC-HRMS and GC/MS/MS indicates the suitability of the Agilent 7000 Triple Quadrupole GC/MS system for the routine screening of dl-PCB congeners in foodstuffs and animal feed that meets the requirements of European Union legislation.

Additionally, the Agilent 7000 Triple Quadrupole GC/MS system has been shown to determine total ndl-PCB congeners in foodstuffs and animal feed samples at concentration levels of 1 ng/g product and below, which is also in good agreement with results obtained by GC-HRMS.

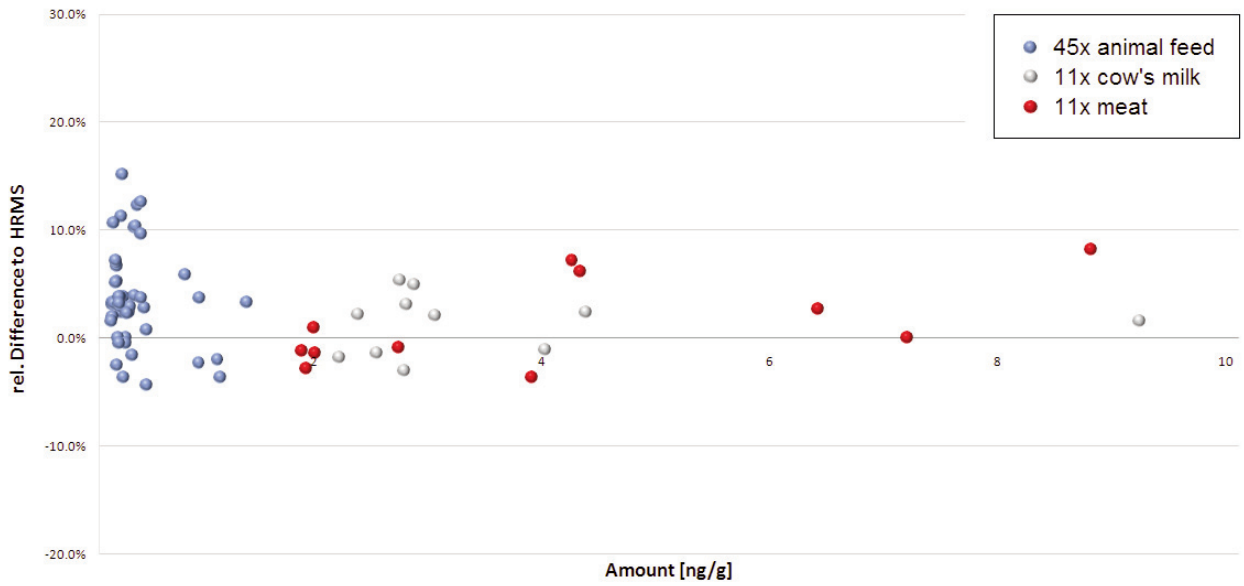

Figure 10. Comparative results for the sum of ndl-PCB congeners (upperbound values) for 67 foodstuffs and animal feed samples analyzed by GC-HRMS and GC/MS/MS that gave values less than 10 ng/g product.

References

1. EPA Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment and Tissue by HRGC/HRMS.
2. Commission Regulation (EC) No 1883/2006 of December 19, 2006, Laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs."
3. C Sandy, "Determination of Polychlorinated Dibenzo-p-dioxins (PCDD) and Polychlorinated Dibenzofurans (PCDF) in Foodstuffs and Animal feed using the Agilent 7000 Triple Quadrupole GC/MS System," Agilent Technologies publication 5990-6594EN (2010).
4. Commission Regulation (EC) No 1881/2006 of December 19, 2006 Setting maximum levels for certain contaminants in foodstuffs.
5. Commission Regulation (EC) No 152/2009 Annex V letter B of January 27, 2009 Laying down the methods of sampling and analysis for the official control of feed.
6. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002, on undesirable substances in animal feed.

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Printed in the USA
March 18, 2011
5990-6950EN

Agilent Technologies

Determination of Polychlorinated Dibenzo-p-dioxins (PCDD) and Polychlorinated Dibenzofurans (PCDF) in Foodstuffs and Animal Feed using the Agilent 7000 Triple Quadrupole GC/MS System

Application Note

Food Safety

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Abstract

A method has been developed on the Agilent 7000 GC Triple Quadrupole GC/MS system for the analysis of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in foodstuffs and animal feed. The method was shown to give linear response over the required concentration range, good repeatability of response and quantitation down to low pg TEQ/g levels.

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Introduction

Polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are highly toxic persistent organic pollutants (POP) with properties that are detrimental to human health and have been linked to causing cancer, endocrine disruption, and reproductive disorders. PCDD and PCDF are not manufactured deliberately but are the byproducts of the combustion of contaminated chemical waste, chemical and pesticide manufacturing, pulp and paper bleaching processes and other sources. PCDD and PCDF are lipophilic chemicals that accumulate in the fatty tissues of animals that form part of the human food chain. It is estimated that more than 80% of human exposure to dioxins derives from food of animal origin.

There have been several incidents of dioxin contamination in the human food chain over the past 20 years. One of the most recent was in December 2008 when contaminated pork and beef products were discovered in the Republic of Ireland [1] during routine testing.

Current legislation in the United States [2] and the European Union, [3,4] requires the confirmation and quantitation of dioxins, furans, and dioxin-like polychlorinated biphenyls (dl-PCBs) in foodstuffs and animal feed by isotope dilution capillary gas chromatography/ high resolution mass spectrometry (GC/HRMS). Additionally, EU Legislation does make provisions for the screening of dioxins in foodstuffs and animal feed by other mass spectrometric techniques or by bioassays. The specific compounds covered by the EU Legislation are shown in Table 1, along with the Toxic Equivalency Factors (TEF) relating the toxicity of each individual analyte to 2,3,7,8 tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), which is assigned a TEF value of 1. The individual concentration of each dioxin, furan, and dl-PCB found in foodstuff and animal feed samples is multiplied with the respective TEF and after summation the total concentration is expressed as the Toxic Equivalent (TEQ) in terms of pg TEQ/g fat .

Table 1. PCDD, PCDF and dl-PCB congeners specified in EU Legislation along with the TEF values stipulated in 1998 and 2005. (WHO_{05} changes indicated in italics)

Compound	TEF WHO_{98}	TEF WHO_{05}	Compound	TEF WHO_{98}	TEF WHO_{05}
Chlorinated dibenzo-p-dioxins			Non-ortho substituted PCBs		
2378-TCDD	1	1	PCB-77	0.0001	0.0001
12378-PeCDD	1	1	PCB-81	0.0001	*0.0003*
123478-HxCDD	0.1	0.1	PCB-126	0.1	0.1
123678-HxCDD	0.1	0.1	PCB-169	0.01	*0.03*
123789-HxCDD	0.1	0.1			
1234678-HpCDD	0.01	0.01			
OCDD	0.0001	*0.0003*			
Chlorinated dibenzofurans			Mono-ortho substituted PCBs		
2378-TCDF	0.1	0.1	PCB-105	0.0001	*0.00003*
12378-PeCDF	0.05	*0.03*	PCB-114	0.0005	*0.00003*
23478-PeCDF	0.5	*0.3*	PCB-118	0.0001	*0.00003*
123478-HxCDF	0.1	0.1	PCB-123	0.0001	*0.00003*
123678-HxCDF	0.1	0.1	PCB-156	0.0005	*0.00003*
234678-HxCDF	0.1	0.1	PCB-157	0.0005	*0.00003*
123789-HxCDF	0.1	0.1	PCB-167	0.00001	*0.00003*
1234678-HpCDF	0.01	0.01	PCB-189	0.0001	*0.00003*
1234789-HpCDF	0.01	0.01			
OCDF	0.0001	*0.0003*			

The maximum levels for PCDD, PCDF, and dl-PCB in certain foodstuffs as prescribed by EU legislation are given in Table 2.

Table 2. Maximum Levels for PCDD, PCDF and Dioxin-like PCB in Certain Foodstuffs, as Specified in EU Regulation (EC) No 1881/2006

	Maximum levels	
Foodstuff	Sum of Dioxins (WHO-PCDD/F-TEQ)	Sum of Dioxins and dl-PCB (WHO-PCDD/F-PCB-TEQ)
Meat and meat products (excluding edible offal) of the following animals :		
Bovine animals and sheep	3.0 pg/g fat	4.5 pg/g fat
Poultry	2.0 pg/g fat	4.0 pg/g fat
Pigs	1.0 pg/g fat	1.5 pg/g fat
Raw milk and dairy products, including butter fat	3.0 pg/g fat	6.0 pg/g fat
Hens' eggs and egg products	3.0 pg/g fat	6.0 pg/g fat

This application note describes a sensitive and reproducible method that meets the requirements of EU Legislation for the screening of PCDD and PCDF in foodstuffs using the Agilent 7000 Triple Quadrupole GC/MS/MS system.

Experimental

Calibration Standards

Native PCDD and PCDF calibration mixtures and their ^{13}C-isotope labeled internal standards were obtained from Cambridge Isotope Laboratories and Wellington Laboratories Inc.

Sample Preparation and Analysis

The most frequently used methods for the determination of PCDD/PCDF and dl-PCB in foodstuffs and animal feed combine fat extraction (for example, Soxhlet or extraction with organic solvents) with cleanup steps using different column chromatographies, such as silica gel coated with sulphuric acid, florisil, alumina, and active carbon. The isotope labeled analogues of all PCDD/PCDF with 2,3,7,8-chlorine substitution were added at the beginning of the extraction. The extract was collected as three fractions containing dioxins (2), mono-ortho-PCB and indicator PCB (1a), and non-ortho PCBs (1b), by eluting with various solvents. After addition of a syringe spike ($^{13}C_{12}$ -1,2,3,4-TCDD), the extracts were evaporated under a gentle stream of nitrogen, reconstituted with toluene, and analyzed with GC/MS/MS. The dioxin fraction was reconstituted with 20 µL of toluene, the non-ortho PCB fraction in 40 µL of toluene and the mono-ortho and indicator PCB fraction in 250 µL of toluene.

A flow diagram summarizing the sample preparation steps is shown in Figure 1.

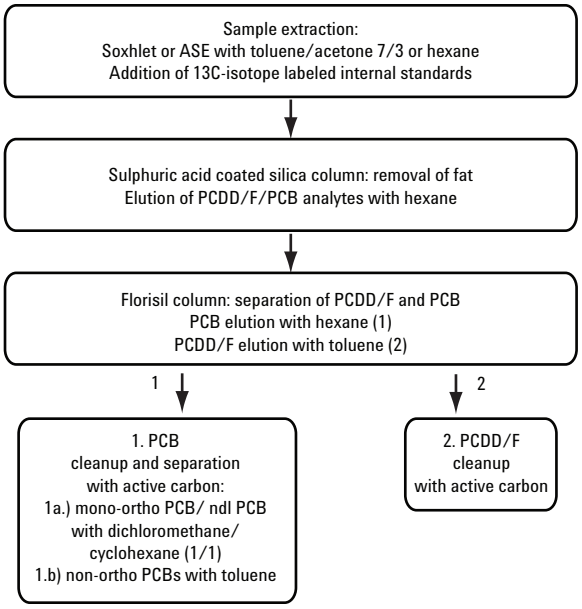

Figure 1. Flow diagram of the sample extraction and cleanup procedures.

The analysis was performed on an Agilent 7000 Triple Quadrupole GC/MS system with an Agilent 7890 GC. The 7890 GC was configured with a carbon dioxide cooled Multimode Inlet (MMI), a 2 m × 0.25 mm id uncoated deactivated capillary column linked to a pressure controlled tee (PCT) and an Agilent J&W DB-5ms UI 60 m × 0.25 mm, 0.25 µm capillary column. The chromatographic method was retention time locked (in direct connect mode) using PCB 105 to a retention time of 34.0 minutes.

The instrument conditions are listed in Table 3. A schematic diagram of the GC/MS/MS system is shown in Figure 2. The 7000 Triple Quadrupole GC/MS was operated in MS/MS-EI (electron ionization) Multiple Reaction Monitoring (MRM) mode. Each analyte and its associated ^{13}C-Internal standard was measured using two precursor ions and two different product ions. A full list of the analyte retention times and MRM settings are given in Table 4. The MRM settings consist of five time segments, each segment monitoring the tetra, penta, hexa, hepta, and octa dioxin and furan isomers, respectively. Dwell times were set to 75 ms for the native analytes and to 25 ms for all internal standards.

An Agilent 7693 Automatic Liquid Sampler with the sampler tray cooled to 5 °C was used to make 2-µL pulsed cold splitless injections using a 10-µL syringe.

Table 3. Gas Chromatograph and Mass Spectrometer Conditions

GC Conditions	
Column (1)	2.0 m x 0.25 mm uncoated siltek deactivated fused silica
Pressure controlled tee	Agilent p/n G3186B
Column (2)	Agilent J&W DB-5ms UI 60 m × 0.25 mm, 0.25 µm (122-5562UI)
Back Flush time	15.0 minutes after injection
Back flush flow rate	Column (1) - 5.0 mL/min, concurrent back flush
Injection	2 µL cold pulsed splitless using CO_2 cooled Multi Mode Inlet (MMI)
Inlet temperature program	100 °C (0.05 min), 600 °C/min to 300 °C
Injection Pulse Pressure	30 psi until 1.0 min
Purge Flow to Split Vent	40 mL/min at 1.5 min
Carrier Gas	Helium, Column (1) constant flow 0.9 mL/min Helium, Column (2) constant flow 1.0 mL/min
RTL Compound	PCB 105, Locked RT = 34.0 minutes
Oven program	130 °C (2.0 min hold), 10 °C/min to 200 °C (16 min), 5 deg °C/min to 235 °C (7 min), 5 °C/min to 350 °C
MS Transfer line temp	300 °C
MS Conditions	
Tune	EI Autotune
Gain	100
MS1 Resolution	Wide
MS2 Resolution	Wide
Dwell Times	Natives 75 ms, Labeled compounds 25 ms
Collision Energies	Table 4
Collision cell gas flows	Nitrogen at 1.5 mL/min, helium at 2.25 mL/min
MS Temperatures	Ion source 280 °C, quadrupoles 150 °C
Solvent delay	25.0 minutes

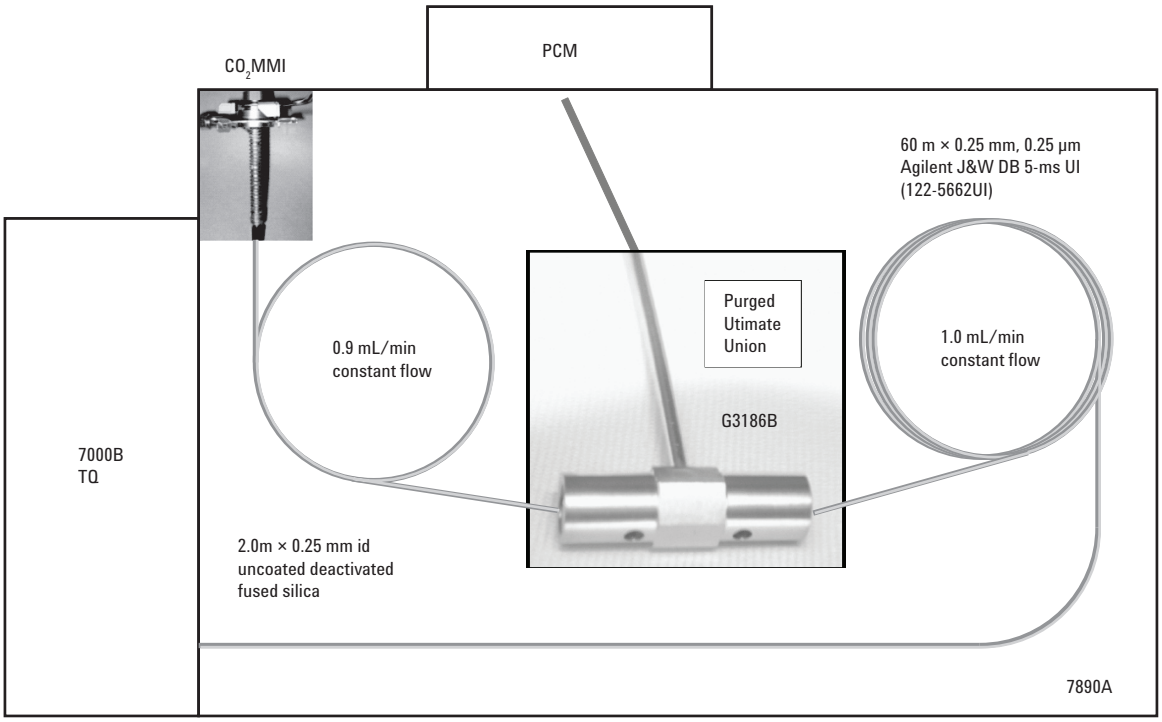

Figure 2. Schematic diagram of the GC/MS/MS hardware.

Table 4. MS/MS Settings for PCDD, PCDF and ^{13}C-Internal Standards

TS	Segment start time (min)	Peak number	Analyte	RT (min)	Quant precursor	Product	CE (V)	Qual precursor	Product	CE (V)
1	25.0									
		1	13C-2378-TCDF	35.43	315.9	251.9	33	317.9	253.9	33
		2	2378-TCDF	35.47	303.9	240.9	33	305.9	242.9	33
		3	13C-1234-TCDD	35.77	331.9	267.9	24	333.9	269.9	24
		4	13C-2378-TCDD	36.79	331.9	267.9	24	333.9	269.9	24
		5	2378-TCDD	36.80	319.9	256.9	24	321.9	258.9	24
2	40.0									
		6	13C-12378-PeCDF	42.55	351.9	287.9	35	349.9	285.9	35
		7	12378-PeCDF	42.56	339.9	276.9	35	337.9	274.9	35
		8	13C-23478-PeCDF	44.00	351.9	287.9	35	349.9	285.9	35
		9	23478-PeCDF	44.02	339.9	276.9	35	337.9	274.9	35
		10	13C-12378-PeCDD	44.45	365.9	301.9	25	367.9	303.9	25
		11	12378-PeCDD	44.48	355.9	292.9	25	353.9	290.9	25
3	46.0									
		12	13C-123478-HxCDF	48.04	385.8	321.9	35	387.8	323.9	35
		13	123478-HxCDF	48.06	373.8	310.9	35	375.8	312.9	35
		14	13C-123678-HxCDF	48.21	385.8	321.9	35	387.8	323.9	35
		15	123678-HxCDF	48.22	373.8	310.9	35	375.8	312.9	35
		16	13C-234678HxCDF	48.96	385.8	321.9	35	387.8	323.9	35
		17	234678-HxCDF	48.97	373.8	310.9	35	375.8	312.9	35
		18	13C-123478-HxCDD	49.17	403.8	339.8	25	401.8	337.9	25
		19	123478-HxCDD	49.19	389.8	326.9	25	391.8	328.8	25
		20	13C-123678-HxCDD	49.30	403.8	339.8	25	401.8	337.9	25
		21	123678-HxCDD	49.32	389.8	326.9	25	391.8	328.8	25
		22	13C-123789HxCDD	49.63	403.8	339.8	25	401.8	337.9	25
		23	123789-HxCDD	49.65	389.8	326.9	25	391.8	328.8	25
		24	13C-123789-HxCDF	50.04	385.8	321.9	35	387.8	323.9	35
		25	123789-HxCDF	50.06	373.8	310.9	35	375.8	312.9	35
4	51.0									
		26	13C-1234678-HpCDF	51.84	419.8	355.8	36	421.8	357.8	36
		27	1234678-HpCDF	51.86	409.8	346.8	36	407.8	344.8	36
		28	13C-1234678-HpCDD	53.11	437.8	373.8	25	435.8	371.8	25
		29	1234678-HpCDD	53.13	423.8	360.8	25	425.8	362.8	25
		30	13C-1234789-HpCDF	53.69	419.8	355.8	36	421.8	357.8	36
		31	1234789-HpCDF	53.70	407.8	344.8	36	409.8	346.8	36
5	55.0									
		32	13C-OCDD	56.23	469.7	405.8	26	471.7	407.8	26
		33	OCDD	56.24	457.7	394.8	26	459.7	396.8	26
		34	13C-OCDF	56.41	453.7	389.8	35	455.7	391.8	35
		35	OCDF	56.42	441.7	378.8	35	443.7	380.8	35

Capillary flow technology and backflushing have proven to be invaluable tools in improving method robustness and chromatographic integrity for GC/MS analysis of samples with high matrix content [5]. Backflushing removes high-boiling matrix components from the system that would otherwise remain behind from injection to injection, causing retention time shifts, loss of chromatographic peak shapes, and eventual contamination of the mass spectrometer ion source.

The 2-m precolumn and pressure controlled tee (PCT) were used to provide concurrent backflushing of the precolumn during the chromatographic run. Concurrent backflushing is a technique that works well in methods employing long (60 m) capillary columns that cannot be efficiently backflushed in postrun mode using a post-column connection to the PCT. The flow rate in the precolumn is reversed once all the analytes of interest have moved in to the 60-m analytical column. This is implemented by automatically reducing the pressure at the MMI 15 minutes after the sample injection takes place, which was determined experimentally by a sequence of standard injections with varying backflush times.

Results and Discussion

Chromatography

The chromatographic separation of the native PCDD and PCDF congeners is shown in Figure 3. The peak numbers refer to the entries in Table 4. The chromatographic run time for each sample was 60 minutes.

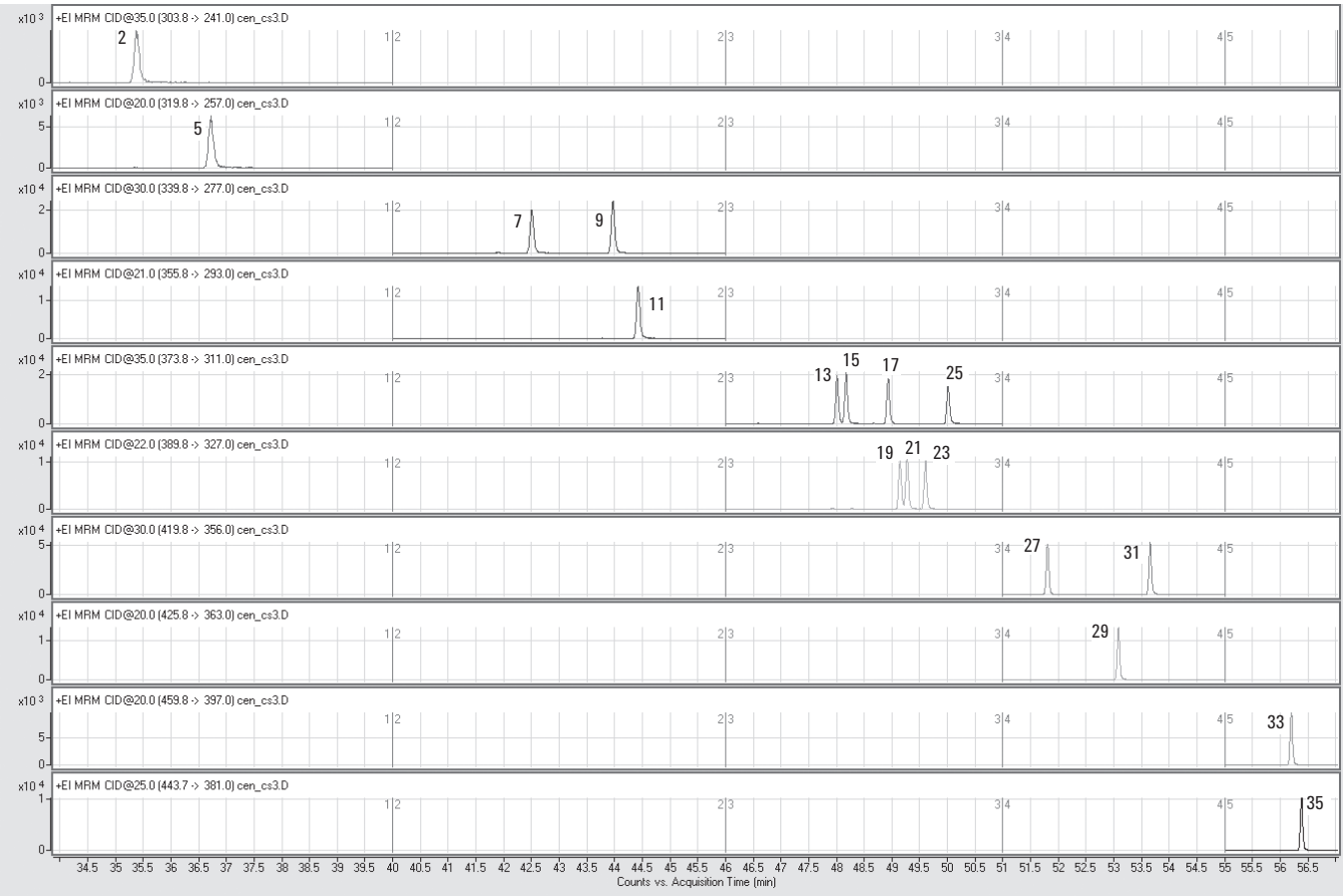

Figure 3. MRM chromatograms of native PCDD and PCDF congeners. (Peak numbers refer to analytes listed in Table 4).

Linearity of Response and Sensitivity

The PCDD and PCDF were measured using ^{13}C-labeled internal standard (ISTD) calibration. The seven-point ISTD calibration curves for 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD are shown in Figures 4 and 5, respectively. Excellent linearity is shown for 2 µL injections of the calibration standards over the concentration range of 0.05 pg/µL to 5 pg/µL with R^2 values > 0.999. The insets in Figures 4 and 5 show the R^2 values for the average of response factors for these two dioxin congeners.

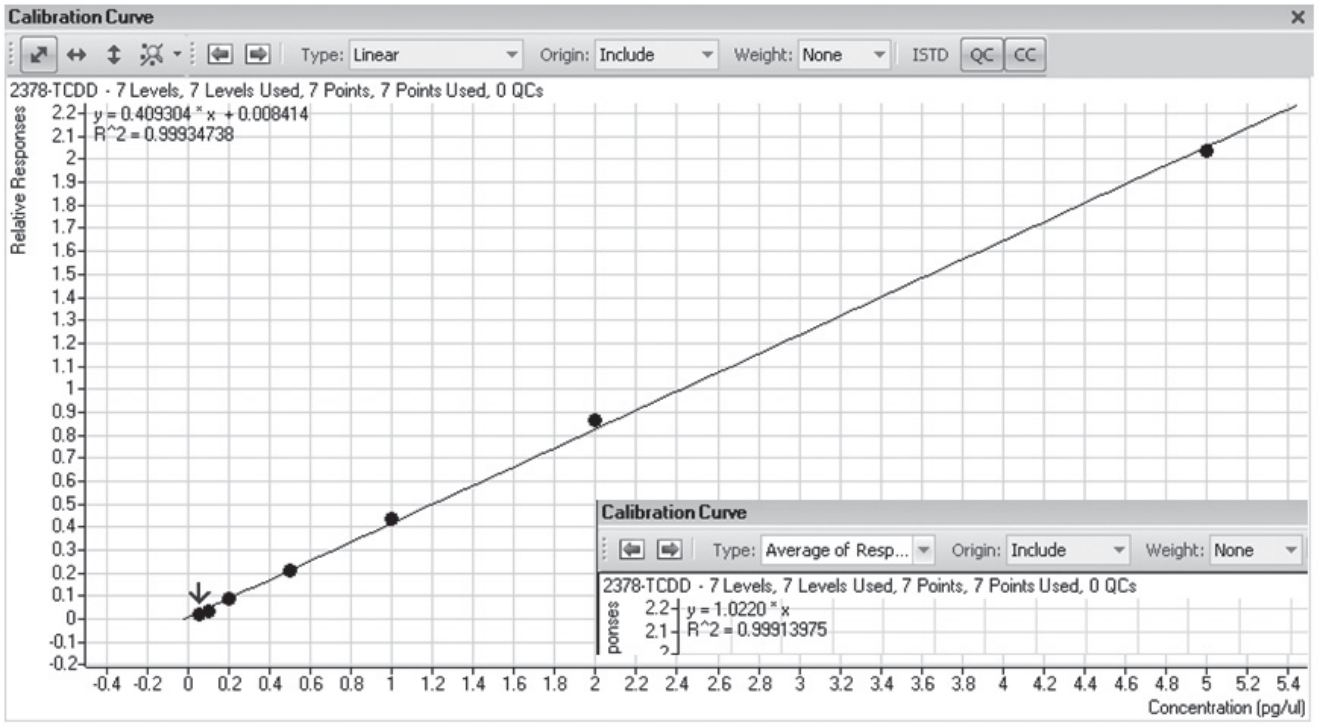

Figure 4. Calibration curve for 2,3,7,8-TCDD with both linear fit and average of response factors (inset).

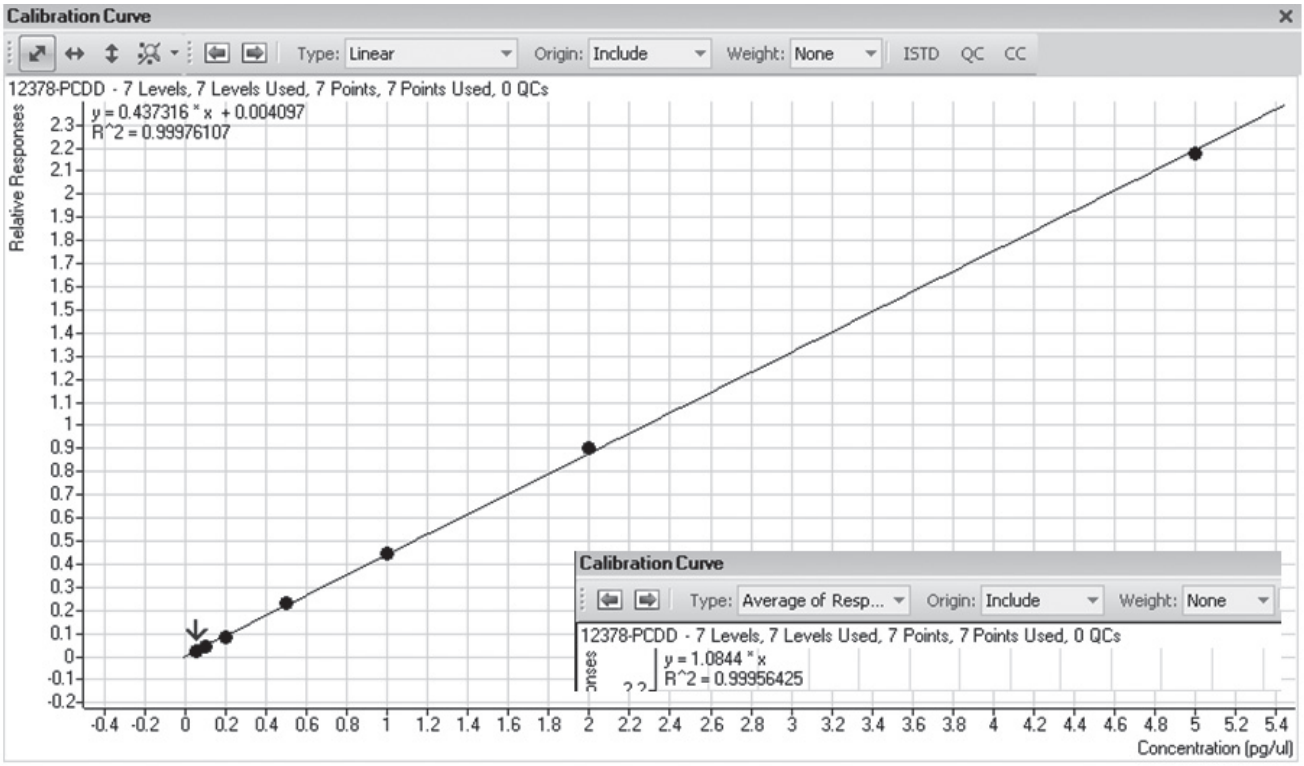

Figure 5. Calibration curve for 1,2,3,7,8-PeCDD with both linear fit and average of response factors (inset).

The linear calibration curve fits for all 17 PCDD and PCDF congeners are shown in Table 5.

*Table 5. Linear Correlation Coefficients for Seven-Point ISTD Calibration Curves over the Range 100 fg – 10 pg Injected. * (OCDD 500 fg – 50 pg injected)*

Analyte	R^2	Analyte	R^2
2378-TCDD	0.99934	2378-TCDF	0.99984
12378-PeCDD	0.99976	12378-PeCDF	0.99909
123478-HxCDD	0.99994	23478-PeCDF	0.99995
123678-HxCDD	0.99905	123478-HxCDF	0.99971
123789-HxCDD	0.99977	123678-HxCDF	0.99983
1234678-HpCDD	0.99945	234678-HxCDF	0.99953
OCDD*	0.99780	123789-HxCDF	0.99972
		1234678-HpCDF	0.99971
		1234789-HpCDF	0.99991
		OCDF	0.99907

The selected reaction monitoring (MRM) chromatograms for the native PCDD and PCDF congeners for the lowest calibration standard (0.1 pg on-column) are shown in Figure 6.

Peak Area Precision and Peak Area Ratio Precision

The peak area precision (raw peak area) for the native PCDD and PCDF congeners was determined by spiking a pork fat extract with native PCDD and PCDF at a concentration of 100 fg/µL and ^{13}C-ISTD at 1pg/µL, respectively. A sequence of replicate 2-µL cold pulsed splitless injections (n = 15) was made. The %RSD values for the peak areas of native PCDD/PCDF and ^{13}C-ISTD are shown in Figure 7. All native congeners gave precision values less than 10% except for 1,2,3,4,6,7,8-HpCDD, which gave a value of 11.9 %. This slightly higher result may be attributed to the somewhat lower absolute response of this particular analyte. The ^{13}C-ISTD gave %RSD values of 5% or lower.

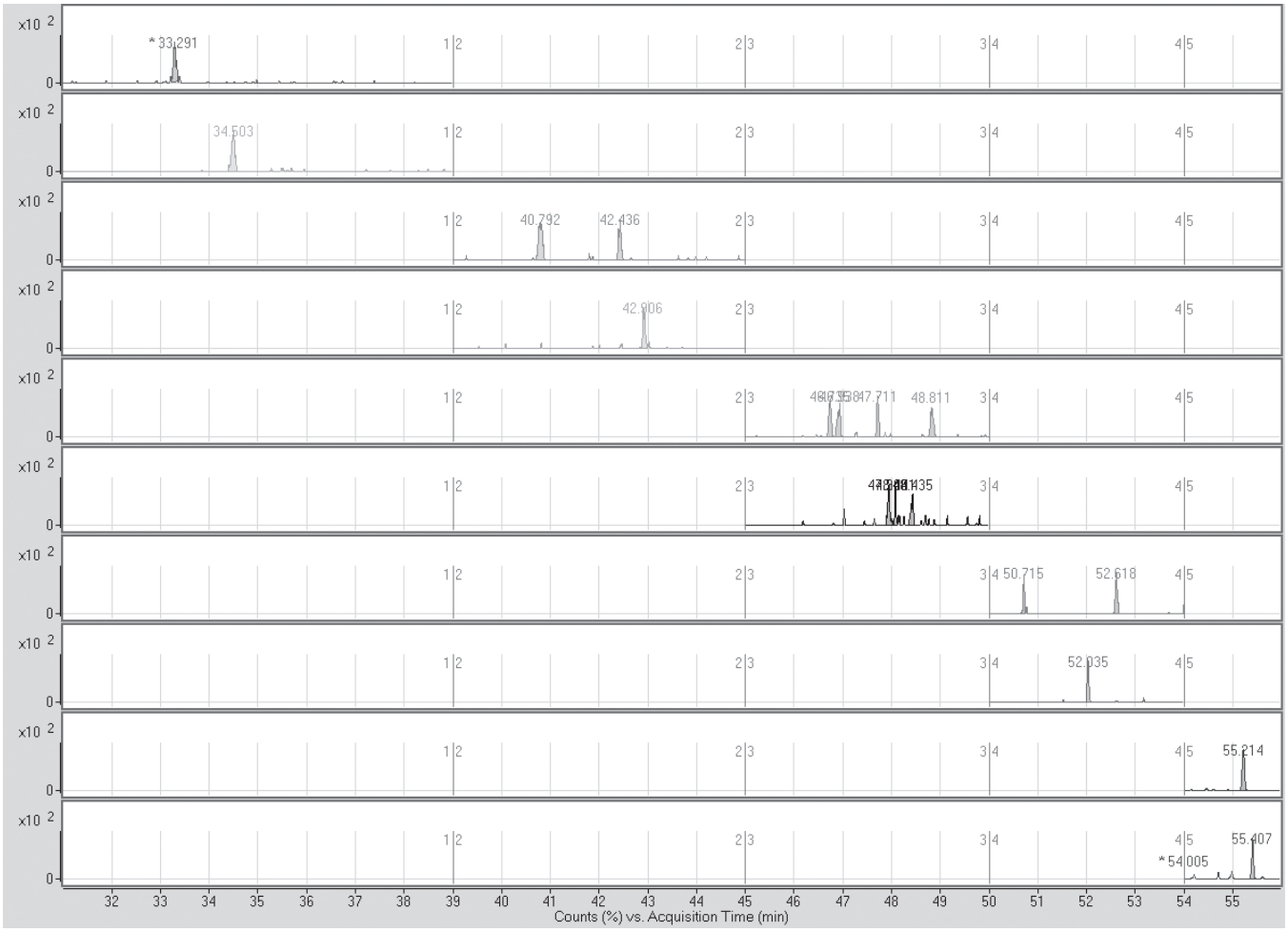

Figure 6. MRM chromatograms of native PCDD and PCDF congeners. Lowest calibration standard, 100 fg injected on-column (OCDD 500 fg injected on-column).

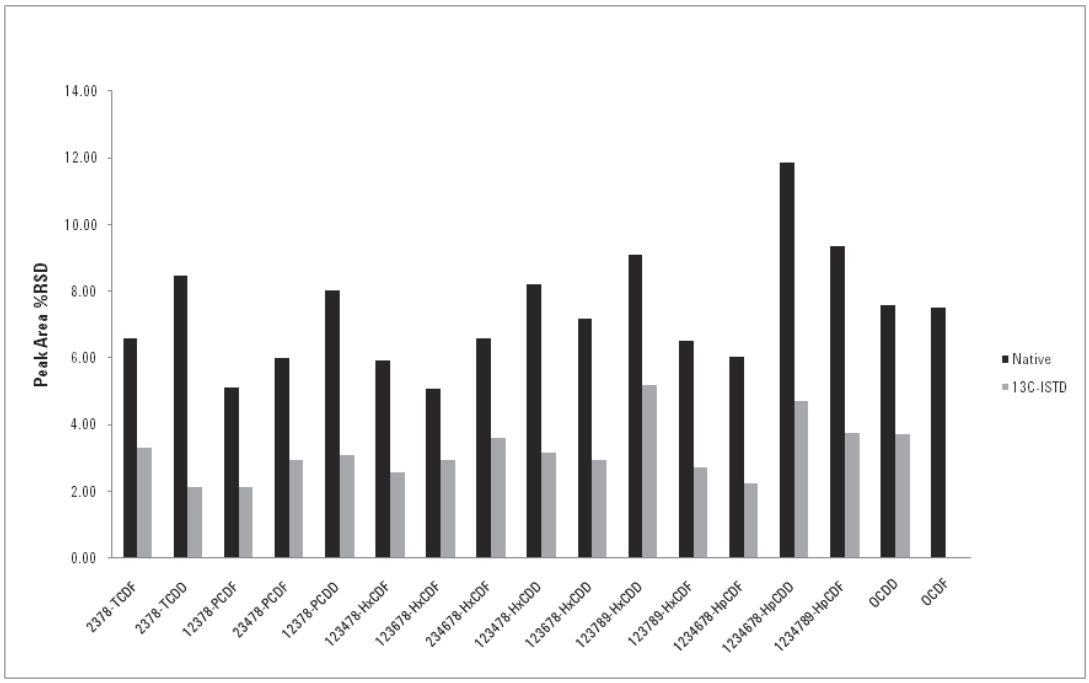

Figure 7. Repeatability of peak areas for native PCDD and PCDF congeners and ^{13}C-ISTD (n=15).

The peak area ratio precision (analyte peak area divided by its ^{13}C-ISTD peak area) was also determined for the 15 replicate injections. The %RSD values for the ratio of peak areas are shown in Figure 8. All analytes gave precision values less than 10% except for 1,2,3,4,6,7,8-HpCDD, which gave a %RSD value of 13.6 %.

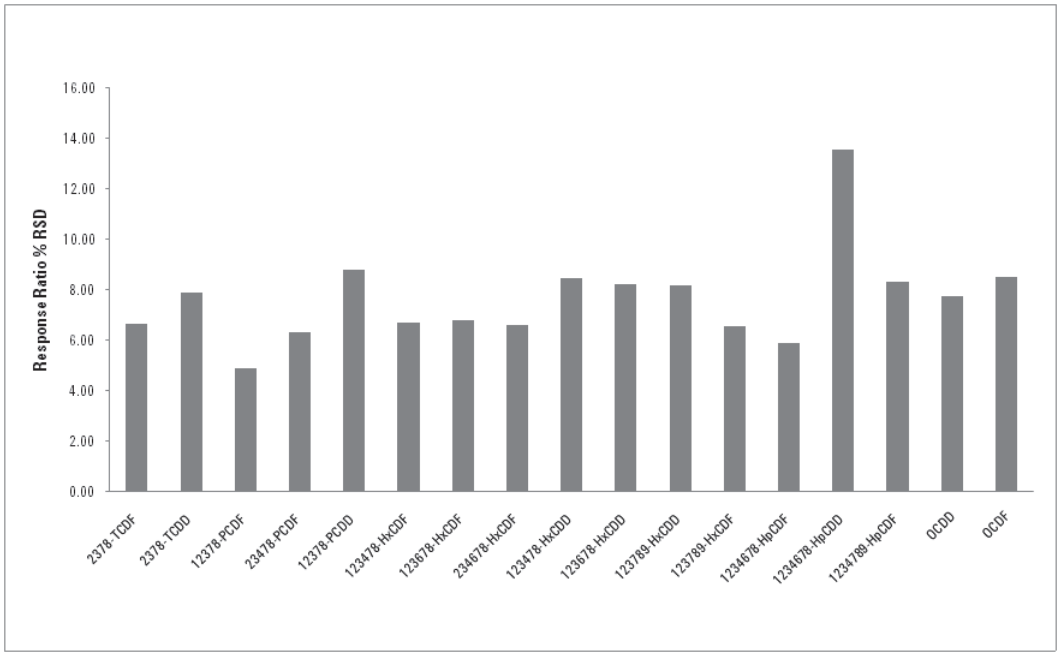

Figure 8. Repeatability of response ratios for native PCDD and PCDF congeners (n=15).

Sample Analysis

The MRM chromatograms for the tetra- and penta-CDF isomers present in a hen's egg extract are shown in Figure 9. The concentrations of the 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 2,3,4,7,8-PeCDF were determined as 15.5, 3.4, and 3.1 pg/g fat, respectively.

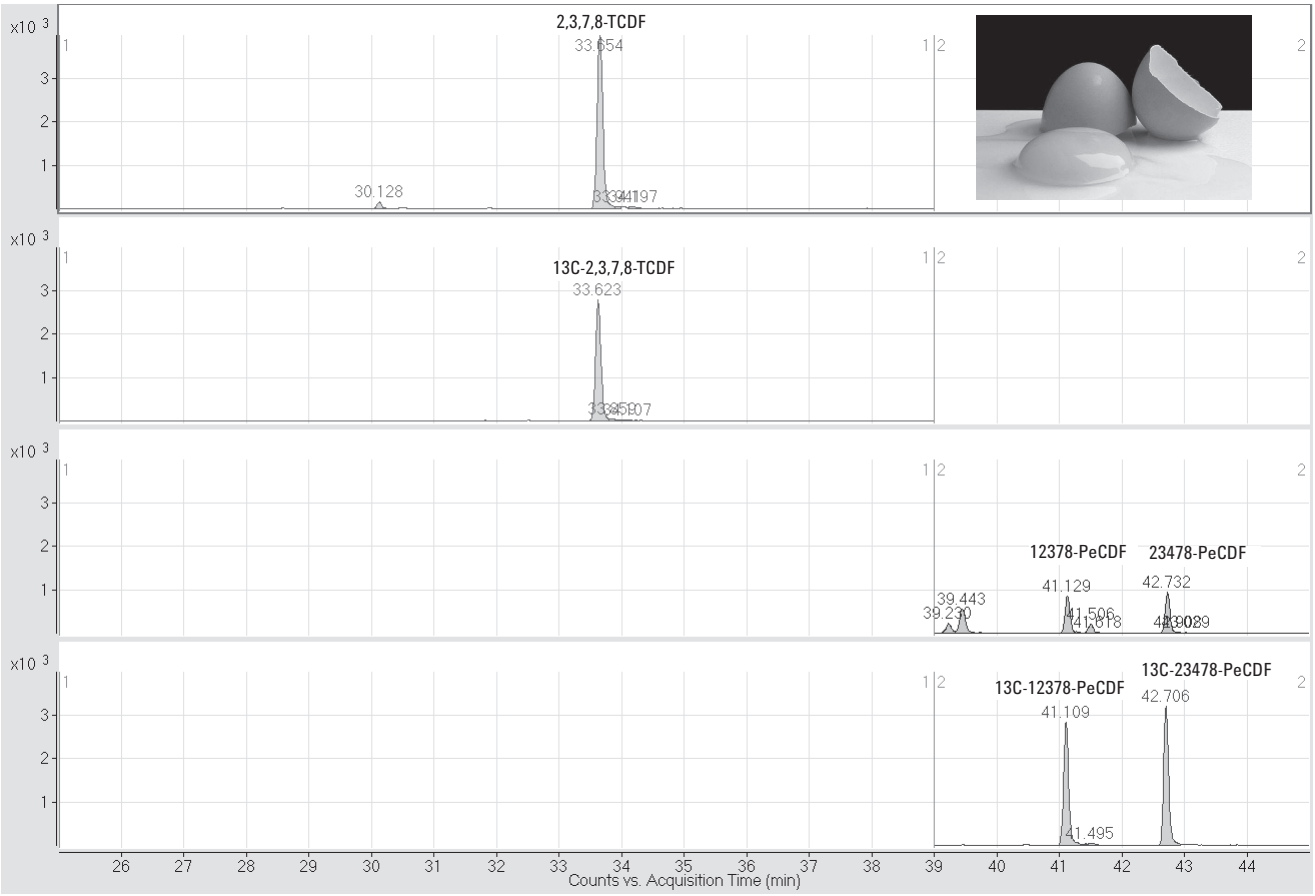

Figure 9. MRM chromatograms of tetra- and penta-CDF congeners and ^{13}C-ISTDs from a hen's egg extract.

An advantage of screening for dioxins and furans in foodstuffs and animal feed by GC/MS/MS, as opposed to using bio-assay, is that each congener is individually quantified. This allows the quantitative contribution of each PCDD and PCDF congener within the sample to be plotted. This, in turn, may provide a valuable clue as to the likely source of the contamination. The quantitative distribution of PCDD and PCDF congeners in a hen's egg extract is shown in Figure 10.

Samples of five different foodstuffs: liver (n=5), beef (n=4), poultry meat (n=6), hens' eggs (n=5), and animal feed (n=31) were extracted and analyzed using a GC High Resolution Mass Spectrometer (GC/HRMS) at a resolution of R=10,000. The same sample vials were then transferred to the Agilent 7000 GC/MS/MS system and reanalyzed.

Figure 11 shows the comparative sample results (upperbound values) of the two sets of measurements expressed as the percentage difference between the results obtained by the GC/HRMS and GC/MS/MS analyses.

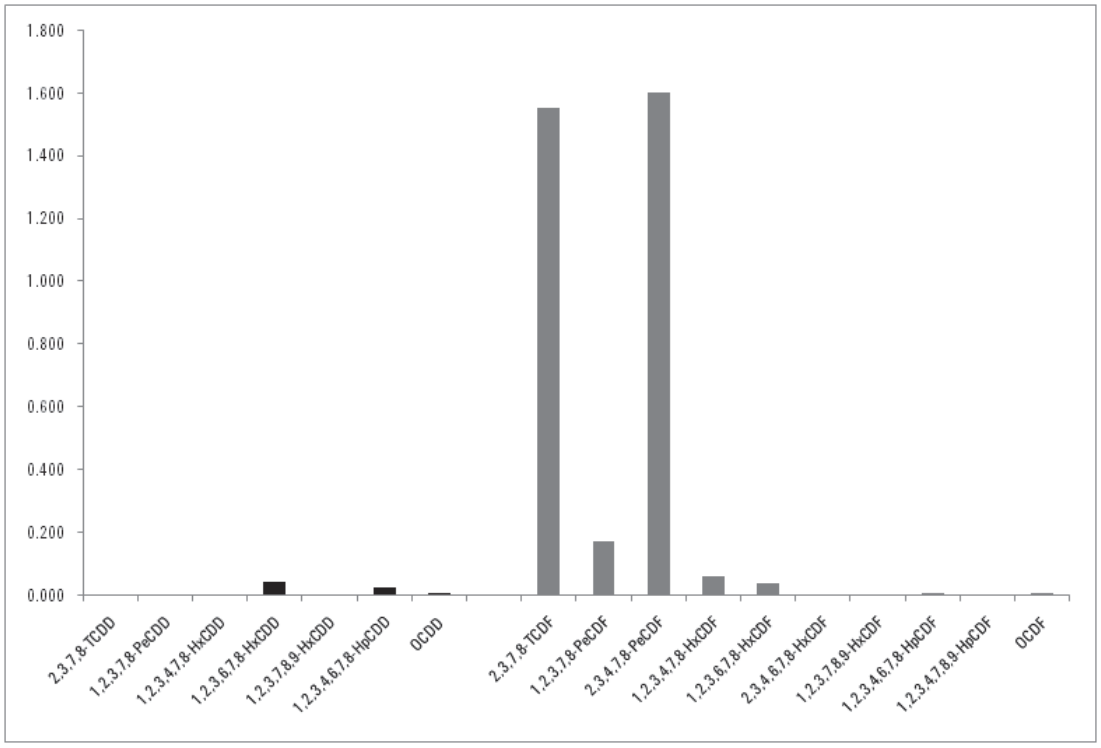

Figure 10. Quantitative distribution of PCDD and PCDF congeners in a hen's egg extract, units are pg TEQ/g fat.

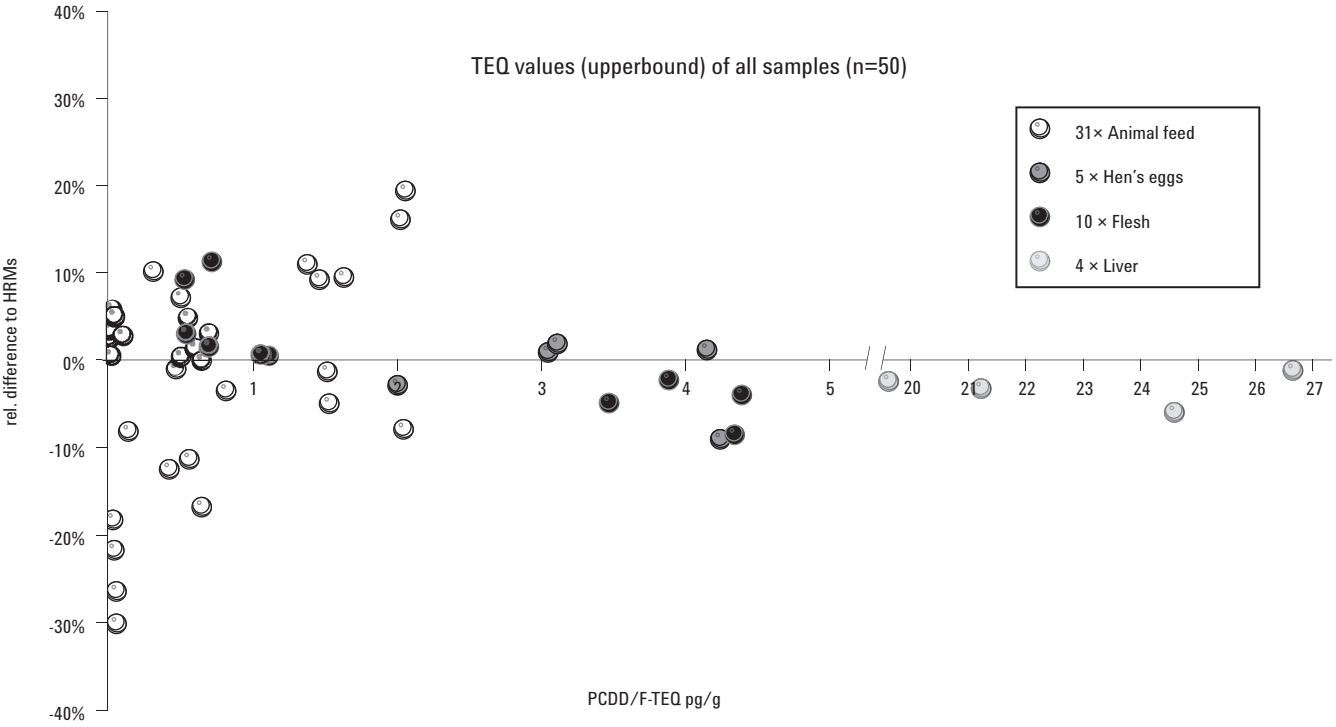

Figure 11. Comparative results (upperbound concentration values) for 50 food and feed samples analyzed by GC/HRMS and GC/MS/MS.

Figure 12 shows the comparative sample results (upperbound concentration values) of the two sets of measurements for those samples that gave values less than 3 pg TEQ/g. Additionally, Figure 12 is annotated with the Maximum Levels (ML) and Action Levels (AL) for poultry meat, hens' eggs, and animal feedstuff as prescribed by European Union Legislation.

Foodstuff samples that exhibited levels of total PCDD and PCDF congeners at upperbound values greater than 3 pg TEQ/g gave quantitative results by GC/MS/MS that were within ± 10% of the value obtained by GC/HRMS.

The agreement between the results obtained on the GC-HRMS and the GC/MS/MS for foodstuff and feedstuff samples at levels between 0.5 and 3 pg/g TEQ were within the range of ± 10 to ± 20%.

Only those animal feedstuff samples with results of 0.1–0.2 pg TEQ/g (well below the EU action level of 0.5 pg TEQ/g) gave result differences > 20% between the GC/HRMS and GC/MS/MS. This greater differential may be attributed to the results being expressed as the upperbound values and the lower limit of detection (LOD) achievable by the GC/HRMS system. In Animal Feedstuff samples, the GC/HRMS gave a range of LODs for the PCDD and PCDF congeners between 0.01–0.06 pg/g, whereas the GC/MS/MS gave 0.02–0.08 pg/g.

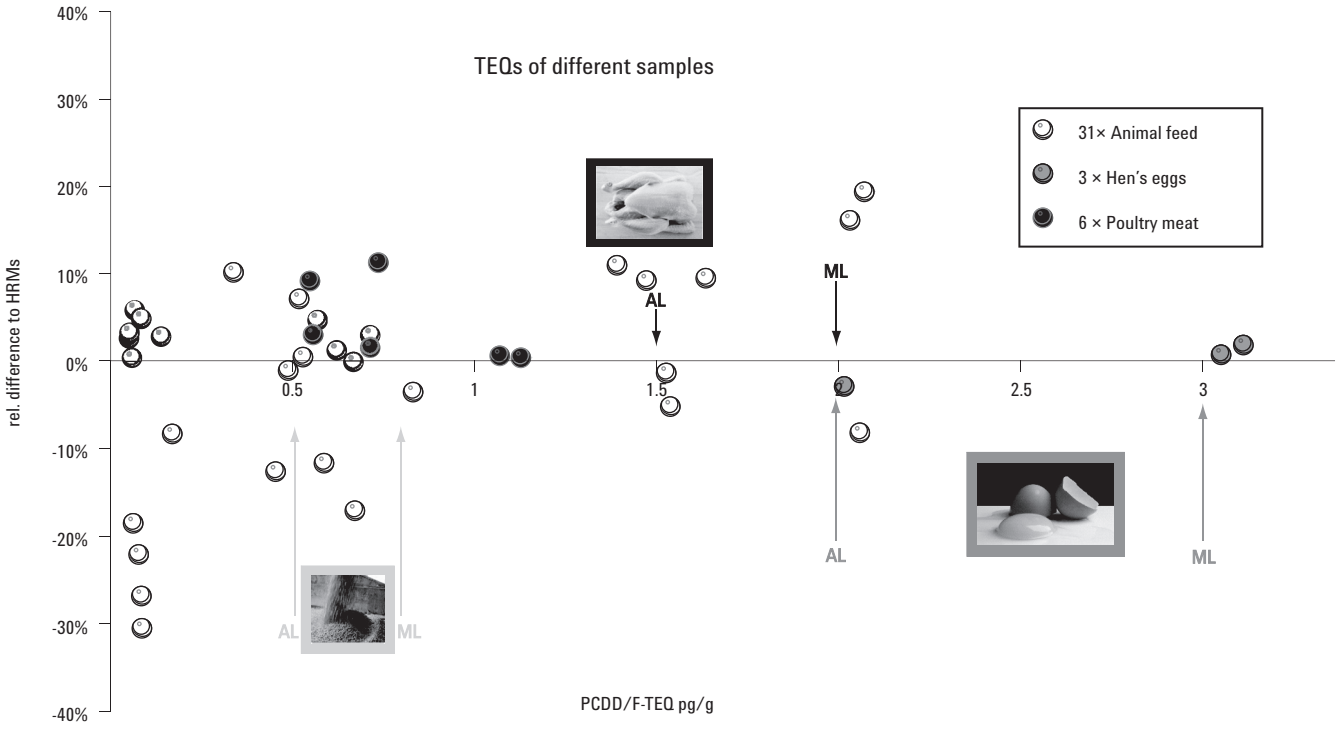

Figure 12. Comparative results (upperbound concentration values) for 40 food and feed samples analyzed by GC/HRMS and GC/MS/MS that gave values less than ~3 pg TEQ/g. ML= EU Maximum Level, AL = EU Action Level.

Conclusion

The Agilent 7000 Triple Quadrupole GC/MS system provides linear, reproducible and sensitive detection of PCDD and PCDF congeners in foodstuffs and animal feed samples down to low pg TEQ/g values. Comparison of analytical results of foodstuff and animal feed extracts by GC/HRMS and GC/MS/MS indicates the suitability of the Agilent 7000 Triple Quadrupole GC/MS system for routine screening of PCDD and PCDF congeners in foodstuffs and feedstuffs that meets the requirements of European Union legislation.

References

1. UK Food Standards Agency Ref 74/2008 - Emerging incident involving presence of dioxins in Irish pork meat.
2. US EPA Method 1613B - Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope dilution HRGC/HRMS.
3. Commission Regulation (EC) No 1883/2006 of 19 December 2006 Laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs.
4. Commission Regulation (EC) No. 152/2009 Annexe V letter B, 27 January 2009, "Laying Down the Methods of Sampling and Analysis for the Official Control of Feed".
5. C. Sandy, "Improving GC-MS Method Robustness and Cycle Times using Capillary Flow Technology and Back Flushing," Agilent Technologies publication 5990-3367EN (2009).

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Printed in the USA
November 15, 2010
5990-6594EN

Agilent Technologies

A reliable and routine GC/MS/MS

Method for the Determination of Chemical Contaminants in Marine Shellfish

FOOD SAFETY

Need a sensitive, reliable and robust method for the routine determination of organo-chlorine pesticides, polyaromatic hydrocarbons and polychlorinated biphenyls in marine shellfish?

Chemical contaminants that are released into the marine environment maybe ingested (absorbed) by fish and shellfish and thus become introduced in to the human food chain. Lipophillic chemicals such as Organo-chlorine pesticides (OCPs) and Polychlorinated biphenyls (PCBs) can bio-accumulate in the fatty tissues of marine fish and shellfish, the longer an organism is exposed to a contaminated environment, the higher the likely levels of contaminants.

The Clean Seas Environmental Monitoring Program (CSEMP) is an initiative designed to monitor the levels of chemical contamination in the United Kingdom's coastal and estuarine areas. The major drivers for this program are:

- To meet the mandatory monitoring requirements under Oslo and Paris Convention (OSPAR) Joint Assessment and Monitoring Program (JAMP)
- Compliance with EC Directives

Agilent Technologies has partnered with a leading European Analytical Laboratory to develop a sample preparation method based on a modified QuEChERS extraction along with a GC/MS/MS method for the determination of selected OCPs, PAHs and PCBs in marine shellfish (Mussel) tissue. The GC/MS/MS method provides reproducible and sensitive determination of OCPs, PCBs and PAHs that employs large volume (solvent vent) injection using a Multimode inlet (MMI) and post-column, post-run backflush in order to remove high boiling matrix components that would otherwise remain in the column between analyses and subsequently cause degradation of chromatographic performance and contamination of the mass spectrometer ion source.

The analytical method meets the detection limit requirements of 0.1 µg/Kg for OCPs and PCBs, and 0.5–1.0 µg/Kg for PAHs.

Compounds

- 16 Organo-chlorine pesticides
- 19 Polyaromatic hydrocarbons
- 7 Polychlorinated biphenyls

Method for the Determination of Chemical Contaminants in Marine Shellfish

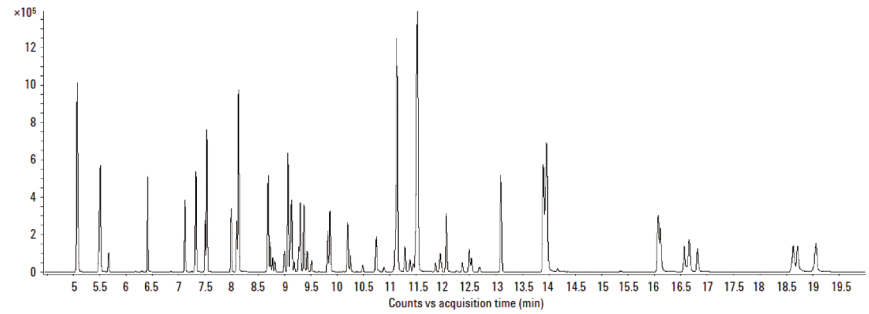

Figure 1. *TIC MRM chromatogram of a calibration standard mixture of OCPs, PAHs and PCBs*.*

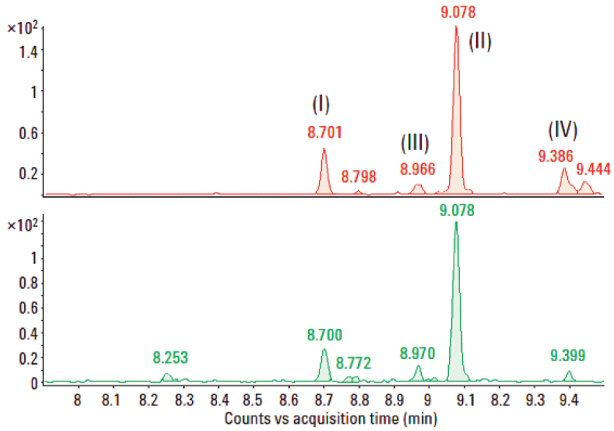

Figure 2. *MRM Chromatograms for (I) incurred a-HCH and (II) incurred g-HCH in mussel sample, Concentrations 0.06 and 0.30 µg/Kg, respectively. Peaks (III) and (IV) are traces of incurred b-HCH and d-HCH, respectively.*

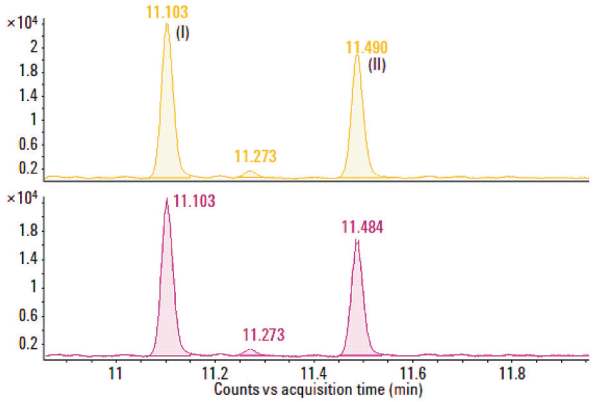

Figure 3. *MRM Chromatograms for (I) incurred Fluoranthene and (II) incurred Pyrene in mussel sample, Concentrations 8.64 and 5.83 µg/Kg, respectively.*

* Full analytical details are available in Agilent Technologies publication 5990-7714EN.

Key Benefits

- Sample extraction based on a modified QuEChERS method.
- Recoveries for all analytes in the range of 85.4% to 123.9%.
- Large volume (solvent vent mode) injection using a multimode inlet ensuring required detection limits are met.
- Retention time locked chromatographic method for ease of set up and on-going maintenance.
- Capillary flow technology provides post-column, post-run backflush to ensure chromatographic method robustness and prevent contamination of the MS ion source with high-boiling matrix.
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Printed in USA, April 13, 2011
5990-7908EN

Agilent Technologies

Agilent 6460 LC-QQQ – Highly Sensitive and Robust Analysis for Lipophilic Marine Toxins in Shellfish

Application Note

Environmental, Food Safety

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Abstract

Marine biotoxins are increasingly threatening the human health in many parts of the world. While the toxins are formed by microscopic planktonic algae of several genera usually at very low concentrations, they can be accumulated in bivalve molluscs to reach toxic doses. The consumption of contaminated shellfish or fish can lead to human poisoning or even death. In animals and humans there are four recognized symptom types of shellfish poisoning: Diarrhetic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP) and amnesic shellfish poisoning (ASP).

The official standard reference method in the EU (Commission Regulation EC No. 2074/2005) for monitoring of lipophilic biotoxins is the mouse bioassay (MBA). Recently the MBA has been considered to be inadequate (The EFSA Journal, 2009, 1306, 1-1) by the European Food Safety Authority (EFSA) because of high variability, insufficient detection capability and limited specificity. A specific, alternative method for the determination of marine biotoxins with low limits of detection (LOD) has been requested.

This document describes a highly sensitive LC-MS/MS method for the determination of lipophilic marine toxins, including ocadaic acid (OA), dinophysistoxins (DTX toxins) and polyether toxins like azaspiracids, pectenotoxins and yessotoxins. Preliminary tests have shown that this method is also applicable to further lipophilic toxins like domoic acid, gymnodimine or spirolides, if reference compounds are available.

Agilent Technologies

Introduction

Marine biotoxins are formed as secondary metabolites by marine planctonic algae typically at very low concentrations. During an algal bloom the concentrations of the toxins can reach toxic levels in particular due to the accumulation of the toxins in bivalve mollusks. In the last two decades the number and intensity of harmful algae blooms has increased and a bigger number of toxic compounds have been found in the marine food chain (Marine biotoxins. FAO Food and Nutrition Papers (80) 2004). A more sensitive and more reliable method for the determination of lipophilic marine toxins has been requested by the European Food Safety Authority (EFSA) since the current official standard reference method in the EU, the mouse bioassay has been considered to be inadequate because of high variability, insufficient detection capability and limited specificity.

Your Challenges

The challenge is to have a sensitive and robust analytical method available for the determination of lipophilic marine toxins, including ocadaic acid, dinophysistoxins (DSP toxins) and polyether toxins like azaspiracids, pectenotoxins and yessotoxins in seafood. The method should have the potential to be extended to further lipophilic toxins like domoic acid, gymnodimine or spirolides. The limits of detection (LODs) need to be below the maximum residue limits (MRLs) which are specified for azaspiracids, the sum of okadaic acid, dinophysistoxins and pectenotoxins, and yessotoxins under EU legislation. Quantitation and confirmation of compounds at a trace level can be complicated by the matrix. As reference material for many compounds is not available tools are required to quantify several compounds based on the response of others.

Our integrated approach

Modern methods for the analysis of marine biotoxins are based on physicochemical techniques like LC-MS/MS. Triple quadrupole mass spectrometry allows for drastic reduction or elimination of matrix interferences. The Multiple Reaction Monitoring (MRM) is based on the detection of a secondary "product ion" produced by the collisional dissociation of an analyte "precursor ion". Whereas the analyte precursor ion (isolated in MS1 by a SIM mechanism) has the same selectivity as SIM, the resultant product ions (isolated in MS2 by a SIM mechanism) are more likely to be unique to the target compounds leading to an increased selectivity of the MRM. The combination of unique product ions (more selectivity) and the elimination of background noise results in consistently low limits of detection even for complex matrices. The method described here is a highly sensitive and specific method for the analysis of shellfish samples for lipophilic marine toxins using the Agilent 6460 Triple Quadrupole LC-MS system in MRM mode in combination with the Agilent 1200 SL Rapid Resolution HPLC and the Agilent MassHunter Workstation software. The described sample clean-up procedure is extremely simple, and thus the method is highly applicable to routine analysis. It allows for the analysis of OA, DTX-1, DTX-2 including their esters after hydrolysis, YTX, OH-YTX, PTX-1, PTX-2, AZA-1, AZA-2, and AZA-3. Due to the lack of commercially available standards some of the toxins have to be quantified using the calibration response of other compounds using the *CopyCalibrationLevel.quant.script.*

Methods and Operation

Modern methods for analysis of marine biotoxins are based on LC-MS/MS with Triple Quadrupole systems. The sample preparation is simply done by liquid extraction and subsequent filtration. Separation is based on HPLC and quantification is done by LC-ESI-MS/MS (MRM –positive and negative mode).

Analysis steps
Liquid Phase Extraction
HPLC Analysis with linear gradient
Detection by LC-ESI-MS/MS (MRM).

Sample preparation steps
An amount of 2 g of cooked, grinded shellfish tissue is weighed. Addition of 9 mL of methanol (80 %). This extraction procedure is done twice. The two extracts are combined and filled up to 50 mL in a volumetric flask. An aliquot is filtered to remove all remaining particles (filter RC 0.45 µm) and 10 µL of this sample extract is injected for the LC-MS/MS analysis.

Structures of analytes (example)

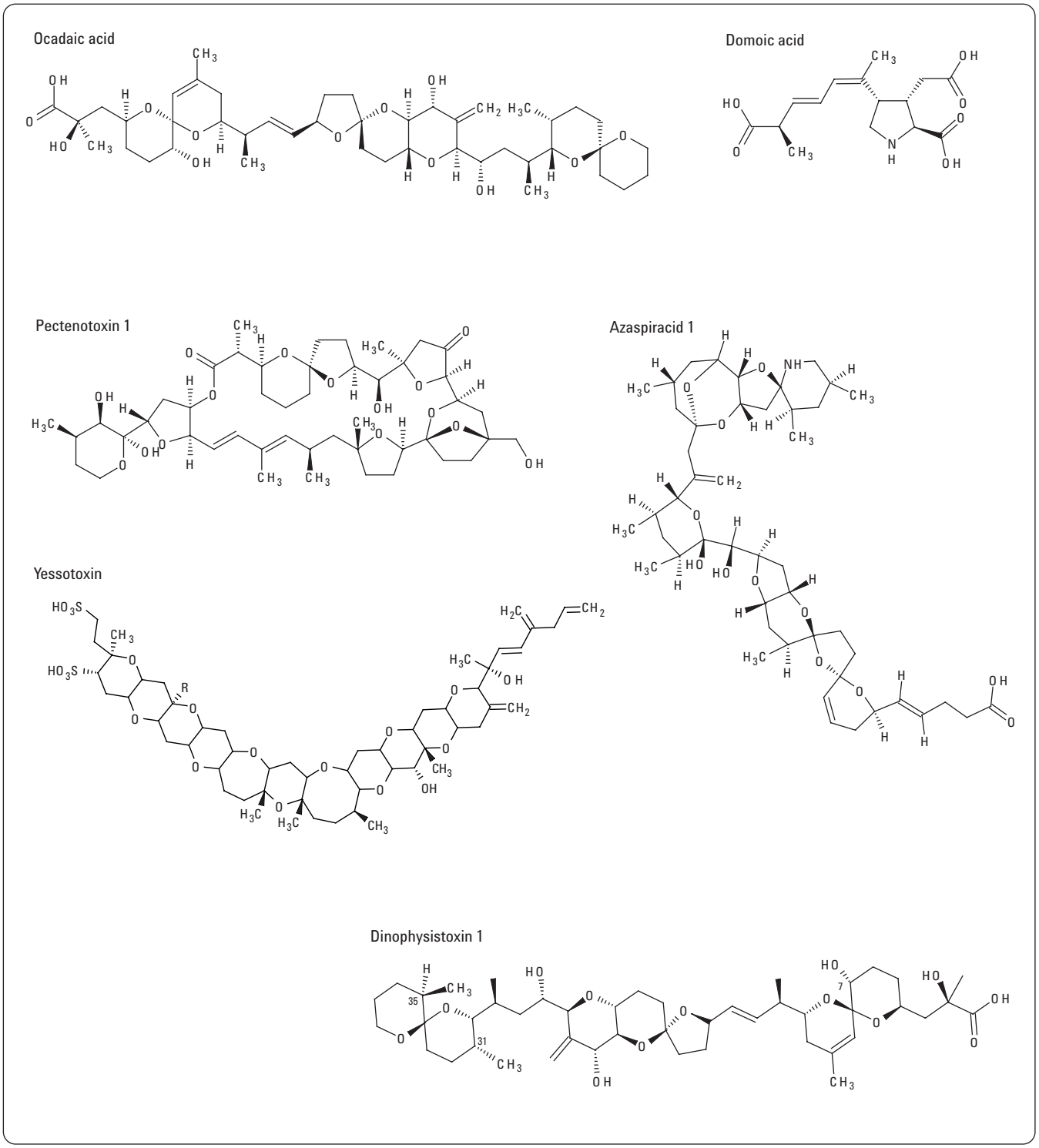

Figure 1
Chemical formulas of some marine toxins.

LC-MS/MS method

The total run time required to determine lipophilic marine toxins is less than 30 min. – The method runs in positive ionization mode for OA, DTX-1, DTX-2, PTX-1, PTX-2, AZA-1, AZA-2 and AZA-3. YTX is done in a separate run which requires a different column and mobile phase. The extract (10 µL) is injected directly to the LC-MS/MS system. For the LC method a solvent mixture of 0.1 % formic acid in Water (A) and MeOH (B) for positive ionization and 2 mM ammonium acetate in water (A) and MeOH (B) for negative ionization is used as mobile phase on a linear gradient. The column (Phenomenex Luna 5 µm C18(2) 100 Å 150 x 2.0 mm [pos. mode], ZORBAX Eclipse Plus C 8 4.6 x 75 mm 3.5 µm [neg. mode]) is held in an oven at 30 °C with a flow-rate of 0.2 mL/min.

Gradient* Time [min]	Solvent ratio B [%]
0	5
10	85
22	85
23	5
30	5

Table 1
Gradient settings.
*Applicable for positive and negative ionization mode.

Sample Preparation Steps

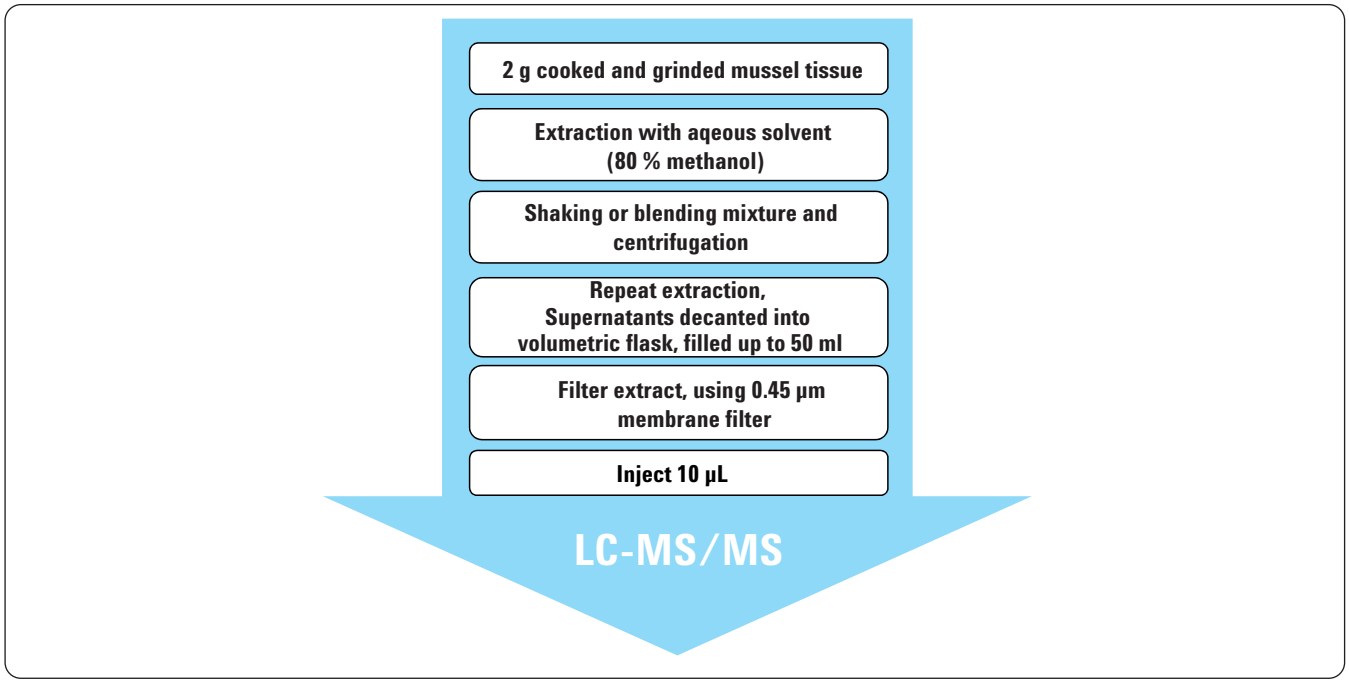

Figure 2
Sample preparation procedure for the determination of marine toxins in shellfish.
According to McNabb, P., A.I. Selwood, and P.T. Holland, Multiresidue method for determination of algal toxins in shellfish. J AOAC Int, 2005. 88: p. 761-772
Chapela, M.J., et al., Lipophilic toxins analyzed by liquid chromatography-mass spectrometry and comparison with mouse bioassay in fresh, frozen, and processed molluscs. J Agric Food Chem, 2008. 56(19): p. 8979-86.
Moutfort, D.O., T. Suzuki, and P. Trueman, Protein phosphatase inhibition assay adapted for determination of total DSP in contaminated mussels. Toxicon, 2001. 39: p. 383-390

Mass Spectrometer Settings and Jet Stream Parameter

Agilent 6460 QQQ ESI JetStream Source parameter

Gas Temperature:	300 °C
Gas Flow:	5 L/min
Nebulizer:	45 psi
Sheath Gas Temp:	250 °C
Sheath Gas Flow:	11 L/min
Capillary:	+ 3500 V
	- 3500 V
Nozzle Voltage:	+/- 500 V
Delta EMV	400

Agilent 6410 QQQ ESI-Source parameter

Ionization:	ESI
Gas Temperature:	300 °C
Gas Flow:	10 L/min
Nebulizer:	43 psi
Capillary:	+ 4500 V
	- 5200 V
Delta EMV:	400

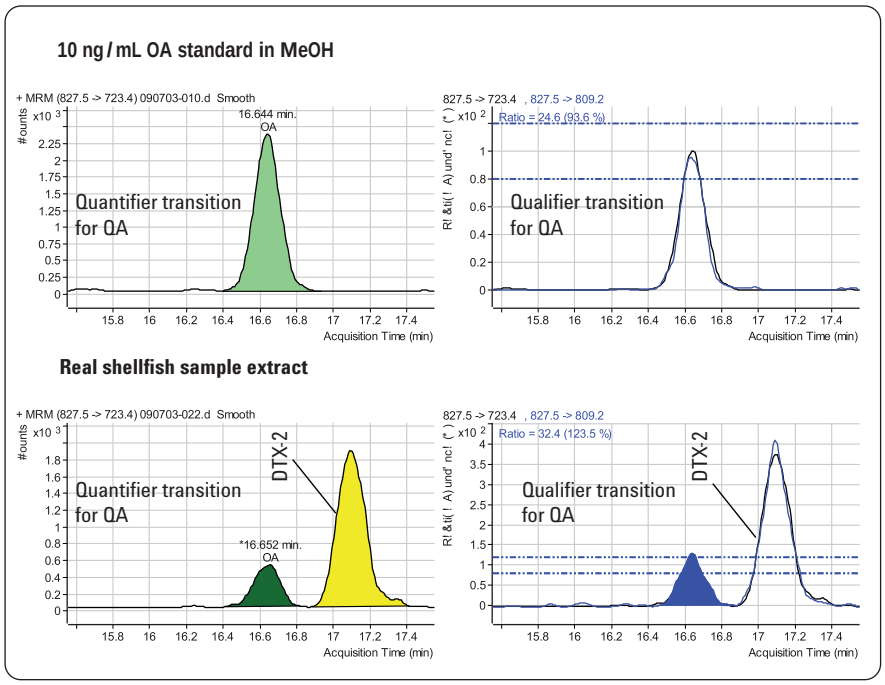

Figure 3
OA standard and real shellfish sample, containing OA and DTX-2 (same sample as shown in figure 4).
OA 23 µg/kg and DTX-2 130 µg/kg.

Analyte	Polarity	Prec Ion m/z	Prod Ion m/z	Frag [V]	CE [eV]	Quantifier
OA and DTX-2	pos	827.5	723.4	220	55	X
	pos	827.5	809.2	220	45	
DTX-1	pos	841.5	737.2	220	55	X
	pos	841.5	823.2	220	45	
PTX-1	pos	897.5	555.3	230	70	X
	pos	897.5	853.5	230	60	
PTX-2	pos	881.5	539.3	230	70	X
	pos	881.5	837.5	230	60	
PTX-2sa*	pos	899.5	855.5	230	60	X
	pos	899.5	557.3	230	70	
YTX	neg	1141.5	1061.3	135	35	X
	neg	1141.5	925.5	135	60	
Homo-YTX*	neg	1155.4	1075.5	135	35	X
OH-YTX	neg	1157.4	1077.5	135	35	X
OH-Homo-YTX*	neg	1171.4	1091.5	135	35	X
AZA-1	pos	842.5	824.5	200	40	X
	pos	842.5	806.5	200	55	
AZA-2	pos	856.5	838.5	200	40	X
	pos	856.5	820.5	200	55	
AZA-3	pos	828.5	810.5	200	40	X
	pos	828.5	792.5	200	55	

*Transitions based on literature information

Table 2
MRM transitions and MS settings.

Results

The method has been validated within an international collaborative study. The collaborative study was conducted in the framework of the working group §64 LFGB "Phycotoxins", which is hosted by the federal Office of Consumer Protection and Food Safety (BVL).

Compound	LOD	LOQ
[1]OA	6 µg/kg	20 µg/kg
[1]DTX-1 & 2	6 µg/kg	20 µg/kg
[2]AZA-1 to 3	6 µg/kg	20 µg/kg
[1]PTX-1 & 2	6 µg/kg	20 µg/kg
[3]YTX	10 µg/kg	35 µg/kg

[1] MRL in raw mussel material for sum of OA, DTX-1 & 2, PTX-1 & 2: 160 µg/kg OA equivalents

[2] MRL in raw mussel material for sum of azaspiracids: 160 µg/kg AZA-1 equivalents

[3] MRL in raw mussel material for sum of yessotoxins: 1000 µg/kg YTX equivalents

Table 3
LODs and LOQs of method for lipophilic marine toxins in cooked, grinded mussels.

Reproducibility S.D. is in the range from approx. 10 % to 35 % (depends on matrix, concentration, analyte). Extraction recovery is in the range from 75 % to 102 % (depends on analyte and matrix).

Benefits

- Highly sensitive and selective determination of marine toxins in shellfish with Agilent 6460 Triple Quadrupole LC-MS system and Agilent 1200 SL Rapid Resolution HPLC
- Simple and cost-effective sample preparation and easy workflow for routine sample analysis with high reliability
- Compliance with recent EFSA guidelines
- Flexibility to add other lipophilic toxins

Chromatogram of real shellfish samples

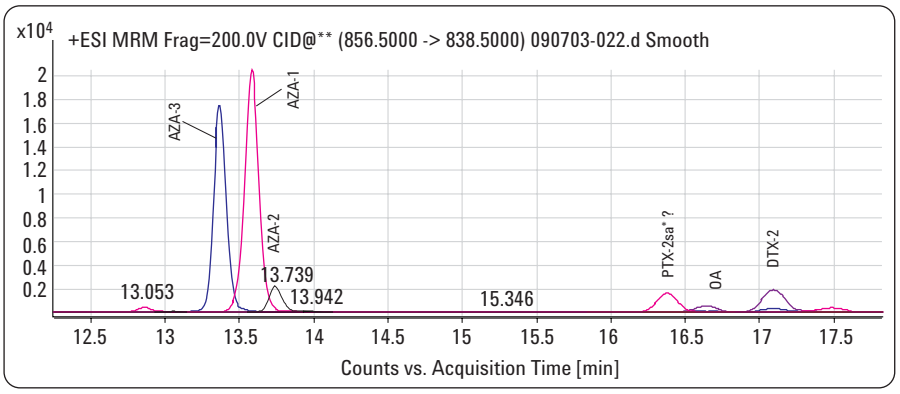

Figure 4
Real blue mussel sample extract.
Concentration: 96 µg/kg AZA-1, 22 µg/kg AZA-2, 50 µg/kg AZA-3, 23 µg/kg OA and 130 µg/kg DTX-2.
*PTX-2sa, tentatively assigned on the basis of transitions from literature, no standard available for PTX-2sa.

Chromatogram of real shellfish samples

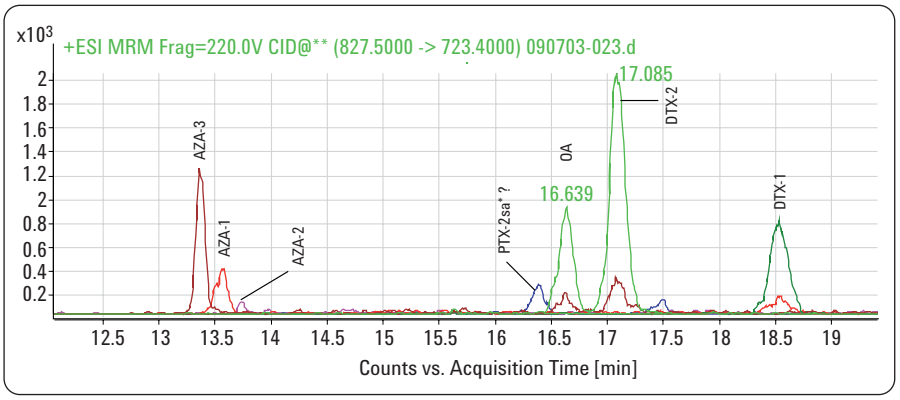

Figure 5
Real blue mussel extract, AZA-1 to -3 < LOQ 20 µg/kg.
Concentration: 37 µg/kg OA, 120 µg/kg DTX-2 and 69 µg/kg DTX-1.
*PTX-2sa, tentatively assigned on the basis of transitions from literature, no standard available for PTX-2sa.

Chromatogram of a QC sample

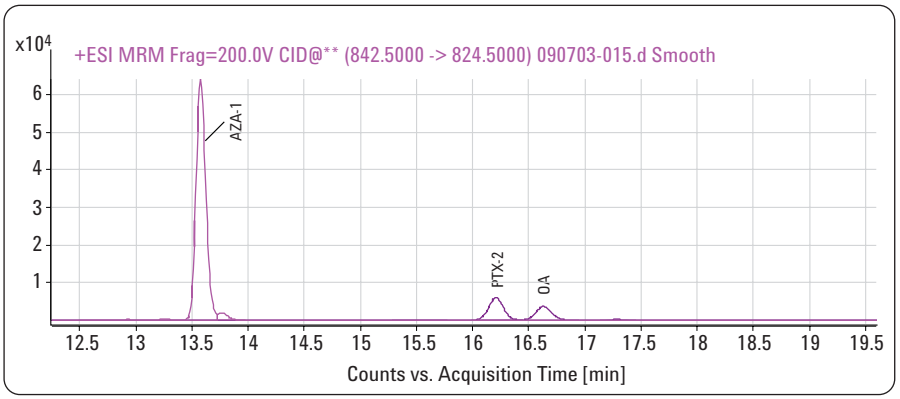

Figure 6
QC-Sample, 15 µg/kg for AZA-1, PTX-2 and OA, spiked in blue mussel extract.

Calibration curve (matrix matched)

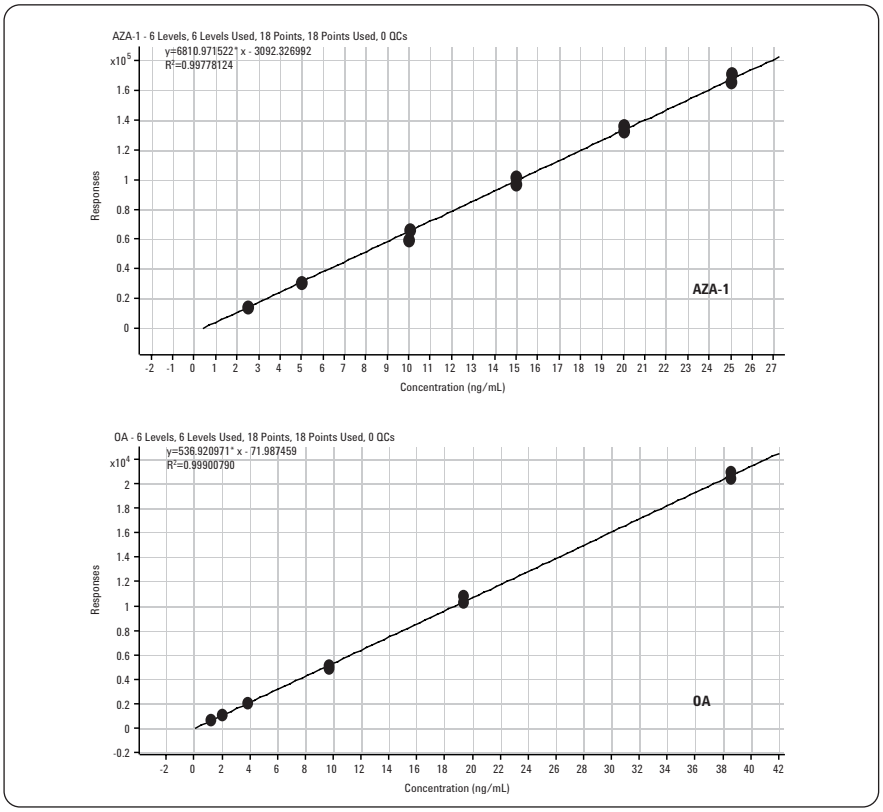

Figure 7
Calibration curve for AZA-1 and OA spiked in mussel extract, linear curve fit, origin included weighting none. Calibration range AZA-1 2.5 to 25 ng / mL and OA 1.1 to 38 ng / mL.

Calibration curve (in methanol)

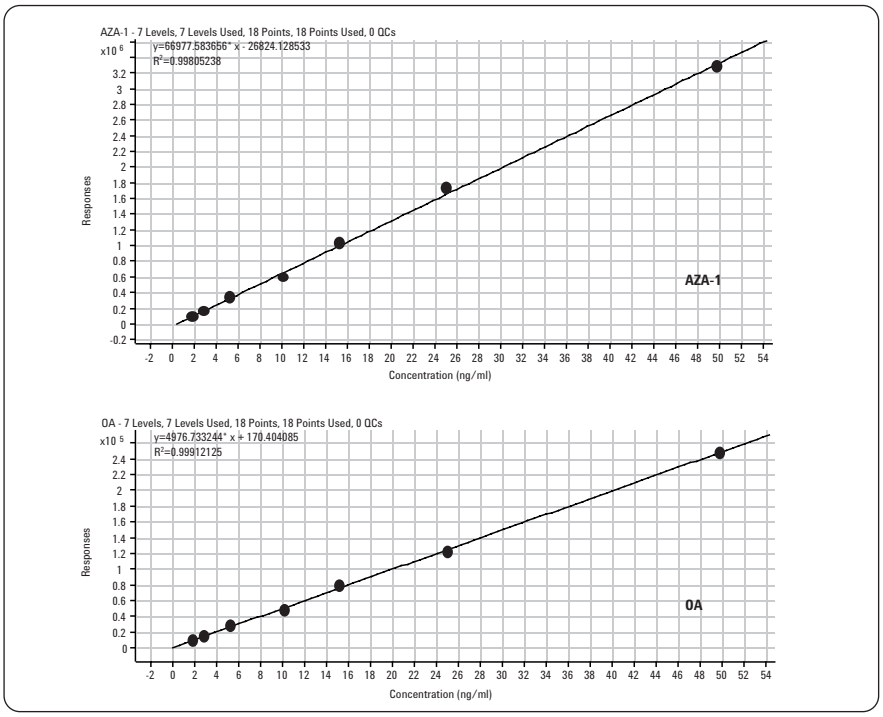

Figure 8
Calibration curve for AZA-1 and OA in MeOH, linear curve fit, origin included weighting none. Calibration Range 1.5 to 50 ng / mL for both.

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Published September 1, 2010
Publication Number 5990-6377EN

Agilent Technologies

Determination of Chemical Contaminants in Marine Shellfish using the Agilent 7000 Triple Quadrupole GC/MS System

Application note

Food Safety

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Abstract

A sample preparation method based on a modified QuEChERS extraction has been developed along with a GC/MS/MS method for the determination of selected Organo-chlorine pesticides, Polyaromatic hydrocarbons and Polychlorinated biphenyl congeners. The analytical method meets the detection limit requirements for the organic chemical contaminants in marine shellfish tissue (mussel) stipulated in the United Kingdom's Clean Seas Environmental Monitoring Program.

Agilent Technologies

Introduction

The Clean Seas Environmental Monitoring Program (CSEMP) is an initiative designed to monitor the levels of chemical contamination in the UK's coastal and estuarine areas. The major drivers for this program are

- To meet the mandatory monitoring requirements under Oslo and Paris Convention (OSPAR) Joint Assessment and Monitoring Program (JAMP).
- Compliance with EC Directives.

The EC dangerous substance directive (76/464/EEC) requires the analysis of sediment or biota to determine the trend in the substances discharged. Organic compounds in Shellfish are also monitored to meet some requirements of Shellfish Water Directive (79/923/EEC), the Shellfish Hygiene Directive (91/492/EEC) and as amended by 97/61/EC, and Fisheries Products Directive (91/493/EEC) [1].

The program specifies 16 organo-chlorine compounds (OCPs), 28 polyaromatic hydrocarbons (PAHs) and 7 polychlorinated biphenyl congeners (PCBs). The Limit of Detection (LoD) requirements are 0.1 µg/Kg for OCPs and PCBs, and 0.5 – 1.0 µg/Kg for PAHs.

An extraction method for these organic contaminants in marine shellfish tissue (mussel), based on a modified QuEChERS [2], [3] extraction method, has been developed and the extracts from which were analysed by gas chromatography coupled to a triple quadrupole mass spectrometer (GC-QQQ). The chromatographic method includes a post-column pressure controlled tee which facilitates post-column, post-run backflush in order to remove high boiling matrix components that would otherwise remain in the column between analyses and subsequently cause degradation of chromatographic performance and contamination of the mass spectrometer ion source. The effectiveness of post-column back flush has been demonstrated in a previously published Agilent application note [4].

Experimental

Calibration Standards

Calibration mixtures of native PAHs and isotope labelled PAH internal standards were obtained from SPEX Certiprep and Cambridge Isotopes, respectively. Custom made mixture for OCPs and PCB congeners were procured from LGC Promochem. PCB 155 and isotope labelled OCP internal standards were obtained from QMX and CDN Isotopes.

Sample Preparation

2 g amounts of homogenized mussel tissue samples were extracted using a modified QuEChERS extraction method. The extraction and clean up workflow is shown in Figure 1.

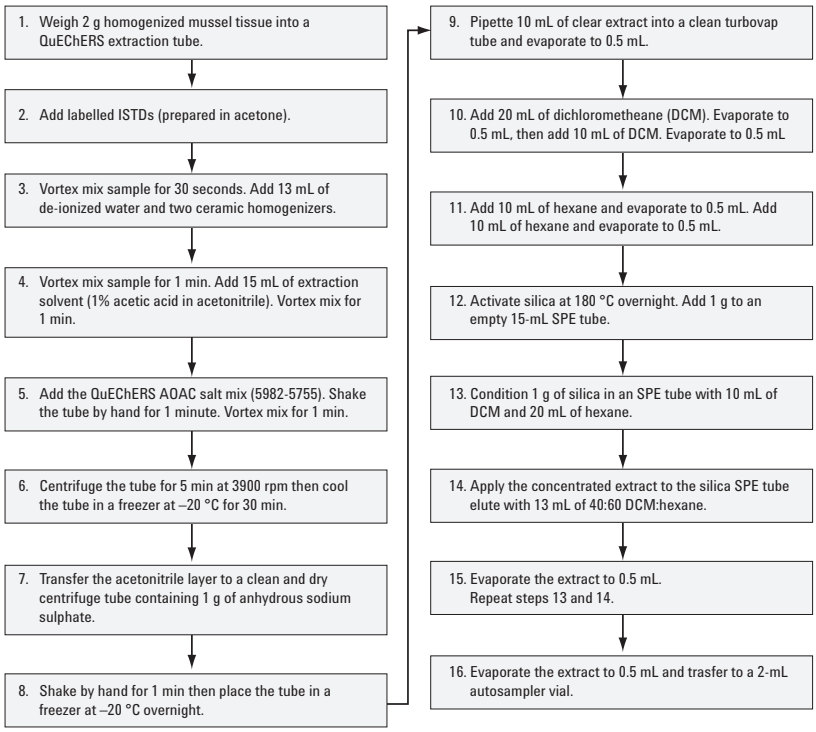

Figure 1. Flow diagram of the sample extraction and clean-up procedure.

GC/MS/MS Analysis

The analyses were performed on an Agilent 7890 GC / 7000 Triple Quadrupole GC/MS system. The 7890 Series GC was configured with a carbon dioxide cooled Multimode Inlet (MMI) and a 15 m × 0.25 mm id, 0.25 µm DB-5MSUI capillary column coupled to a 0.65 mm id × 0.15 mm id, 0.15 µm DB-5MSUI restrictor to the mass spectrometer via a capillary flow pressure controlled tee. A schematic diagram of the GC/MS/MS system configuration is shown in Figure 2.

The analytical column was operated in constant flow mode and the chromatography was retention time locked using PCB 118 as the locking compound at a retention time of 12.370 minutes. The pressure controlled tee was operated in constant pressure mode with helium controlled by a pneumatics control module (PCM).

An Agilent 7693A auto-liquid sampler with was employed and either 1 µL cold splitless injections using a 10 µL syringe (during GC/MS/MS method optimization) or 10 µL solvent vent injections made using a 25 µL syringe (for instrument calibration and sample analyses).

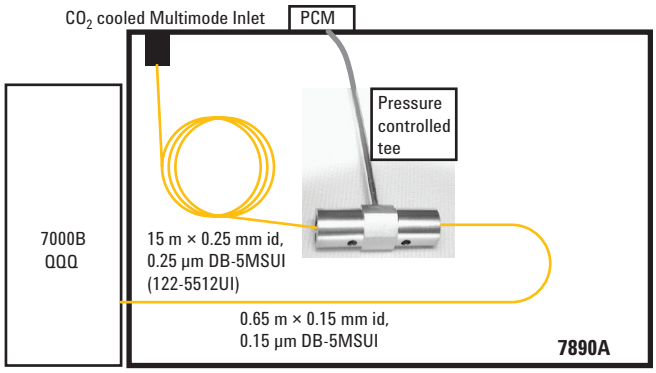

Figure 2. Schematic diagram of GC/MS/MS system configuration.

The GC instrument conditions are listed in Table 1.

The mass spectrometer was operated in electron impact ionization (EI) MS/MS mode using multiple reaction monitoring (MRM) for all the analytes and their associated internal standards. Mass spectrometer operating conditions are given in Table 2 and the full list of analytes with their respective retention times, monitoring ion transitions, collision energies and, dwell times are shown in Table 3.

Table 1. GC Analysis Conditions

Column (1)	15 m × 0.25 mm id, 0.25 µm DB-5MSUI (122-5512UI)
Column (2)	0.65 m × 0.15 mm id, 0.15 µm DB-5MSUI (cut from 165-6626)
Injection mode (1)	1 µL cold pulsed splitless using CO_2 cooled Multimode Inlet (MMI) and a 10 µL syringe
Inlet temperature program	50 °C (0.05 min), 600 °C/min to 325 °C
Inlet pressure pulse	13.0 psig for 0.75 min
Purge Flow to Split Vent	50 mL/min at 1.0 min
Injection port liner	2 mm id, multi-baffled (5190-2296)
Injection mode (2)	10 µL solvent vent using CO_2 cooled Multimode Inlet (MMI) and a 25 µL syringe
Inlet temperature program	40 °C (0.31 min), 600 °C/min to 325 °C
Inlet Vent pressure	5.0 psig
Inlet vent flow	100 mL/min
Inlet vent time	0.31 min
Outlet pressure	0 psig
Injection speed	100 µL/min
Purge Flow to Split Vent	50 mL/min at 1.0 min
Injection port liner	2 mm id, multi-baffled (5190-2296)
Carrier Gas	Helium, constant flow 1.2 mL/min
Oven temp program	50 °C (1) – 20 – 200 °C/min (0) – 10 °C/min – 300 °C (1.5)
RTL Compound	PCB 118, locked at 12.370 min
Pressure controlled tee	G3186B, operated at 2.0 psig constant pressure
Back flush conditions	Inlet pressure 1.0 psig, PCM pressure 60 psig, time 2.0 min

Table 2. Mass Spectrometer Operating Conditions

MS Transfer line temp	325 °C
MS Source	300 °C
MS Quad 1 , 2 temp	150 °C , 150 °C
Collision cell gases	Nitrogen 1.5 mL/min, Helium 2.25 mL/min
MS1 / MS2 Resolution	Wide/wide
MRM settings	See Table 3
Electron energy	-70 eV
Ionization mode	Electron impact (EI)
EI Autotune	Gain normalized
Gain factor	5

Table 3. MS/MS Settings for OCPs, PAHs, PCB Congeners and Labelled Internal Standards

TS	Time	Analyte	RT (min)	Precursor	Product	Dwell (ms)	CE(V)	Precursor	Product	Dwell (ms)	CE(V)
1	4.0	d3-135-TCB	5.050	182.9	147.9	25	35	182.9	110.9	25	35
		135-TCB	5.068	179.9	144.9	25	35	179.9	108.9	25	35
		d8-Napthalene	5.479	136.0	108.0	25	25				
		Naphthalene	5.504	128.0	102.0	25	22	128.0	127.0	25	20
		HCBD	5.658	224.9	189.9	25	22	224.9	187.9	25	22
2	6.8	d8-Acenapthylene	7.308	160.0	132.0	25	30	160.0	108.0	25	30
		Acenaphthylene	7.321	152.0	151.0	25	40	152.0	150.0	25	40
		d10-Acenapthene	7.494	164.0	162.0	25	30	164.0	160.0	25	30
		Acenaphthene	7.525	154.0	152.0	25	40	153.0	152.0	25	40
3	7.8	d10-Fluorene	8.099	176.0	174.0	15	30				
		Fluorene	8.131	166.0	165.0	15	30				
		d6-HCH - alpha	8.699	224.0	187.0	15	15	224.0	150.0	15	15
		HCH - alpha	8.730	181.0	145.0	15	15	181.0	109.0	15	30
		HCB	8.770	283.9	248.8	15	25	283.9	213.9	15	35
		HCH- beta	8.990	181.0	145.0	15	15	181.0	109.0	15	30
		d6-HCH- gamma	9.077	224.0	187.0	15	15	224.0	150.0	15	15
		HCH - gamma	9.107	218.8	183.0	15	5	181.0	109.0	15	30
		Dibenzothiophene	9.110	184.0	152.0	15	40	184.0	139.0	15	40
		d10-Phenanthrene	9.274	188.0	184.0	15	40	188.0	160.0	15	40
		Phenanthrene	9.299	178.0	176.0	15	34				
		Anthracene	9.367	178.0	176.0	15	34				
		HCH - delta	9.428	181.0	145.0	15	15	181.0	109.0	15	30
4	9.6	PCB 28	9.820	256.0	186.0	20	26	258.0	186.0	20	26
		PCB 52	10.250	289.9	220.0	20	28	291.9	222.0	20	28
		Aldrin	10.480	298.0	263.0	20	8	263.0	191.0	20	30
		Isodrin	10.880	262.9	193.0	20	35	262.9	191.0	20	35
5	11.0	d10-Fluoranthene	11.103	212.0	210.0	15	45	212.0	208.0	15	45
		Fluoranthene	11.128	202.0	201.0	15	30	202.0	200.0	15	50
		PCB 155	11.280	357.8	287.9	15	28	359.8	289.9	15	28
		op-DDE	11.375	248.0	176.0	15	30	246.0	211.0	15	20
		PCB 101	11.437	323.9	253.9	15	28	325.9	255.9	15	28
		d10-Pyrene	11.486	212.0	210.0	15	45	212.0	208.0	15	45
		Pyrene	11.512	202.0	201.0	15	30	202.0	200.0	15	45

Table 3. MS/MS Settings for OCPs, PAHs, PCB Congeners and Labelled Internal Standards (Continued)

TS	Time	Analyte	RT (min)	Precursor	Product	Dwell (ms)	CE(V)	Precursor	Product	Dwell (ms)	CE(V)
5		pp-DDE	11.857	248.0	176.0	15	30	246.0	211.0	15	20
		C13-Dieldrin	11.933	269.8	200.0	15	40	269.8	198.0	15	40
		Dieldrin	11.940	262.8	193.0	15	30	262.8	191.0	15	30
		op-DDD	11.956	237.0	165.0	15	20	235.0	200.0	15	8
6	12.15	Endrin	12.265	281.0	245.0	25	20	263.0	193.0	25	35
		PCB 118 (RTL compound)	12.370	323.9	253.9	25	28	325.9	255.9	25	28
		pp-DDD	12.500	237.0	165.0	25	20	235.0	199.1	25	8
		op-DDT	12.543	237.0	165.0	25	20	235.0	199.1	25	20
		PCB 153	12.698	357.8	287.9	25	28	359.8	289.9	25	28
		C13-pp-DDT	13.091	247.0	177.0	25	20	247.0	211.0	25	20
		pp-DDT	13.099	237.0	165.0	25	20	235.0	199.1	25	20
		PCB 138	13.112	357.8	287.9	25	28	359.8	289.9	25	28
7	13.5	Benzo[a]anthracene	13.897	228.0	226.0	40	38				
		d12-Chrysene	13.915	240.0	236.0	40	35				
		Chrysene / Triphenylene	13.965	228.0	226.0	40	38				
		PCB 180	14.175	393.8	323.9	40	30	395.8	325.9	40	30
8	15.0	Benzo[b+j]fluoranthene	16.06	252.0	250.0	75	42	250.0	248.0	75	40
		d12-Benzo[k]fluoranthene	16.084	264.0	260.0	75	40				
		Benzo[k]fluoranthene	16.116	252.0	250.0	75	42	250.0	248.0	75	40
		Benzo[e]pyrene	16.561	252.0	250.0	75	42	250.0	248.0	75	40
		d12-Benzo[a]pyrene	16.616	264.0	260.0	75	40				
		Benzo[a]pyrene	16.654	252.0	250.0	75	42	250.0	248.0	75	40
		Perylene	16.814	252.0	250.0	75	42	250.0	248.0	75	40
9	18.0	d12-Indeno[123-cd]pyrene	18.600	288.0	284.0	75	50				
		Indeno(123-cd)pyrene	18.631	276.0	274.0	75	42				
		d14-Dibenz[a,h]anthracene	18.662	292.0	288.0	75	50				
		Dibenz[a,h]anthracene	18.712	278.0	276.0	75	38				
		d12-Benzo[g,h,i]perylene	19.020	288.0	284.0	75	45				
		Benzo[ghi]perylene	19.064	276.0	274.0	75	38				

Results and Discussion

Chromatography

The total ion chromatogram (TIC) for all MRM transitions of all analytes is shown in Figure 3. For additional clarity, labelled TIC MRM chromatograms of the OCPs, PAHs and PCB congeners are shown in Figures 4, 5, and 6, respectively.

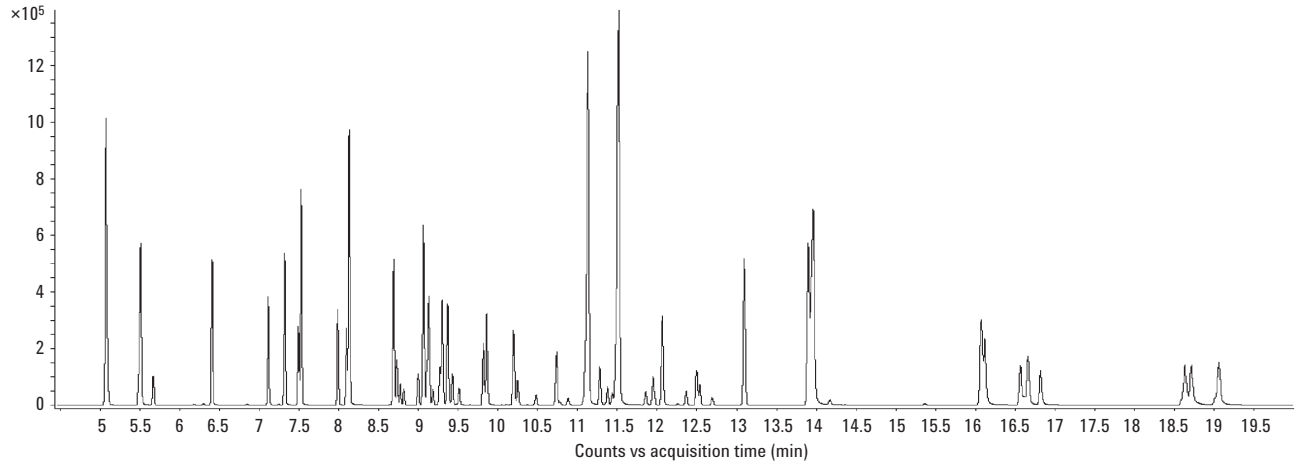

Figure 3. TIC MRM Chromatogram for a calibration standard.

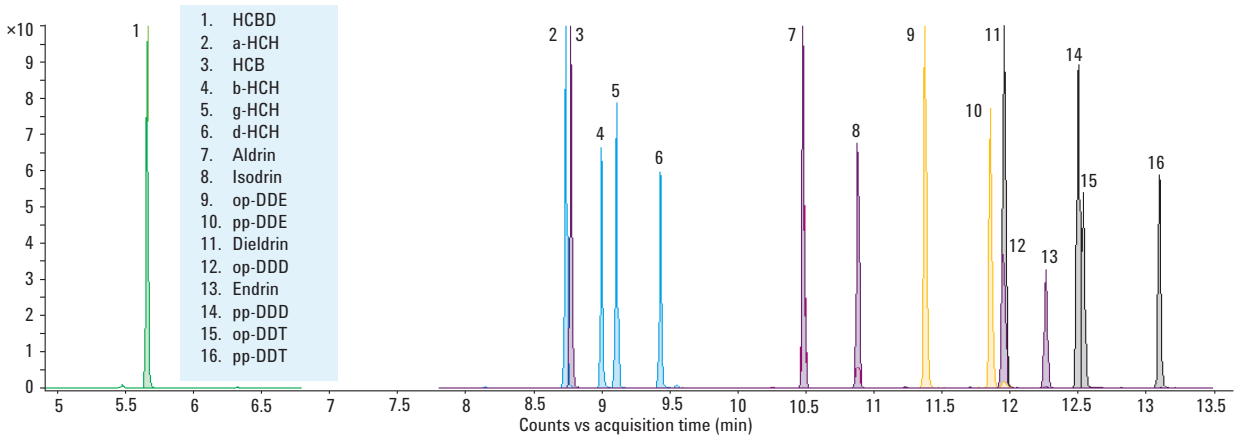

Figure 4. TIC MRM Chromatogram for OCP analytes.

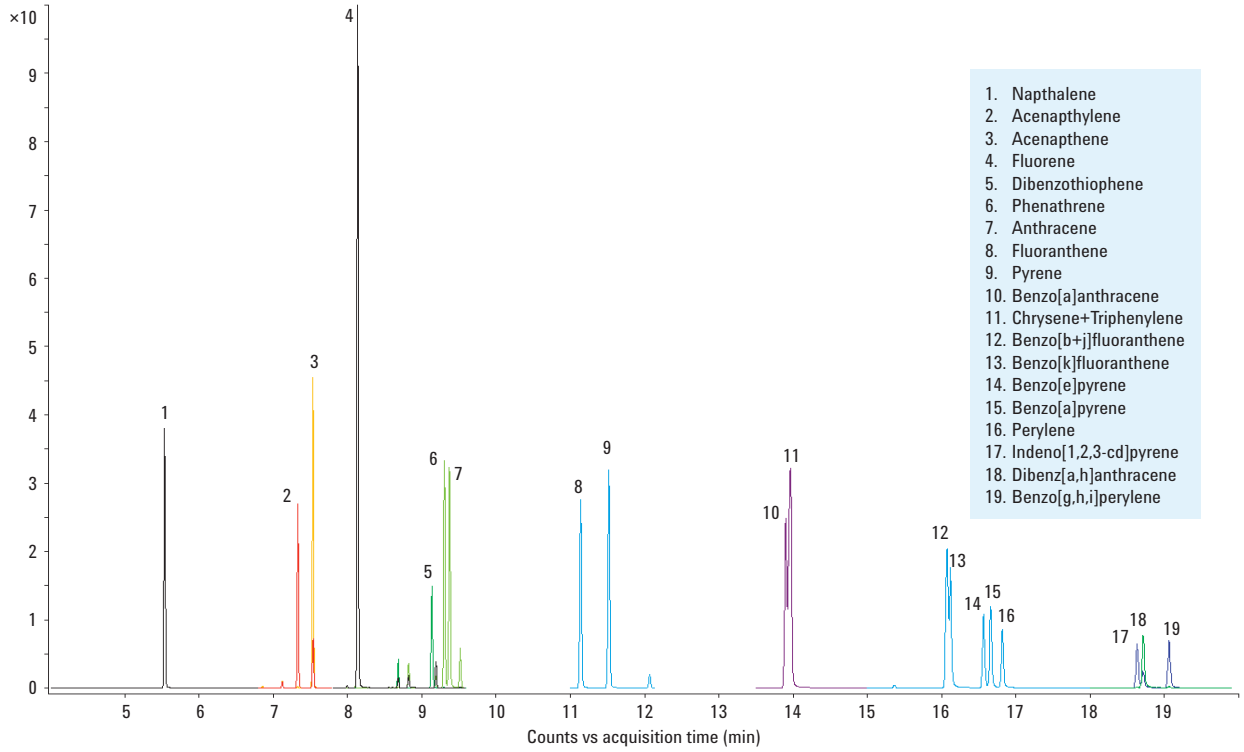

Figure 5. TIC MRM Chromatogram for PAH analytes.

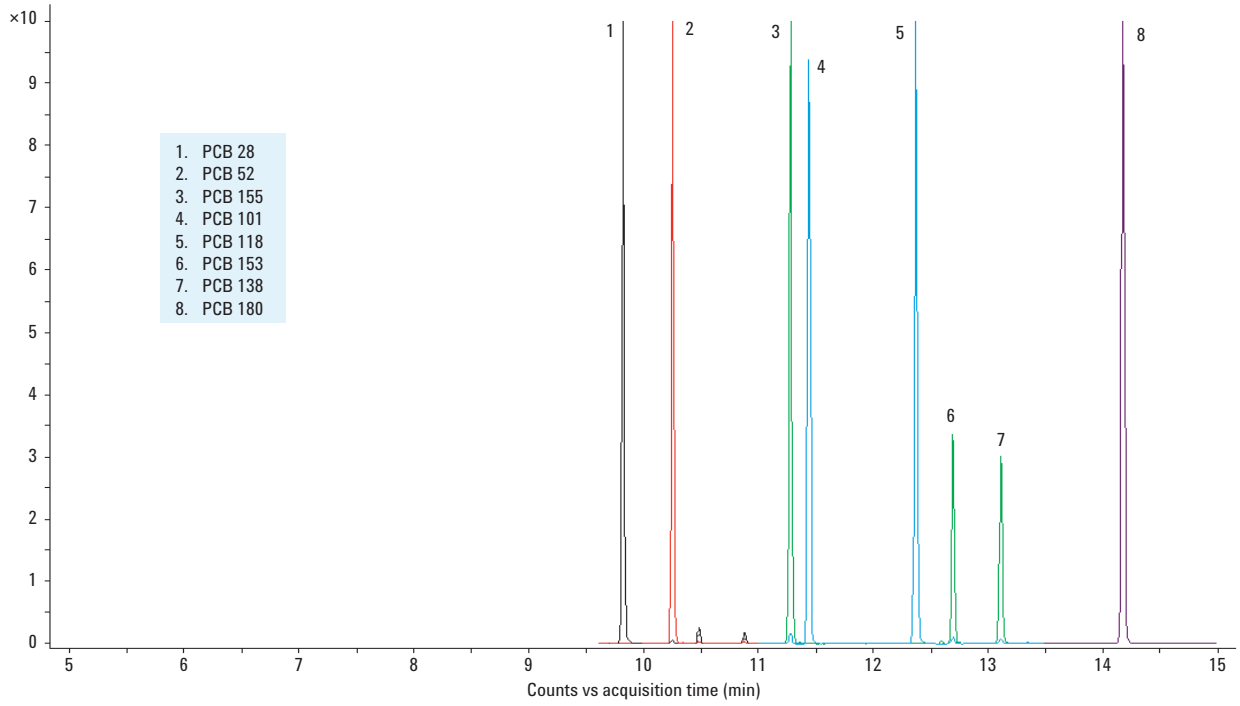

Figure 6. TIC MRM Chromatogram for PCB congeners.

Analyte calibration curves

The GC-MS/MS system was calibrated using a five-point internal standard (ISTD) calibration. The calibration standards for target analytes were prepared in hexane at concentrations of 0.4, 2.0, 8.0, 80.0, and 200.0 pg/µL. All ISTDs were added at 80.0 pg/µL. Calibration curves were created using 10 µL solvent vent mode injections. The calibration curves for all analytes gave correlation coefficients greater than 0.999. Table 4 shows curve fit types and correlation coefficient values.

Table 4. Curve Fits and Correlation Coefficients for ISTD Calibration Curves

Analyte	Curve fit	R^2
HCBD	Quadratic	0.9994
a-HCH	Linear	0.9996
HCB	Linear	0.9998
b-HCH	Linear	0.9995
g-HCH	Linear	0.9999
d-HCH	Linear	0.9991
Aldrin	Quadratic	0.9999
Isodrin	Quadratic	0.9999
op-DDE	Linear	0.9998
p,p-DDE	Linear	0.9993
Dieldrin	Quadratic	0.9992
op-DDD	Quadratic	0.9999
Endrin	Linear	0.9997
pp-DDD	Linear	0.9997
o,p-DDT	Linear	0.9992
p,p-DDT	Linear	0.9995
Napthalene	Linear	0.9997
Acenapthylene	Linear	0.9997
Acenapthene	Linear	0.9999
Fluorene	Linear	0.9997
Dibenzothiophene	Quadratic	0.9999
Phenanthrene	Linear	0.9999
Anthracene	Linear	0.9997
Fluoranthene	Linear	0.9992
Pyrene	Linear	0.9996
Benzo[a]anthracene	Linear	0.9998
Chrysene+Triphenylene	Quadratic	0.9999
Benzo[b+j]fluoranthene	Linear	0.9998
Benzo[k]fluoranthene	Quadratic	0.9997
Benzo[e]pyrene	Linear	0.9996
Benzo[a]pyrene	Linear	0.9998
Perylene	Linear	0.9999
Indeno[123-cd]pyrene	Quadratic	0.9996
Dibenz[a,h]anthracene	Quadratic	0.9999
Benzo[g,h,i]perylene	Quadratic	0.9997
PCB 28	Linear	0.9998
PCB 52	Linear	0.9998
PCB 101	Linear	0.9999
PCB 118	Linear	0.9996
PCB 153	Linear	0.9998
PCB 138	Linear	0.9998
PCB 180	Linear	0.9994

Example calibration graphs over the range of interest for g-HCH, PCB 118 and Benzo[a]pyrene are shown in Figure 7.

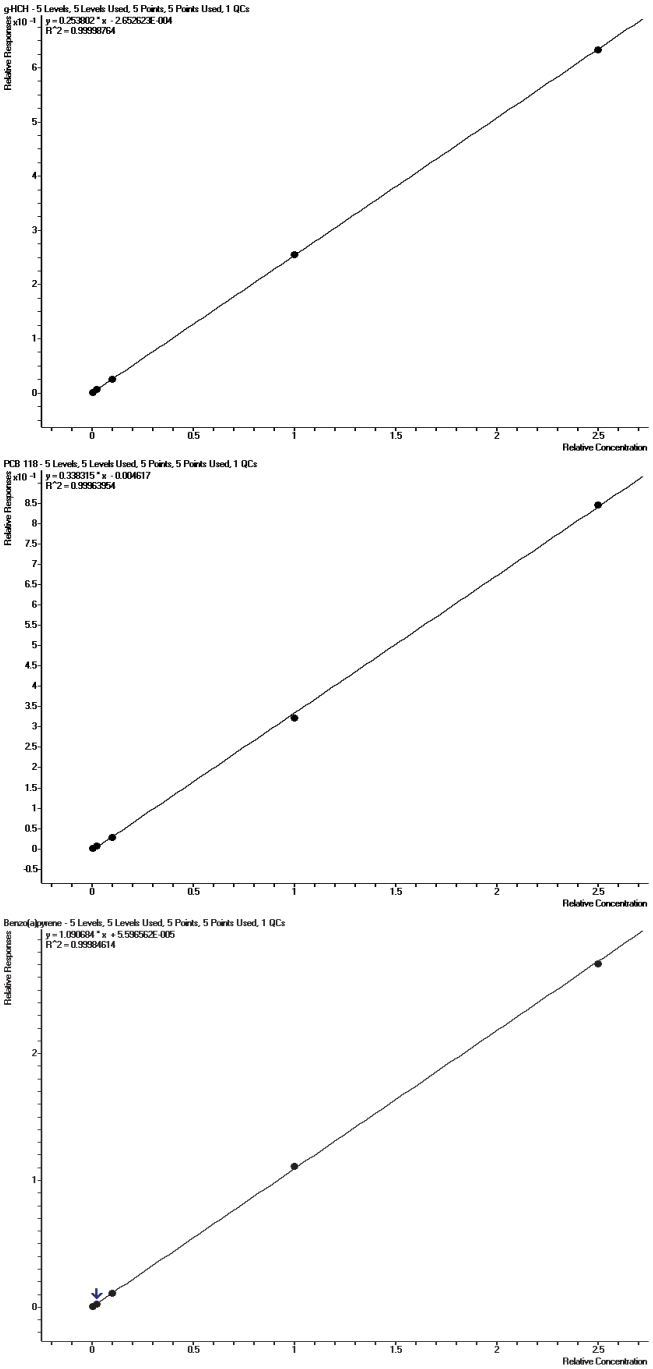

Figure 7. Five point ISTD calibration curves for g-HCH (Top), PCB 118 (middle) and Benzo[a]pyrene (bottom).

Recovery of target analytes and Quantitative Reproducibility

Five sample aliquots (2g) from homogenized mussel tissue were weighed into QuEChERS extraction tubes. The samples were spiked with an acetone solution of target analytes at a level equivalent to 4 µg/Kg (8 ng/2g sample) and internal standards. The extraction tubes were then vortex mixed for 1 minute and the samples were extracted using the procedure given in Figure 1.

Relative percent standard deviations (RSD%) and Spike Recoveries (Recovery %) were calculated for each of the target analytes as given below;

$$RSD\% = \frac{SD}{Mean} \times 100$$

$$\text{Recovery } \% = \frac{Mean(spiked)}{[Mean(unspiked) + Spike]} \times 100$$

where SD is the Standard Deviation.

The list of target analytes, grouped by chemical class (OCPs, PAHs and PCBs) plus their associated internal standards, quantitative reproducibility values and percentage recovery values are shown in Table 5. Percent recovery values for the OCPs, PAHs and PCB congeners from spiked mussel tissue are also shown graphically in Figure 8, (a), (b) and (c), respectively.

Table 5. Target Analytes, Their Associated ISTDs, RSD% Values for Quantitative Reproducibility and Recovery% Values

Analyte	ISTD	RSD% [n=5]	Recovery%
HCBD	d3-135-TCB	11.1	85.4
a-HCH	d6-g-HCH	3.9	115.1
HCB	d6-a-HCH	13.3	92.0
b-HCH	PCB-155	7.0	116.8
g-HCH	d6-g-HCH	2.3	114.1
d-HCH	PCB-155	6.7	123.9
Aldrin	PCB-155	15.8	108.9
Isodrin	PCB-155	13.9	108.7
op-DDE	PCB-155	3.5	120.4
p,p-DDE	PCB-155	4.8	121.5
Dieldrin	^{13}C-Dieldrin	4.0	93.4
op-DDD	PCB-155	4.0	119.9
Endrin	^{13}C-Dieldrin	7.7	112.7
pp-DDD	^{13}C-pp-DDT	6.1	101.6
o,p-DDT	^{13}C-pp-DDT	3.5	104.1
p,p-DDT	^{13}C-pp-DDT	1.1	100.0
Napthalene	d8-Napthalene	3.7	107.7
Acenapthylene	d8-Acenapthylene	7.4	98.5
Acenapthene	d10-Acenapthene	5.0	102.5
Fluorene	d10-Fluorene	8.4	100.9
Dibenzothiophene	d10-Fluorene	8.5	105.6
Phenanthrene	d10-Phenanthrene	7.8	102.6
Anthracene	d10-Phenanthrene	5.6	100.2
Fluoranthene	d10-Fluoranthene	0.9	101.0
Pyrene	d10-Pyrene	7.0	92.3
Benzo[a]anthracene	d12-Chrysene	4.1	103.5
Chrysene+Triphenylene	d12-Chrysene	1.1	104.5
Benzo[b+j]fluoranthene	d12-Benzo[k]fluoranthene	24.0	107.7
Benzo[k]fluoranthene	d12-Benzo[k]fluoranthene	5.4	104.1
Benzo[e]pyrene	d12-Benzo[a]pyrene	1.6	105.0
Benzo[a]pyrene	d12-Benzo[a]pyrene	3.3	102.9
Perylene	d12-Benzo[a]pyrene	1.1	106.4
Indeno[123-cd]pyrene	d14-Dibenz[a,h]anthracene	2.8	94.9
Dibenz[a,h]anthracene	d14-Dibenz[a,h]anthracene	2.4	103.0
Benzo[g,h,i]perylene	d14-Dibenz[a,h]anthracene	4.8	103.1
PCB 28	PCB-155	3.9	105.5
PCB 52	PCB-155	3.0	105.8
PCB 101	PCB-155	3.5	112.3
PCB 118	PCB-155	6.1	107.0
PCB 153	PCB-155	3.6	107.6
PCB 138	PCB-155	4.5	109.9
PCB 180	PCB-155	4.8	110.1

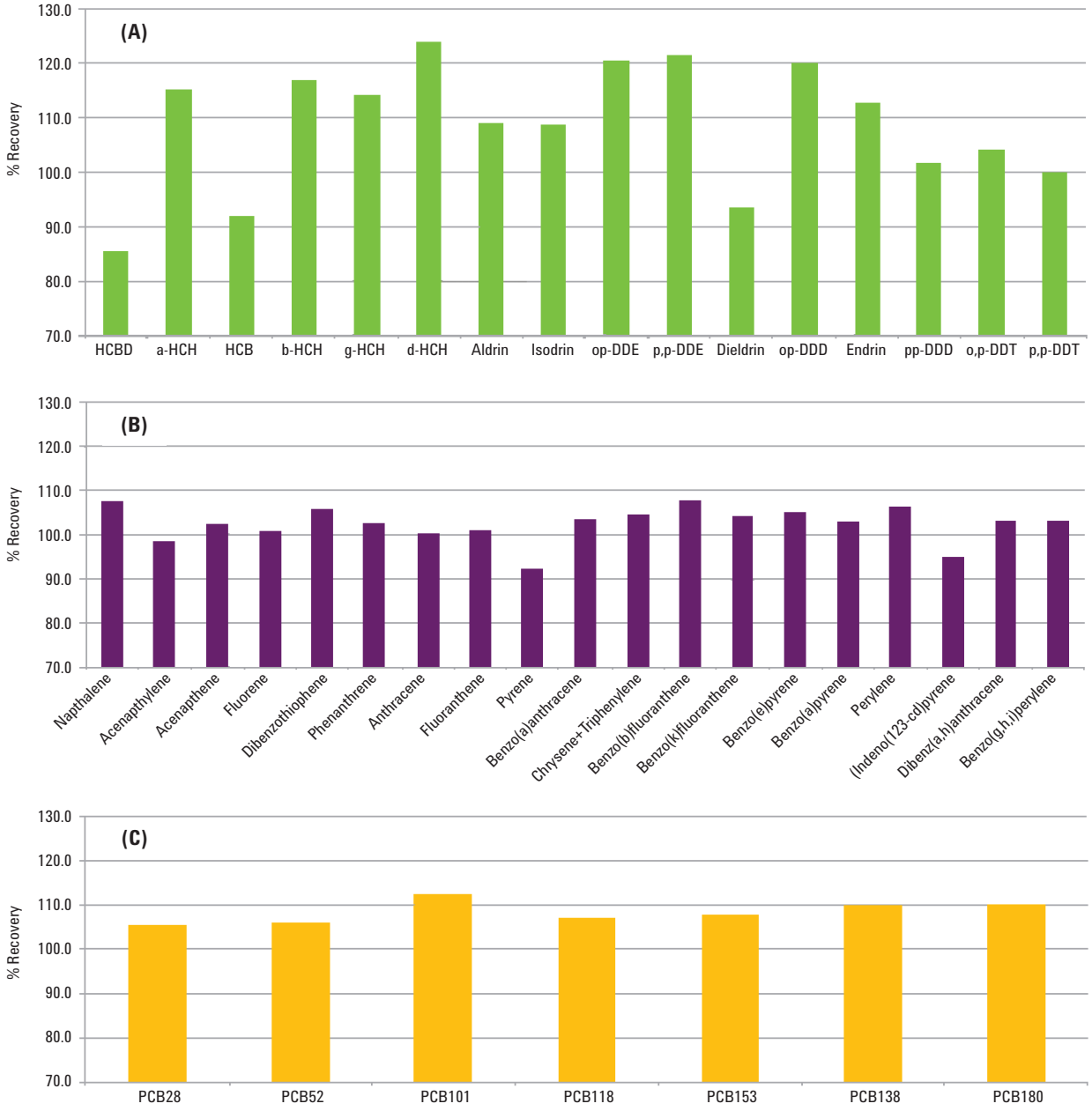

Figure 8. Graphical representation of analyte percent recovery values for (a) OCPs, (b) PAHs and (c) PCB congeners in spiked mussel tissue.

Sample Analysis

Marine mussel samples were sourced from local commercial shell fish suppliers, homogenized, extracted and analysed using the sample preparation and GC/MS/MS conditions as described. MRM chromatograms for the incurred HCH isomers quantified in a mussel sample are shown in Figure 9, the incurred Fluoranthene and Pyrene PAHs in Figure 10 and, the incurred PCB 180 congener in Figure 11, respectively.

Conclusion

A sample preparation method based on a modified QuEChERS extraction and clean up regime has been developed and applied to the extraction of OCPs, PAHs and PCB congeners from marine mussel tissue. The quantitative GC/MS/MS method demonstrated good reproducibility and recoveries for all analytes were in the range of 85.4% – 123.9% in spiked mussel tissue.

The Agilent 7000 Triple Quadrupole GC/MS system provided reproducible and sensitive detection of OCPs, PAHs and PCB congeners in mussel tissue down to concentration levels of 0.1 µg/Kg. The performance of the extraction/clean-up and analysis by GC/MS/MS meets the requirements of the CSEMP legislation.

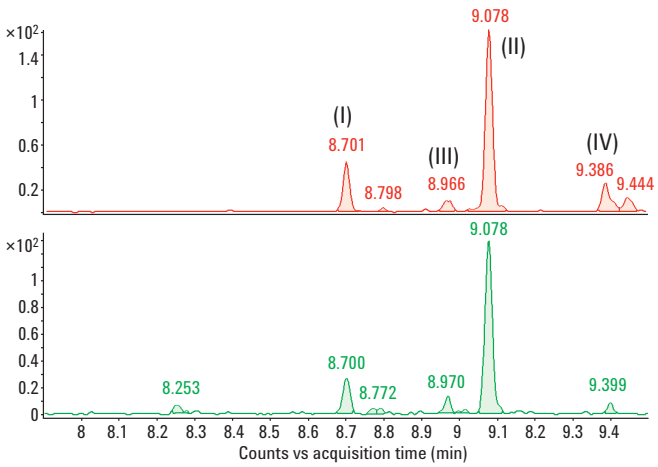

Figure 9. MRM Chromatograms for (i) incurred a-HCH and (ii) incurred g-HCH in mussel sample, Concentrations 0.06 and 0.30 µg/Kg, respectively. Peaks (iii) and (iv) are traces of incurred b-HCH and d-HCH, respectively.

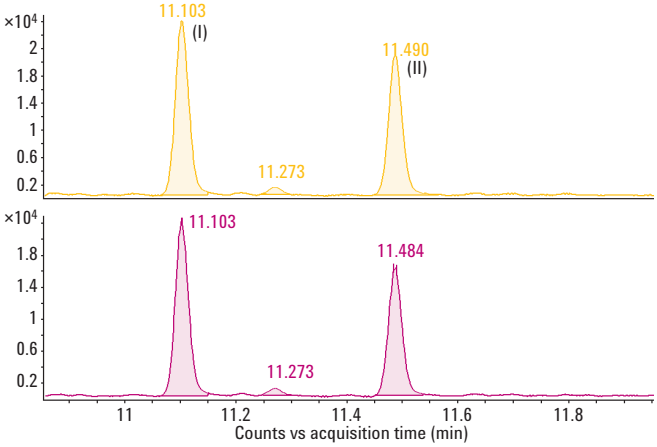

Figure 10. MRM Chromatograms for (i) incurred Fluoranthene and (ii) incurred Pyrene in mussel sample, Concentrations 8.64 and 5.83 µg/Kg, respectively.

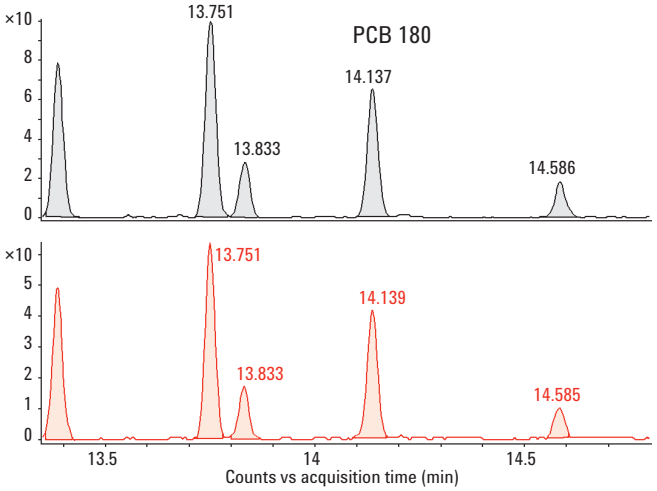

Figure 11. MRM Chromatograms for incurred PCB 180 in mussel sample, Concentration 0.14 µg/Kg.

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Acknowledgement

The authors would like to acknowledge the considerable assistance given by Dr Joan Stevens, Sample Preparation Applications Scientist, Chemistries and Supplies Division, Agilent Technologies Inc., USA, during the development of the sample extraction and clean up procedure.

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Application Note

Food Safety

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Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation approach described in the European Committee Standard (EN) for extraction and cleanup of 16 multiple class pesticide residues of interest in apple. The method employed involves initial extraction in an aqueous/acetonitrile system, an extraction/partitioning step after the addition of salt, and then a cleanup step utilizing dispersive solid phase extraction (dispersive SPE). The two different dispersive SPE clean-up approaches (1 mL and 6 mL sample volume) are evaluated simultaneously after sample extraction. The target pesticides in the apple extracts are then determined by liquid chromatography coupled to an electrospray ionization tandem mass spectrometer (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode. The method is validated in terms of recovery and reproducibility. The 5 ng/g of limits of quantitation (LOQ) for pesticides in apple established in this application is well below their regulatory maximum residue limits (MRLs). The spiking levels for the recovery experiments are 10, 50, and 200 ng/g. Excluding pymetrozine, recoveries of the pesticides ranged between 73 and 111% (87% on average), and RSDs below 20% (5.8% on average).

Agilent Technologies

Introduction

Multi-residue analysis of pesticides in fruits, vegetables, and other foods is a primary function of many regulatory, industrial, and contract laboratories throughout the world. Because of the wide variety of pesticides and complexity of food matrices, the sample must be initially cleaned up using a compatible sample preparation technique before injection into the detection system. It is unquestionable that the most efficient approach to pesticide analysis involves the use of multiclass, multi-residue methods. Once the preliminary analytical quality requirements of accuracy, precision, sensitivity, selectivity, and dynamic scope have been met to suit the need for a particular analysis, other practice considerations should be evaluated. These additional considerations include high sample throughput, ruggedness, ease of use, low cost, labor, minimal toxic solvent usage, and waste generation.

The QuEChERS method was introduced first by USDA scientists in 2003 [1]. The method was then modified to address some problematic pesticides by using a buffered extraction system. [2] There is also a European variation, the prEn method 15662: 2007 [3], [4]. In summary, the method uses acetonitrile extraction, followed by the salting out of water from the sample using anhydrous magnesium sulfate ($MgSO_4$), NaCl and buffering citrate salts to induce liquid-liquid partitioning. For cleanup, a dispersive solid-phase extraction (dispersive SPE) is conducted using a combination of primary secondary amine (PSA) to remove fatty acids from other components and anhydrous $MgSO_4$ to reduce the remaining water in the extract. After mixing and centrifugation, the upper layer is ready for analysis.

The EN methodology is similar in principal to the AOAC method, but has several differences. First, the extraction buffered system in the EN method uses sodium chloride, sodium citrate dehydrate, and disodium citrate hydrogenate sesquihidrate instead of sodium acetate in the extraction step. Second, in the dispersive SPE step, the EN method uses 25 mg PSA per mL of extract rather than 50 mg PSA per mL of extract used by the AOAC method.

In this study, 16 pesticides are used to demonstrate the performance of Agilent SampliQ QuEChERS EN Buffered Extraction kit (p/n 5982-5650) and EN dispersive SPE kit (p/n 5982-5021 and 5982-5056) for General Fruits and Vegetables, suitable for common fruit and vegetable applications. Most of the pesticides are from the original 'representative pesticides' list [1]. According to their experience, a method working well for these representative pesticides should work equally well for nearly all of the other pesticides that are routinely monitored in multiclass, multi-residue methods. These pesticides are from nine different pesticide classes, including acidic, basic, neutral, base-sensitive, and acid-labile pesticides. Furthermore, the selected pesticides are suitable for LC/MS/MS analysis. The MRLs of these pesticides have been set for 10 ng/g or higher. Table 1 shows the chemical and regulatory information of these pesticides.

Experimental

Reagents and Chemicals

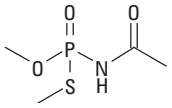
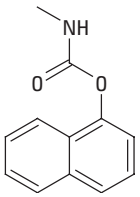
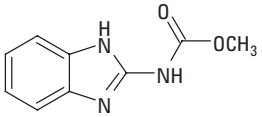
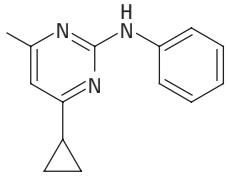
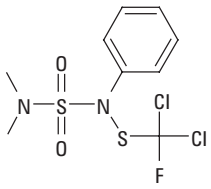
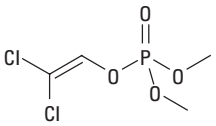
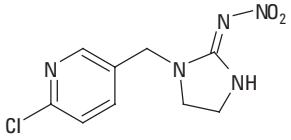
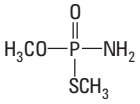
All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN), methanol (MeOH) were from Honeywell (Muskegon, MI, USA). Dimethyl sulfoxide (DMSO) was from Sigma-Aldrich (St Louis, MO, USA). Ammonium acetate (NH_4OAc) was from Fisher Chemicals (Fair Lawn, NJ, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards and internal standard (triphenyl phosphate, TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), ChemService (West Chester, PA, USA), Ultra Scientific (North Kingstown, RI, USA), or AlfaAesar (Ward Hill, MA, USA).

Solutions and Standards

A 1M NH_4OAc pH 5 stock solution was made by dissolving 19.27 g NH_4OAc powder in 250 mL Milli-Q water. The pH was adjusted to 5 with HAc and monitored with a pH meter. The solution was stored at 4 °C. 20:80 MeOH/H_2O containing 5 mM NH_4OAc pH 5 was made by combining 200 mL MeOH and 800 mL Milli-Q water, adding 5 mL of 1M NH_4OAc pH 5 stock solution.

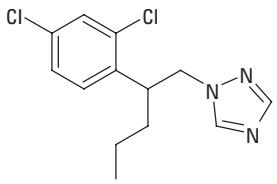
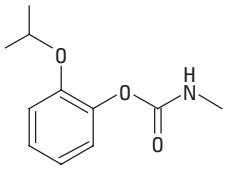
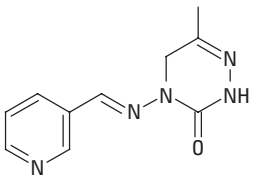
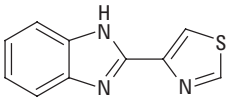
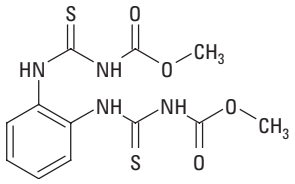
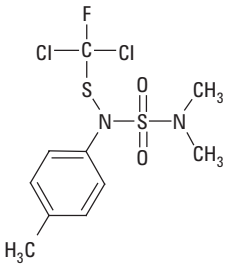
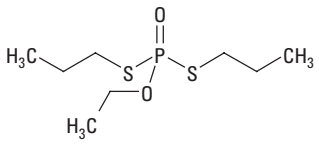
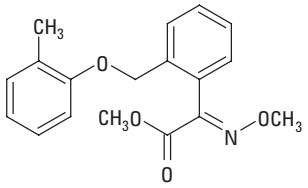
A 5 mM NH_4OAc in ACN solution was prepared by adding 5 mL of 1M NH_4OAc pH 5 stock solution to 1 L ACN, mixing well and sonicating 5 min. A 1% FA in ACN solution was prepared by adding 1 mL of FA to 100 mL of ACN.

Table 1. Pesticides Chemical and Regulatory Information [5–7]

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
Acephate	Organophosphate	–0.89	8.35		20
Carbaryl	Carbamate	2.36	10.4		50
Carbendazim	Benzimidazole	1.48	4.2		100
Cyprodinil	Anilinopyrimidine	4	4.44		50
Dichlofluanid	Sulphamide	3.7	NA		5000
Dichlorvos	Organophosphate	1.9	NA		10
Imidacloprid	Neonicotinoid	0.57	NA		500
Methamidophos	Organophosphate	–0.79	NA		10

(Continued)

Table 1. Pesticides Chemical and Regulatory Information [5–7]

Name	Class	Log P	pKa	Structure	MRLs in apple (ng/g)*
Penconazole	Triazole	3.72	1.51		50
Propoxur	Carbamate	0.14	NA		1000
Pymetrozine	Pyridine	-0.19	4.06		20
Thiabendazole	Benzimidazole	2.39	4.73 12.00		50
Thiophanate-methyl	Benzimidazole	1.45	7.28		100
Tolylfluanid	Sulphamide	3.9	NA		3000
Ethoprophos	Organophosphate	2.99	NA		5
Kresoxim-methyl	Strobilurin	3.4	NA		50

*The MRLs numbers list in the table are for apple or lowest level in other fruit and vegetables. They could be higher in different commodities.

Standard and internal standard (IS) stock solutions (2.0 mg/mL for all, except 0.5 mg/mL for carbendazim) were made in MeOH, 0.1% FA in ACN, or DMSO, respectively, and stored at –20 °C. Three QC spiking solutions of 1,5, and 20 µg/mL were made fresh daily in 1:1 ACN/H_2O with 0.1% FA. A 10 µg/mL standard spiking solution in 1:1 ACN/H_2O with 0.1% FA was made for preparation of calibration curves in the matrix blank extract by appropriate dilution. A 10 µg/mL IS spiking standard of TPP was made in 1:1 ACN/H_2O (0.1% FA).

Equipment and Material

- Agilent 1200 HPLC with Diode Array Detector (Agilent Technologies Inc., Santa Clara, CA, USA).
- Agilent 6410 Triple Quadrupole LC/MS/MS system with Electrospray Ionization (Agilent Technologies Inc., Santa Clara, CA, USA).
- Agilent SampliQ QuEChERS extraction kit, p/n 5982-5650, and dispersive SPE tubes, p/n 5982-5021 and 5982-5056 (Agilent Technologies Inc., Wilmington, DE, USA).
- CentraCL3R Centrifuge (Thermo IEC, MA, USA)
- Bottle top dispenser (VWR, So. Plainfield, NJ, USA)
- Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)
- Grinder (St. Joseph, MI, USA)

Instrument Condition

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl, 3.0 x 150 mm, 3.5 µm (p/n 959963-312)
Flow rate:	0.3 mL/min
Column temperature:	30 °C
Injection volume:	10 µL
Mobile phase:	A: 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H2O B: 5 mM ammonium acetate, pH 5.0 in ACN
Needle wash:	1:1:1:1 ACN/MeOH/IPA/H_2O (0.2% FA.)

Gradient:

Time	% B	Flow rate (mL/min)
0	20	0.3
0.5	20	0.3
8.0	100	0.3
10.0	100	0.3
10.01	20	0.5
12.0	100	0.5
13.0	STOP	

Post run:	4 min
Total cycle time:	17 min

MS conditions

Positive mode	
Gas temperature:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 psi
Capillary:	4000 V

Other conditions relating to the analytes are listed in Table 2.

Table 2. Instrument Acquisition Data Used for the Analysis of 16 Pesticides by LC/MS/MS

Analyte	MRM channels (m/z)	Fragmentor (V)	CE (V)	RT (min)
Acephate	1) 184.0 > 94.9 2) 184.0 > 111.0	60	3 15	2.55
Methamidophos	1) 142.0 > 94.0 2) 142.0 > 124.9	60	8 8	2.54
Pymetrozine	1) 218.1 > 105.0 2) 218.1 > 78.0	115	20 50	2.97
Carbendazim	1) 192.1 > 160.0 2) 192.1 > 105.0	95	18 40	5.07
Dichlorvos	1) 221.0 > 109.0 2) 221.0 > 95.0	110	13 40	6.57
Thiophanate methyl	1) 343.1 > 151.0 2) 343.1 > 117.9	105	17 65	7.08
Propoxur	1) 210.1 > 111.0 2) 210.1 > 92.9	50	12 15	6.89
Carbaryl	1) 202.0 > 145.0 2) 202.0 > 115.0	50	3 40	7.30
Cyprodinil	1) 226.1 > 93.0 2) 226.1 > 108.0	120	35 35	9.23
Dichlorfluanid	1) 333.0 > 123.0 2) 333.0 > 223.9	85	28 5	9.40
Ethoprophos	1) 243.1 > 130.9 2) 243.1 > 172.9	80	15 15	8.50
Penconazole	1) 284.1 > 158.9 2) 284.1 > 172.9	80	32 32	8.95
Tolyfluanid	1) 347.0 > 136.9 2) 347.0 > 238.0	60	25 3	9.73
Thiabendazole	1) 202.1 > 175.0 2) 202.1 > 131.0	110	27 38	5.65
Imidacloprid	1) 256.1 > 209.1 2) 256.1 > 175.0	60	12 18	5.53
TPP	1) 327.1 > 77.0 2) 327.1 > 151.9	70	45 45	9.49
Kresoxim methyl	1) 314.0 > 222.1 2) 314.0 > 235.0	70	10 10	9.44

1) Quantifier transition channel
2) Qualifier transition channel

Sample preparation

Sample comminution

In order to get the most reliable statistical results, it is important to spend the necessary effort and time on conducting proper sampling and homogenization procedures. Organically grown, pesticide free apples were purchased from a local grocery store. Approximately three pounds of apples were chopped into small, bean sized cubes. Skin was included, but the pit was discarded. Then, the chopped apple cubes were put into a clean plastic bag and frozen at –20 °C overnight. The bag was massaged occasionally to make sure the cubes were frozen loosely to avoid clumping. The following day, a portion of frozen apple cubes were removed and thoroughly blended. Certain precautions were exercised while blending the sample. First, the chopped apple cubes remained in the freezer until the point of blending. Only the portion of apple cubes necessary for homogenizing were removed; the rest was kept in the freezer until the next comminution. Dry ice was added while comminuting to keep the temperature low. Second, the blender container was kept dry to prevent clumping. In between blending, the container was rinsed and dried. Third, samples were comminuted thoroughly to get the best sample homogeneity. There were not any pieces of apple visible in the final sample.

Extraction/Partitioning

A 10 g (±0.05g) previously homogenized sample was placed into a 50 mL centrifuge tube. QC samples were fortified with 100 µL of appropriate QC spiking solution. A 100 µL of IS spiking solution (10 µg/mL of TPP) were added to all of samples except the control blank to yield a 100 ng/g concentration in sample. Tubes were capped and vortexed for 1 min. Ten milliliters of ACN was added to each tube using the dispenser. Tubes were then capped and shaken by hand for 1 min. To each tube, an Agilent SampliQ QuEChERS EN extraction salt packet (p/n 5982-5650), containing 4 g anhydrous $MgSO_4$, 1g NaCl, 1g sodium citrate, and 0.5 g disodium citrate sesquihydrate, was added directly. No powders were left in the threads or rims of the tubes. Tubes were sealed tightly and shaken vigorously for 1 min by hand to ensure that the solvent interacted well with the entire sample and crystalline agglomerates were broken up sufficiently. Sample pH was checked with pH paper, and 5N NaOH was added to adjust the pH to 5-5.5. Sample tubes were centrifuged at 4000 rpm for 5 min.

Dispersive SPE Cleanup

A 1 mL aliquot of the upper ACN layer was transferred into an Agilent SampliQ QuEChERS EN dispersive SPE 2 mL tube (p/n 5982-5021); or 6 mL of aliquot was transferred into an Agilent SampliQ QuEChERS EN dispersive SPE 15 mL tube (p/n 5982-5056). The 2 mL tube contained 25 mg of PSA and 150 mg of anhydrous $MgSO_4$; while the 15 mL tube contained 150 mg of PSA and 900 mg of anhydrous $MgSO_4$. The tubes were capped tightly and vortexed for 1 min. The 2 mL tubes were centrifuged with a micro-centrifuge at 13,000 rpm for 2 min, and the 15 mL tubes with a standard centrifuge at 4000 rpm for 5 min. Ten microlitres of extract were transferred into an autosampler vial. A 10 µL of a 1% FA in ACN solution was added immediately, in addition to 800 µL of water or appropriate standard solution (prepared in water). The samples were capped and vortexed thoroughly, to prepare for LC/MS/MS analysis.

The flow chart in Figure 1 illustrates the sample preparation procedure.

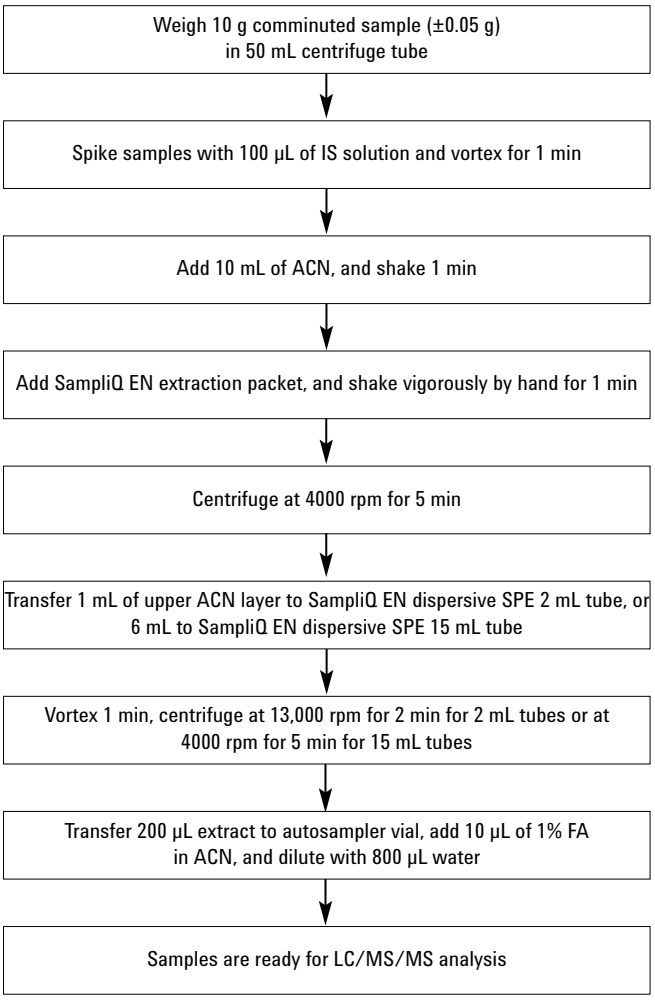


Figure 1. QuEChERS EN sample preparation procedures flow chart.

Results and Discussion

In addition to being fast, easy, cheap, effective, rugged and safe, an additional key feature of the QuEChERS method is the potential for the simultaneous analysis of multi-pesticide residues. With the new design of Agilent's SampliQ QuEChERS kits, the whole procedure is even faster, easier, and offers more time and labor savings, while ensuring consistency. An analyst can process 40–50 samples in just a few hours. Adding a food sample with a high percentage of water directly to the salts may create an exothermic reaction that can affect analyte recovery. Agilent's SampliQ salts and buffers are uniquely prepared in anhydrous packages. This allows addition **AFTER** adding solvent to the sample, as specified in the QuEChERS methology. The final QuEChERS sample still contains food matrix impurities because it is a very simple sample extraction and cleanup procedure. The final apple extract appeared light green. But with the powerful selectivity of LC/MS/MS multiple reaction monitoring mode, the extracted apple blank appeared to be clean and free of coeluting impurities, indicating that the cleaned-up apple extract did not contribute any interferences with the target compounds. Figure 2 shows the chromatograms of a blank apple extract and a 10 ng/g fortified apple extract.

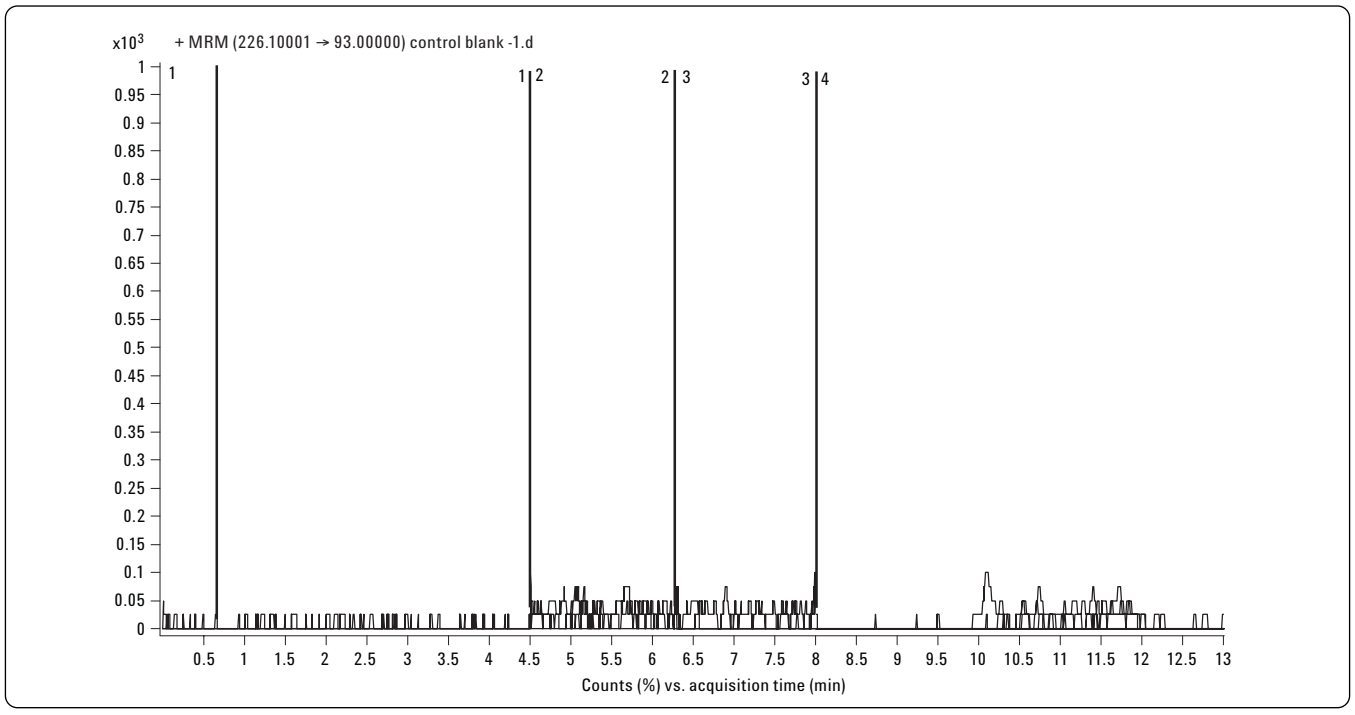


Figure 2a Chromatograms of apple extract blank. No interference was found in the blank.

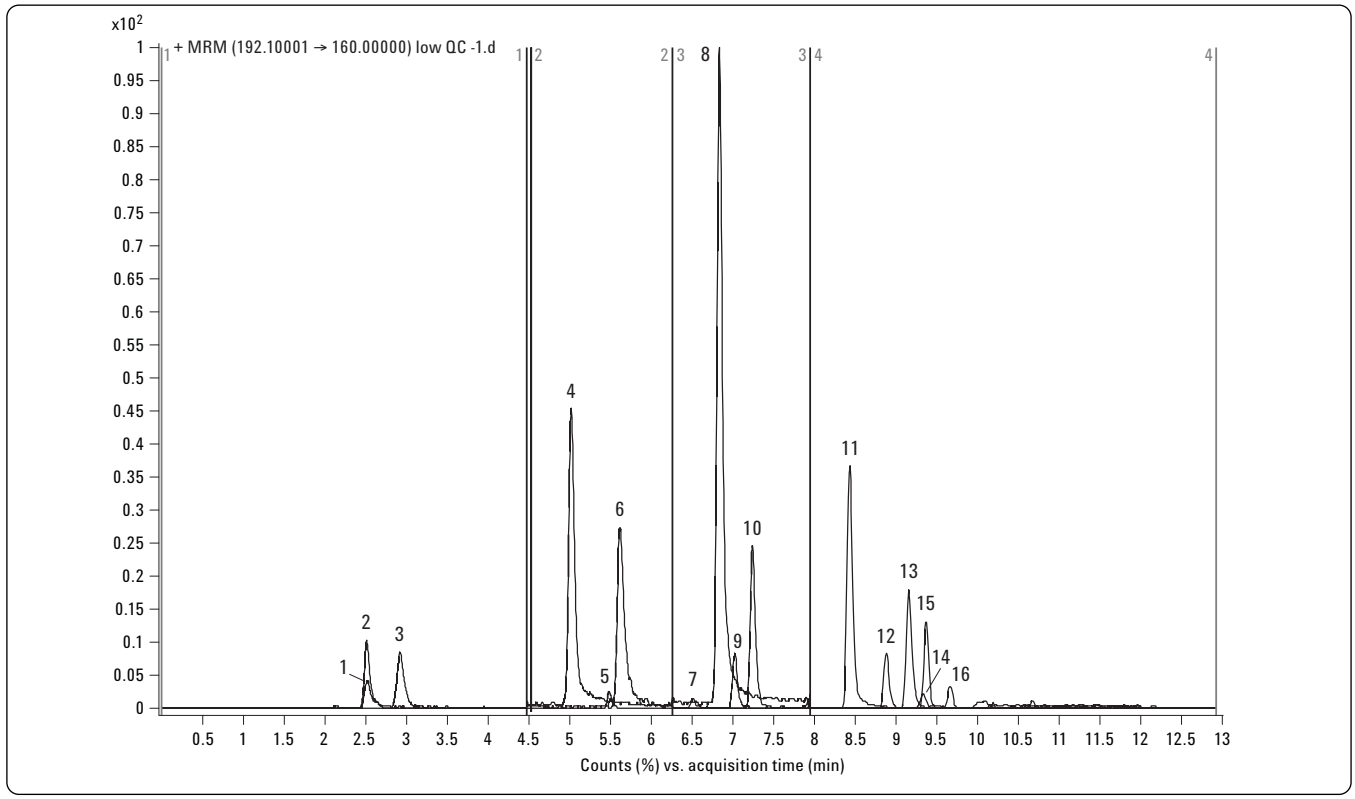


Figure 2b. Chromatogram of 10 ng/g fortified apple extract. Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid, 6. Thiabendazole, 7. Dichlorvos, 8. Propoxur, 9. Thiophanate methyl, 10. Carbaryl, 11. Ethoprophos, 12. Penconazole, 13. Cyprodinil, 14. Dichlofluanid, 15. Kresoxim methyl, 16, Tolyfluanid.

Linearity and Limit of Quantification (LOQ)

The linear calibration range for all of the pesticides was 5 – 250 ng/g. Since two different dispersive SPE sizes (1 mL and 6 mL sample volume) were used for evaluation and comparison, two sets of calibration curves were generated respectively. Matrix blanks were prepared for each size. Calibration curves, spiked in matrix blanks, were made at levels of 5, 10, 50, 100, 200, and 250 ng/g. The TPP was used as an internal standard (IS) at 100 ng/g level. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). Table 1 shows that the 5 ng/g quantification limits LOQ (5 ppb) established for all of the pesticides is significantly lower than the MRLs of these pesticides in fruit and vegetables. Table 3 shows the regression equation and correlation coefficient (R^2) for both 1 mL and 6 mL dispersive SPE.

Table 3. Linearity of Pesticides in Apple Extract

Analytes	1 mL dispersive SPE Regression equation	R^2	6 mL dispersive SPE Regression equation	R^2
Methamidophos	Y = 0.3203X – 0.0005	0.9972	Y = 0.3255X – 0.0018	0.9957
Acephate	Y = 0.1373X – 0.0021	0.9975	Y = 0.1375X – 0.0010	0.9953
Pymetrozine	Y = 0.4688X – 0.0009	0.9961	Y = 0.3821X + 0.0007	0.9782
Carbendazim	Y = 1.4253X + 0.0126	0.9931	Y = 1.3379X + 0.0045	0.9903
Imidacloprid	Y = 0.0647X – 0.0004	0.9944	Y = 0.0636X – 0.0006	0.9974
Thiabendazole	Y = 0.9014X + 0.0127	0.9922	Y = 0.8600X + 0.0050	0.9942
Dichlorvos	Y = 0.0364X + 0.0002	0.9884	Y = 0.0362X + 0.0002	0.9892
Propoxur	Y = 2.4398X – 0.0001	0.9989	Y = 2.4272X + 0.0029	0.9994
Thiophanate methyl	Y = 0.3171X – 0.0015	0.9965	Y = 0.2869X – 0.0020	0.9904
Carbaryl	Y = 0.6378X + 0.0017	0.9989	Y = 0.6363X + 0.0003	0.9988
Ethoprophos	Y = 1.0897X – 0.0030	0.9984	Y = 1.0628X – 0.0001	0.9992
Penconazole	Y = 0.2334X – 0.0012	0.9978	Y = 0.2186X – 0.0003	0.9979
Cyprodinil	Y = 0.4805X + 0.0008	0.9992	Y = 0.4697X – 0.0017	0.9985
Dichlorfluanid	Y = 0.0552X – 0.0003	0.9970	Y = 0.0562X – 0.0012	0.9946
Kresoxim methyl	Y = 0.2958X – 0.0005	0.9978	Y = 0.2762X – 0.0003	0.9966
Tolyfluanid	Y = 0.0860X – 0.0011	0.9918	Y = 0.0845X – 0.0008	0.9968

Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticides standards in comminuted apple sample at levels of 10, 50, and 200 ng/g. These QC samples were quantitated against the matrix spiked calibration curve. The analysis was performed in replicates of six (n = 6) at each level. The recovery and reproducibility (RSD) data of 1 mL and 6 mL dispersive SPE are shown in Tables 4 and 5, respectively. It can be seen from the results, that all of the pesticides but pymetrozine give acceptable recoveries (average of 85.7% for 1 mL and 88.2% for 6 mL) and precision (average of 6.0% RSD for 1 mL and 5.7% RSD for 6 mL). The notorious base-sensitive pesticides such as dichlorfluanid and tolyfluanid showed excellent recovery and precision. Pymetrozine, an acid labile pesticide, shows poor recovery using the European method when compared to the AOAC method [2]. With the AOAC method, an average recovery of 88% with 9.4% average RSD for pymetrozine was obtained [8].

Table 4. Recovery and Repeatability of Pesticides in Fortified Apple With 2 mL EN Dispersive SPE Tube (p/n 5982-5021)

	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
Analytes	**Recovery**	**RSD (n=6)**	**Recovery**	**RSD (n=6)**	**Recovery**	**RSD (n=6)**
Methamidophos	73.0	5.6	75.6	3.1	84.3	5.3
Acephate	92.8	4.2	87.2	5.6	95.6	5.8
Pymetrozine	27.1	18.2	24.9	10.5	28.1	12.3
Carbendazim	85.1	5.9	89.5	3.4	84.1	4.7
Imidacloprid	91.0	3.3	102.7	5.4	107.2	4.9
Thiabendazole	84.8	6.8	90.4	3.5	86.7	4.0
Dichlorvos	83.1	13.9	92.2	5.2	93.1	4.6
Propoxur	97.8	2.6	100.2	2.9	100.7	3.8
Thiophanate methyl	79.9	8.5	79.9	2.9	85.5	5.7
Carbaryl	89.3	2.8	92.5	3.6	95.8	4.1
Ethoprophos	93.7	1.6	93.5	2.9	95.7	3.4
Penconazole	109.2	6.7	108.1	5.7	110.6	4.4
Cyprodinil	98.9	6.9	101.2	2.7	102.9	4.3
Dichlorfluanid	85.1	7.8	92.2	4.4	99.4	5.5
Kresoxim methyl	90.4	4.8	99.6	3.9	103.7	4.5
Tolyfluanid	98.3	13.7	102.0	4.0	106.0	4.4

Table 5. Recovery and Repeatability of Pesticides in Fortified Apple With 15 mL EN Dispersive SPE Tube (p/n 5982-5056)

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Methamidophos	77.6	4.9	77.8	6.4	81.2	2.1
Acephate	86.6	7.6	87.8	5.5	91.5	1.5
Pymetrozine	28.1	24.3	27.5	12.2	29.1	10.4
Carbendazim	89.9	6.8	88.9	3.3	81.8	3.6
Imidacloprid	105.3	10.8	105.2	4.8	106.6	5.0
Thiabendazole	89.8	4.8	87.6	3.1	84.2	1.4
Dichlorvos	97.8	14.5	98.2	5.6	98.1	2.5
Propoxur	99.5	3.8	104.0	2.6	100.9	3.3
Thiophanate methyl	87.4	5.8	88.3	4.7	89.0	7.6
Carbaryl	92.9	6.1	93.7	3.0	93.6	2.4
Ethoprophos	94.8	5.5	99.2	3.0	98.8	3.8
Penconazole	106.8	4.9	111.2	3.0	109.0	4.1
Cyprodinil	102.7	4.5	105.7	3.5	102.4	2.6
Dichlorfluanid	99.7	18.9	97.4	4.5	98.9	5.5
Kresoxim methyl	102.6	12.0	106.1	2.0	106.1	5.6
Tolyfluanid	92.0	9.3	105.5	3.3	105.1	4.3

Figure 3 shows the recovery and precision results comparison of 1 mL dispersive SPE and 6 mL dispersive SPE. The two different dispersive SPE clean-ups were performed by transferring 1 mL or 6 mL of ACN extract from the sample tube after the extraction step. To simplify the comparison, the average recovery and precision of three fortification concentrations were used for all pesticides. The results of two dispersive SPE clean-up approaches appear to be independent of volume used. There was < 10% difference in recovery and < 5% difference for RSD. Both approaches provided efficient sample clean-up, and generated relatively equivalent results.

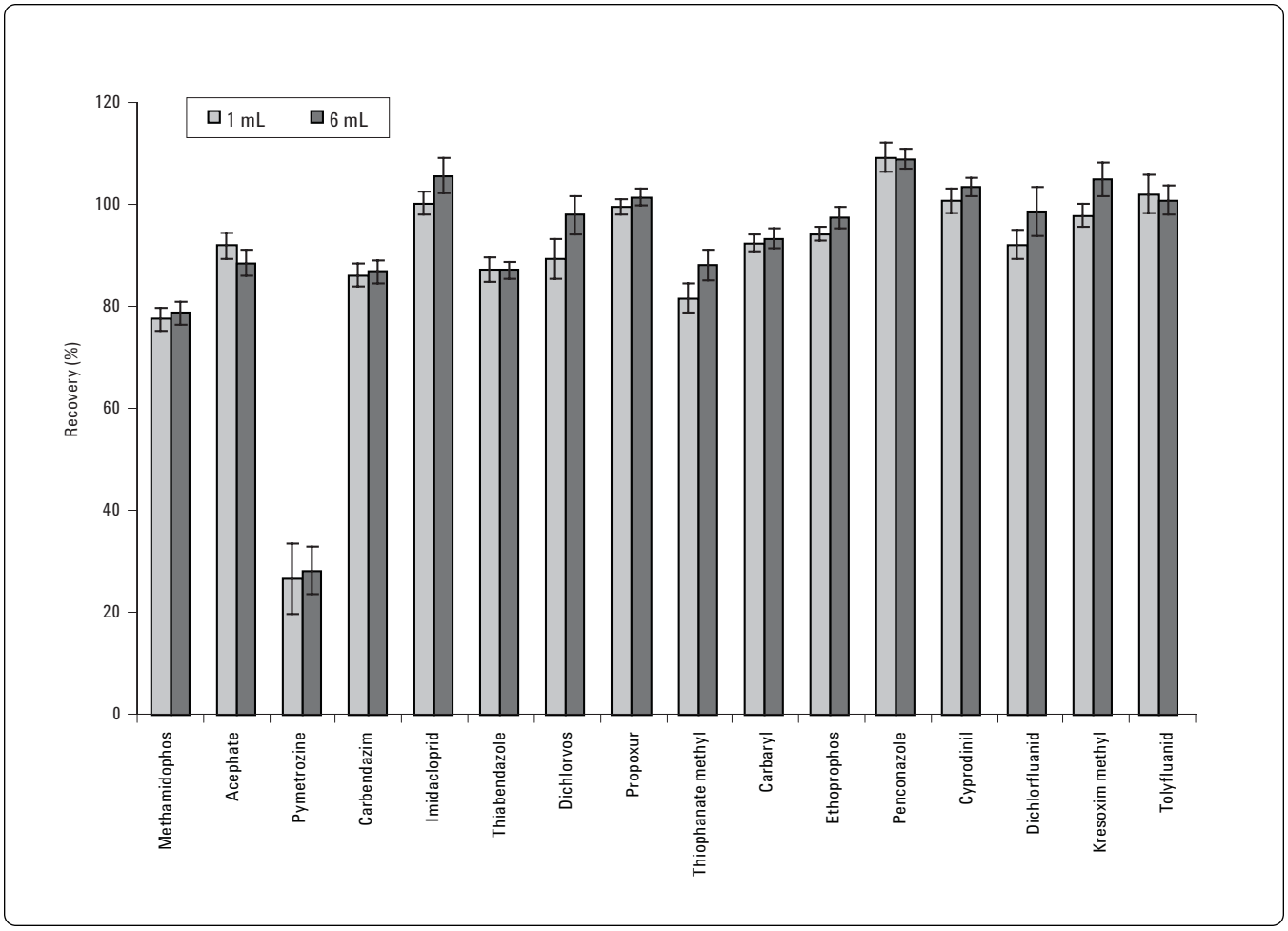


Figure 3. Results comparison of 1-mL dispersive SPE and 6-mL dispersive SPE.

Conclusions

The Agilent SampliQ QuEChERS EN fruit and vegetable kit provides a simple, fast and effective method for the purification and enrichment of selective representative pesticides in apple. The recovery and reproducibility results, based on matrix spiked standards, were acceptable for selected pesticide residue determination in apple. The impurities and matrix effect from apple were minimal and did not interfere with the quantitation of target compounds. The LOQs of the pesticides were lower than their MRLs in fruits and vegetables. As the selected pesticides represented a broad variety of different classes and properties, the Agilent SampliQ QuEChERS EN kit for General Fruits and Vegetables can be used for other pesticides in similar food matrices.

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Printed in the USA
August 6, 2009
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Determining Malachite Green and Leucomalachite Green in Food by LC/MS/MS

Application

Food Safety

Authors

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Abstract

This application note demonstrates a complete method to rapidly and precisely determine residue levels of malachite green and leucomalachite green in fish with the new Agilent 6410 LC/MS triple quadrupole system. Using positive mode electrospray ionization (ESI+) and multiple reaction monitoring (MRM), qualification and quantification were accomplished without the traditional tedious PbO_2 oxidation process. The LC/MS/MS method's LOQ is 0.01 µg/Kg, which easily meets the import requirement of 2 µg/Kg set by Japan and the EU.

Introduction

Malachite green (MG) is a metallic-looking crystal. It dissolves in water easily as a blue-green solution. It is a toxic chemical primarily used as a dye and has been found very effective in treating parasites, fungal infections, and bacterial infections in fish and fish eggs.[1] On uptake, MG is rapidly reduced into leucomalachite green (LMG) and deposited in the fatty tissue of the fish with little MG remaining.

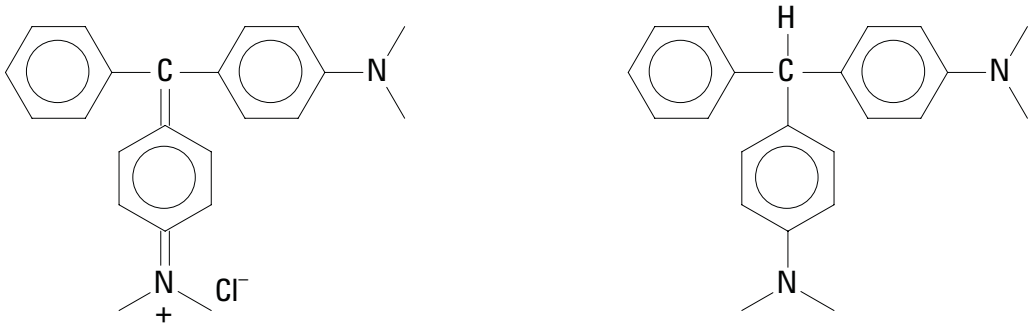
MG can cause significant health risk for humans who eat contaminated fish. For example, it can cause liver tumor formation and is suspected of carcinogenesis.[1] The United States, Japan, China, the European Union, and many other countries have already banned MG in fishery. Due to its low cost and antifungal effectiveness, MG is still being used illegally as indicated in the European Rapid Alert System for Food and Feed.[2]

HPLC with UV detection has been used to analyze MG and LMG. Figure 1 shows the structure of the two compounds. Loss of conjugation by reduction changes the chromaphore of LGM significantly. To obtain the sum of both, the method employs post-column oxidation with PbO_2 to convert LMG to MG, thus providing a sum of both comounds.[3] Most recently, LC/MS has been used to both meet the EU confirmation criteria and provide quantitative results for both compounds without the need for post-column oxidation. In this application, a simple and sensitive method for simultaneously determining MG and LMG is presented.[4, 5] The LC/MS/MS method's LOQ is 0.01 µg/Kg, which easily meets the import requirement set by Japan or the EU.[6]

Experimental

Reagents

MG	Sigma-Aldrich, CAS 569-64-2, USA
LMG	Dr. Ehreastorfer's lab, D-86199, 99% pure, Augsburg, Germany
Acetonitrile	CAS 75-05-8; Burdick & Jackson; Morristown, New Jersey, USA
Acetic acid	Merck, Germany
Ammonium acetate	CAS 631-61-8, Acros Organics, Morris Plains, New Jersey, USA



Malachite green Leucomalachite green

Figure 1. Molecular structure of malachite green and leucomalachite green.

Calibration Solutions

A stock standard solution of MG and LMG in acetonitrile was prepared at 100 µg/mL and stored at −18 °C, avoiding light. The stock solution was diluted in 50:50 acetonitrile:water to make the calibration solutions—10, 50, 100, 500, 1000, 5000, and 10,000 fg/µL.

Sample Preparation

To 5 g tilapia tissue was added 1 mL (0.25 mg/mL) hydroxylamine, 2 mL 1 M toluene sulfonic acid, 2 mL of 0.1 M ammonium acetate buffer (pH 4.5), and 40 mL acetonitrile. The mixture was then homogenized for 2 min. The supernatant was decanted, and to the precipitate was added 20 mL acetonitrile. This was filtered and added to the supernatant. To the combined acetonitrile extracts, 35 mL water and 30 mL methylene chloride were added. The solution was shaken and the methylene chloride layer collected. A second extract of 20 mL methylene chloride was made, and this layer added to the first extract. The methylene chloride was taken to dryness with a gentle stream of nitrogen and the extract reconstituted in 100 µL of acetonitrile

Instrumentation

LC	1100 LC	
Column	C18, 2.1 x 150 mm, 5 µm	
Column temp.	40 °C	
Mobile phase	A − 10 mmol/L ammonium acetate (adjust to pH 4.5 with acetic acid)	
	B − acetonitrile	
Column flow	0.3 mL/min	
Gradient	Time	%B
	0	30
	1	50
	2	95
	8	95
	8.01	30
	13	30
Injection vol.	10 µL	
MS	Agilent 6410 LC/MS Triple Quadrupole	
Ionization	ESI(+)	
Capillary	4000 V	
Nebulizer P.	35 psi	
Drying gas	11 L/min	
Gas temp.	350 °C	
Skimmer	15 V	
OctDc1 (Skim2)	45 V	
Oct RF	500 V	
Q1 resolution	Unit	
Q3 resolution	Unit	
Collision gas	Nitrogen	

The MRM parameters are listed in Table 1.

Table 1. MRM Method Parameters

Time	Compound	Precursor	Product	Dwell (ms)	Fragmentor (V)	Collision Energy (V)
0	MG	329.3	313.3	40	100	40
		329.3	208.2	40	100	40
7	LMG	331.3	316.3	40	100	30
		331.3	239.2	40	100	30

Results and Discussion

To obtain the most sensitive results, optimization of certain fragmentor voltages is important. Figure 2 shows the EICs of both target compounds at fragmentor values of 70 V, 90 V, and 100 V. The results show that the three different fragmentor values have little effect on the intensity of $[M+H]^+$ ions. Thus, 100 V was chosen for this study.

In addition, an optimal collision energy for the MS/MS must be set. Figure 3 shows the MS/MS spectra from three different collisional voltages, (a) 20 V, (b) 30 V, and (c) 40 V. Due to their structural differences, the voltage required for optimum fragmentation of each compound is different. For MG, the optimum fragmentation was observed at 40 V. The ion *m/z* 313 was due to the neutral loss of methane. The ion at *m/z* 208 was due to the neutral loss of N,N-dimethylaniline. For LMG, the optimum fragmentation was observed at 30 V. The ion at *m/z* 316 was due to the loss of a methyl radical. The ion at *m/z* 239 resulted from a subsequent loss of a benzene radical or, more likely, the rearrangement and neutral loss of toluene.

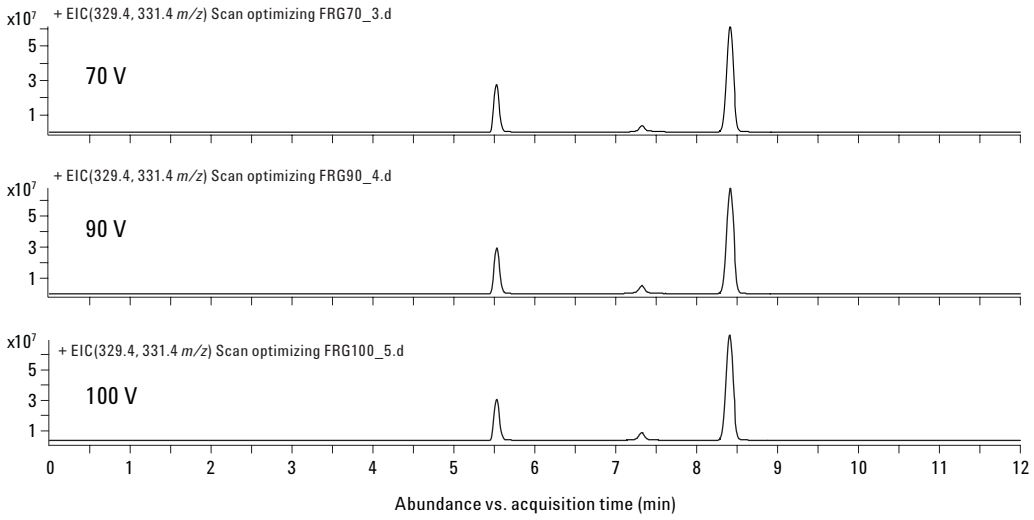


Figure 2. EICs of malachite green and leucomalachite green at fragmentor values of 70 V, 90 V, and 100 V.

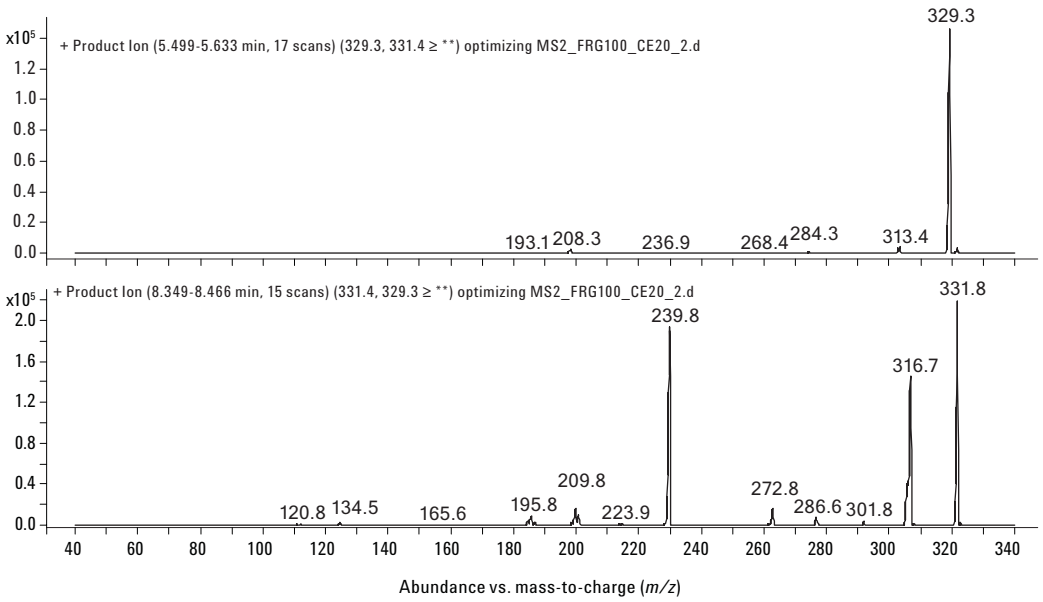


Figure 3a. MS/MS spectra of MG and LMG at collisional voltage of 20 V.

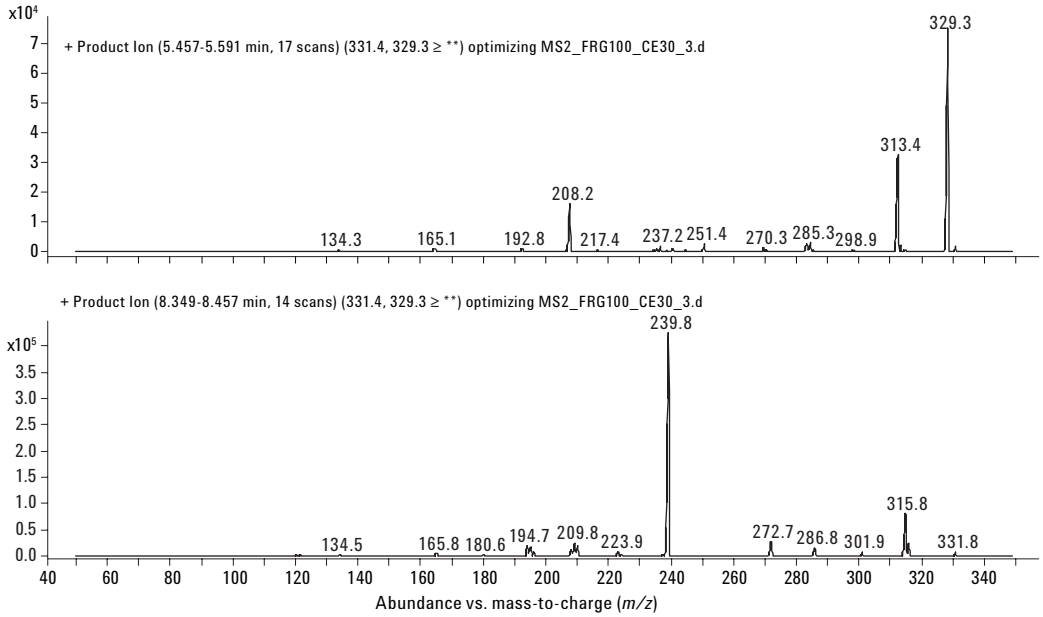


Figure 3b. MS/MS spectra of MG and LMG at collisional voltage of 30 V.

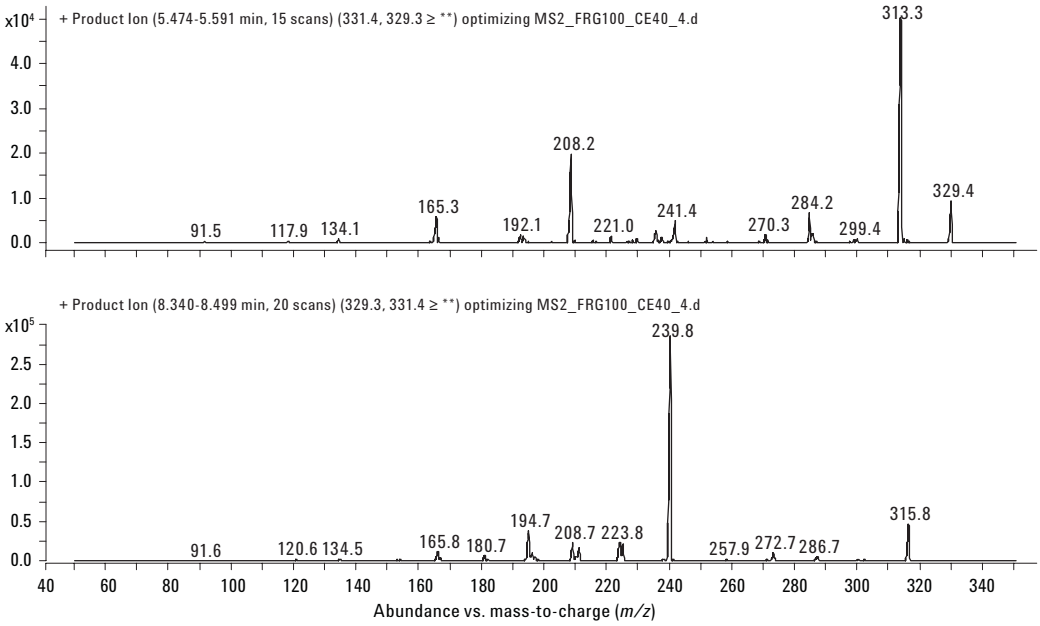


Figure 3c MS/MS spectra of MG and LMG at collisional voltage of 40 V.

Figure 4 shows the calibration curves for both MG (4a) and LMG (4b). Calibration solution concentrations were from 10 to 10,000 fg/µL. The linear calibration range is 100 to 100,000 fg on column for both compounds. The R^2 for both compounds was > 0.999 (origin ignored and no weighting). To demonstrate the sensitivity of the instrument, Figure 5 shows MS/MS spectra of a blank sample extract (5a) and sample extract spiked with 10 ppt of each compound (5b). A sample of tilapia spiked at 100 ppt MG and LMG before extraction was made to demonstrate method performance. The MRM results after extraction and cleanup are shown in Figure 6. The recover-

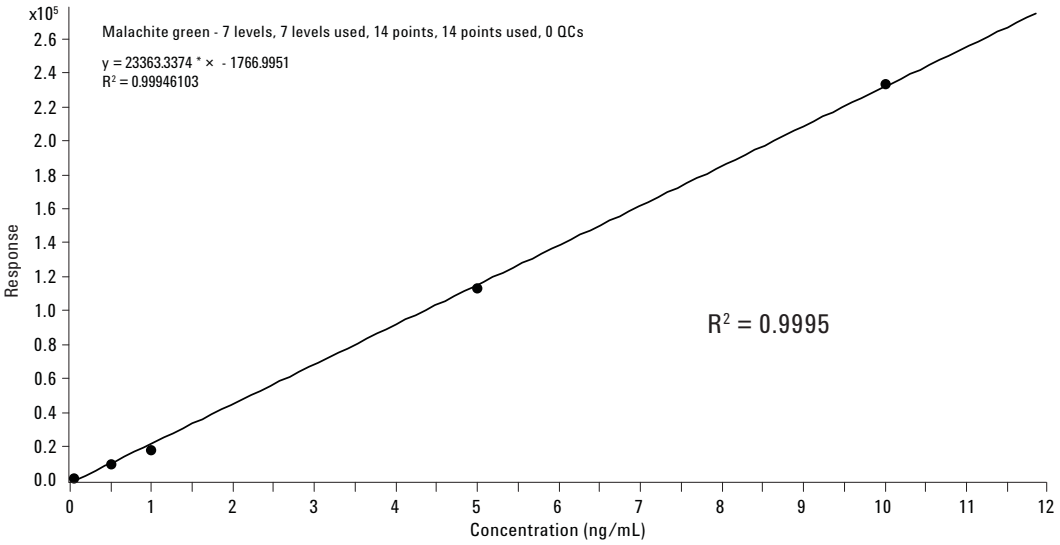


Figure 4a. Calibration curve of malachite green, linear range: 10 ppt to 10 ppb.

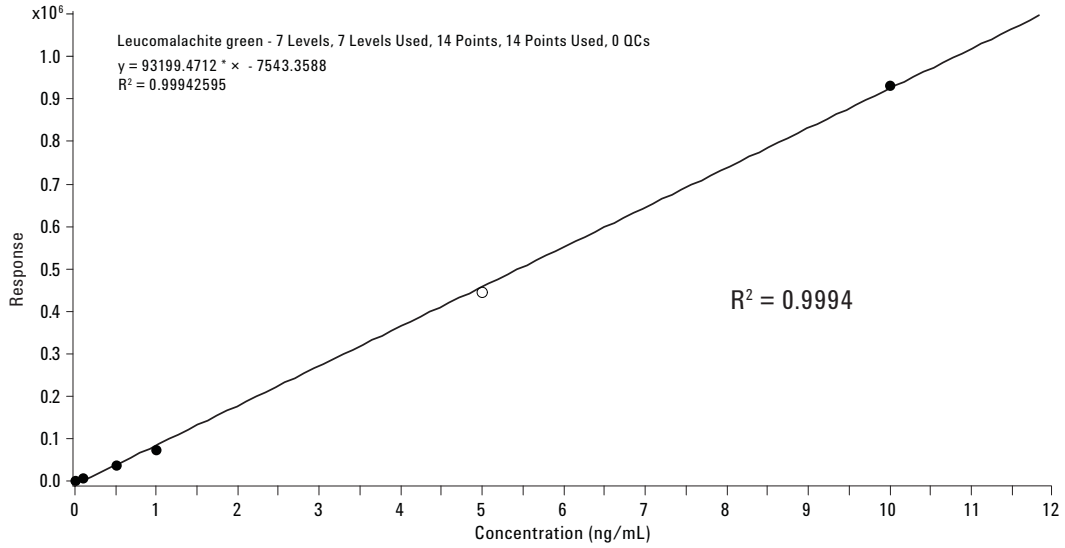


Figure 4b. Calibration curve of leucomalachite green, linear range: 10 ppt to 10 ppb.

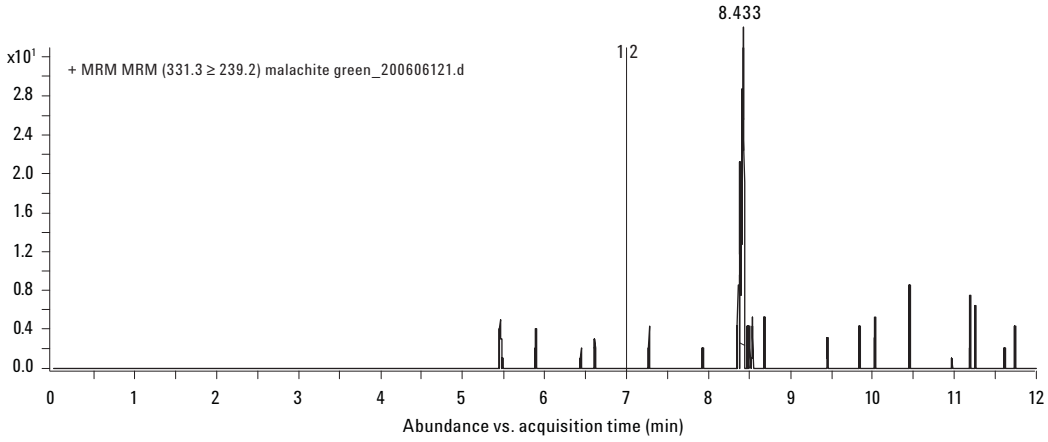
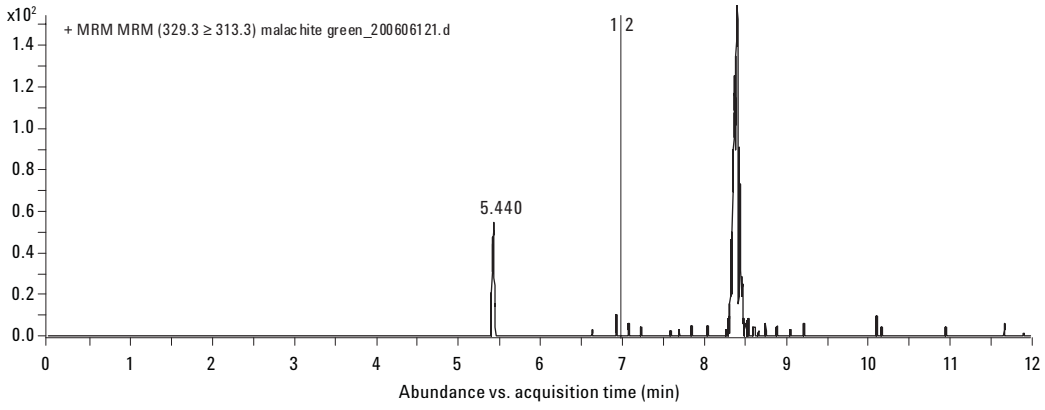


Figure 5a. MG and LMG MRM of a blank sample.



ppt spiked sample.

Figure 5b. MG and LMG MRM of a 10-ppt spiked sample.

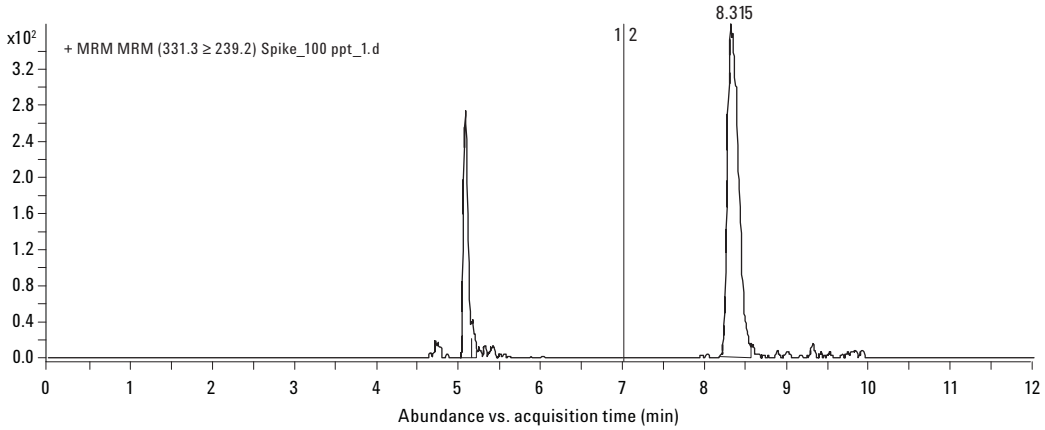


Figure 6. MRM result of talapia extract spiked with 100-ppt MG and LMG.

ies for MG were 48% and 23% for LMG. A mixture of MG and LMG at 100 fg/µL in 50:50 acetonitrile: ammonium acetate was used for the repeatability study for instrument performance. The RSD from eight injections for MG was 3.52% (S/N > 20). The RSD from eight injections for LMG was 2.25% (S/N > 40).

Conclusions

This application note demonstrates a complete method to rapidly and precisely determine residue levels of malachite green and leuco-malachite green in fish. Using positive mode electrospray ionization (ESI+) and multiple reaction monitoring (MRM) technique, the LC/MS/MS method shows detection limit of 10 ppt, which easily meets the import requirement set by Japan or EU.

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Acknowledgement

The authors would like to thank Dr. Yanqin Liu for providing the standard solutions and sample extracts.

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Printed in the USA
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