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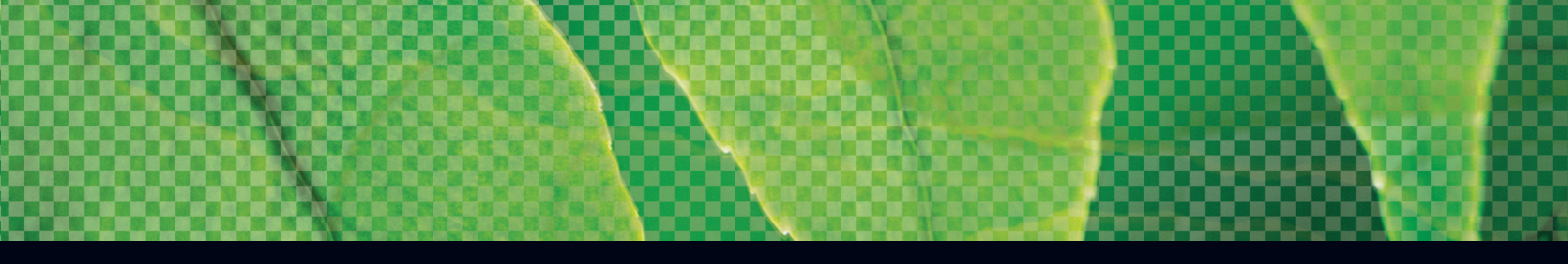

NEW TECHNOLOGIES FOR THE SIMULTANEOUS ANALYSIS OF MULTIPLE PESTICIDE RESIDUES IN AGRICULTURAL PRODUCE

NEW TECHNOLOGIES FOR THE SIMULTANEOUS ANALYSIS OF MULTIPLE PESTICIDE RESIDUES IN AGRICULTURAL PRODUCE

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INTRODUCTION

Pesticides are often used in the production of foodstuffs. The concentrations of individual pesticides permitted in our food are controlled by legislation. There is, therefore, a requirement for surveillance monitoring of pesticide residues in foodstuffs. Analytical methods developed for this purpose must achieve limits of detection at or below the Maximum Residue Limit (MRL).

Given the large number of pesticides in existence and the variety of agricultural produce available, multi-residue pesticide screening methods can offer efficiency advantages over single residue and class specific methods. However, these multi-residue methods are limited both by the chromatographic separation of the analytes and the speed of data acquisition.

Tandem quadrupole mass spectrometry is often used as a detection system due to the high selectivity offered in multiple reaction monitoring (MRM) mode, which compensates for generic sample preparation methods involving minimal sample cleanup. Due to the number of potential analytes, the mass spectrometer chosen should be able to rapidly switch both between MRM channels and between positive and negative ionization modes, thereby offering the potential to achieve greater efficiency in the analysis of multi-component mixtures. Complementing these +/– ionization mode switching capabilities in the Waters® Micromass® Quattro Premier® mass spectrometer is the revolutionary Waters ACQUITY UPLC® system, offering improved chromatographic resolution and shorter analysis times resulting from the use of columns packed with novel 1.7 µm stationary phase particles[1].

In this work, we highlight recent advances in these chromatographic and mass spectrometric technologies via the analysis of a multi-component mixture for surveillance monitoring of pesticides in agricultural produce.

METHOD

Sample Preparation, Extraction and Cleanup Procedure

The raisin sample, Californian sun-dried seedless raisins (Thompson variety) was prepared using a procedure described below involving methanolic extraction and ChemElut™ cleanup, evaporation and reconstitution[2].

The raisin sample was chopped to avoid loss of juice. A 5 g aliquot of the homogenized sample was transferred to a blender cup, to which 9 mL of water was added. After 10 minutes, 20 mL of methanol was added and the sample was blended for 2 minutes. 6 mL of the resultant extract was mixed with 2 mL of a solution of sodium chloride (20 g in 100 mL water). A 5 mL aliquot was then transferred to a ChemElut column containing 5 mL of diatomaceous earth. After 5 minutes, the ChemElut column was eluted with 16 mL of dichloromethane. The eluate was evaporated to dryness and the dry residue was reconstituted in 250 µL of methanol and further diluted with 1 mL of water. The final extract contained the residues of 0.5 g dry sample per mL. The extract was filtered through a 0.45 µm filter into a glass sample vial.

Blank matrix was prepared from organically grown sun-dried seedless raisins (Thompson variety) using the same extraction and cleanup procedure described above. Matrix-matched standards were prepared by spiking all analytes at 0.5, 1, 2.5, 5, 10 pg/L (equivalent to 1, 2, 5, 10, 20 µg/kg, respectively).

LC Conditions

LC system:	ACQUITY UPLC
Mobile phase A:	MeOH/H_2O (1:4 v/v) + 5 mM $CH_3CO_2NH_4$
Mobile phase B:	MeOH/H_2O (9:1 v/v) + 5 mM $CH_3CO_2NH_4$
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm, 1.7 µm

LC Conditions (continued)

Flow rate:	0.45 mL/min	
Injection volume:	20 µL	
Column temp.:	40 °C	
Gradient elution:	Time	%B
	0 min	0%
	8.5 min	100%
	11.0 min	100%
	11.1 min	0%
	13.5 min	0%

MS Conditions

MS system:	Quattro Premier
Ionization mode:	ES+/ES-
Capillary voltage:	0.8 kV (+/- ionization)
Gas flow:	800 L/hr
Source temp.:	120 °C
Desolvation temp.:	400 °C
Cone voltage:	See Table 1
MS/MS:	Operated in MRM mode
Collision voltage:	See Table 1

METHOD DEVELOPMENT AND PERFORMANCE

The work details the development of a multi-residue method for the analysis of 100 pesticide residues by UPLC/MS/MS. The work is based upon a previously developed HPLC/MS/MS method using a Waters Alliance® HT/Quattro Premier system, which had an overall cycle time of 25 minutes (HPLC conditions: XTerra® MS C_{18} Column, 2.1 x 100 mm, 3.5 µm, linear gradient from 0 to 100% B in 17 min).

Comparison of UPLC and HPLC chromatograms is shown below (Figure 1). Peak widths observed for the majority of pesticide residues analyzed under UPLC conditions are approximately 0.1 min (cf 0.3 min under HPLC conditions). The narrower peak widths often resulted in an increase in signal response over that achieved under HPLC/MS/MS conditions (Figure 1).

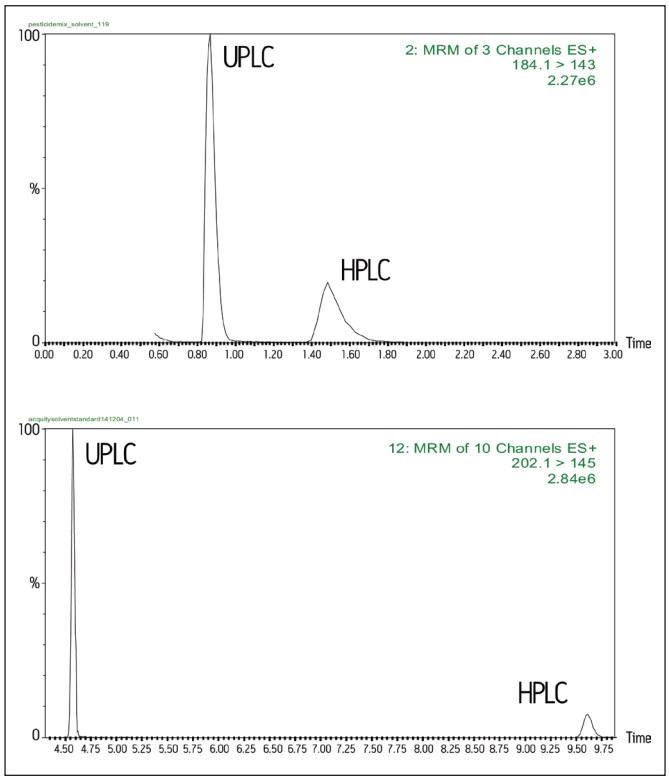

Figure 1. Comparison of UPLC (0.1 min) and HPLC (0.3 min) chromatograms. Data obtained for a) acephate and b) carbaryl (solvent standard) at 10 pg/µl.

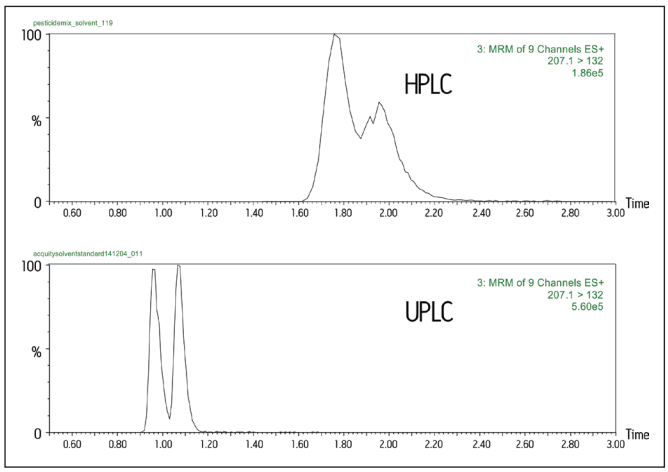

Figure 2. Increased resolution of UPLC over HPLC.

Greater chromatographic resolution is achievable under UPLC conditions (cf. HPLC) and is illustrated in Figure 2. Butoxycarboxim sulfoxide and aldicarb sulfoxide have similar retention properties, with butoxycarboxim sulfoxide eluting first, and the same MRM transition (m/z 207.1>132).

It can be seen that UPLC has the ability to separate complex mixtures. This is confirmed by considering the analysis of 100 pesticide residues in raisin matrix (Figure 3). All 100 pesticides elute within 10 minutes, and the overall cycle time is just 13.5 minutes.

Since the analytical method is intended for surveillance monitoring, it needs to be able to detect tens of pesticide residues; some of which are better detected under negative ES conditions (Table 1). The use of the ACQUITY UPLC system places added demands on the mass spectrometer due to the improved chromatographic resolution and short analysis times. For these reasons, the Quattro Premier tandem quadrupole mass spectrometer was selected as the detector for this application.

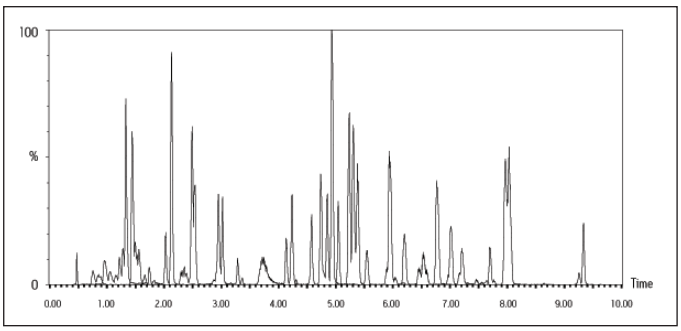

Figure 3. UPLC TIC chromatogram for the analysis of 100 pesticide residues in raisin extract (10 µg/kg).

In order for accurate quantization to be performed, a minimum of 10 data points across each peak must be acquired. This requirement, coupled with the number of target analytes and narrow chromatographic UPLC peaks indicated that it would be advantageous if the MRM functions were arranged into time windows, based on analyte retention times (Figure 4). This system enabled the flexible use of dwell times (Table 1), such that those peaks with lower intensities can have their S/N ratios increased by employing longer dwell times, while retaining a minimal scan time.

In addition to the primary MRM traces monitored for each analyte, confirmation MRM traces were incorporated into the method for the 31 most commonly found residues. In total, 131 MRM transitions were monitored in 26 time windows (Figure 4).

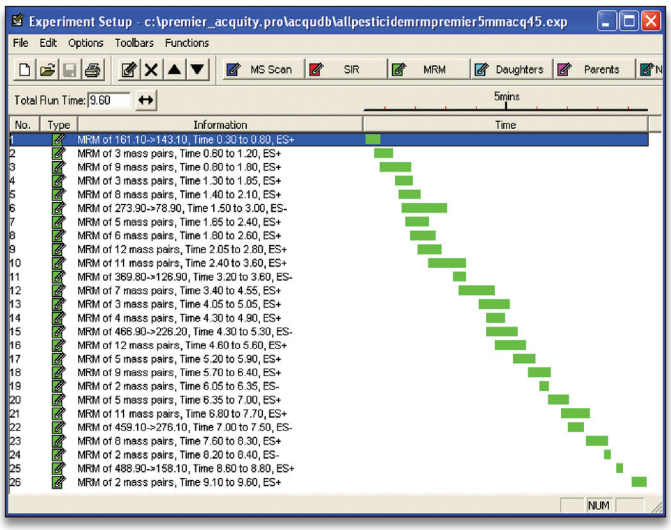

Figure 4. MRM functions arranged into time windows.

Six of the pesticides included within the method ionize under negative ES mode. The Quattro Premier can switch rapidly between positive and negative ionization modes, so that closely eluting analytes under both modes can be achieved within a single analytical run as illustrated above right (Figure 5), thereby minimizing the need to perform separate analyses.

Analysis of standard solutions enabled LODs (based on 3 x S/N) to be determined (Table 1). All are well below the necessary reporting level of individual pesticides in food (10 µg/kg, 5 pg/L), indicating that this method could be applied to the analysis of pesticide residues in a variety of matrices.

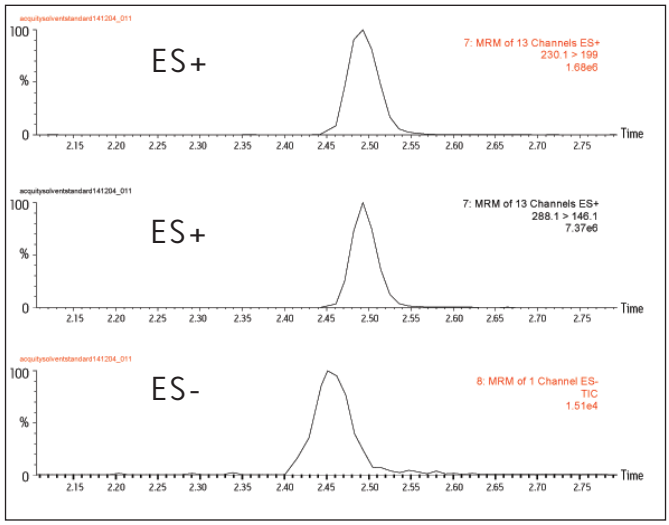

Figure 5. Chromatographic traces for dimethoate and vamidothion (ES+) and bromoxynil (ES-).

APPLICATION

The analytical method was applied to the analysis of pesticide residues in raisins. The chromatogram (Figure 3) obtained for the analysis of a raisin sample containing the pesticides spiked at a level equivalent to the MRL demonstrates good signal response for all analytes at this reporting level. Since the analytical method is intended for surveillance monitoring, it needs to be able to detect tens of pesticide residues; some of which are better detected under ES- conditions (Table 1).

Good linearity in calibration was demonstrated over the range analyzed, 1-20 µg/kg (Figure 6).

Inclusion of a second transition within the surveillance method enables unambiguous confirmation of the presence of a residue within the sample, without the need to perform a second confirmatory analytical run (Figure 7) resulting in further efficiency gains. Two pesticide residues (imidacloprid and tebufenozide) were confirmed present within the raisin sample at levels below the MRL, 4.4 and 3.4 µg/kg, respectively.

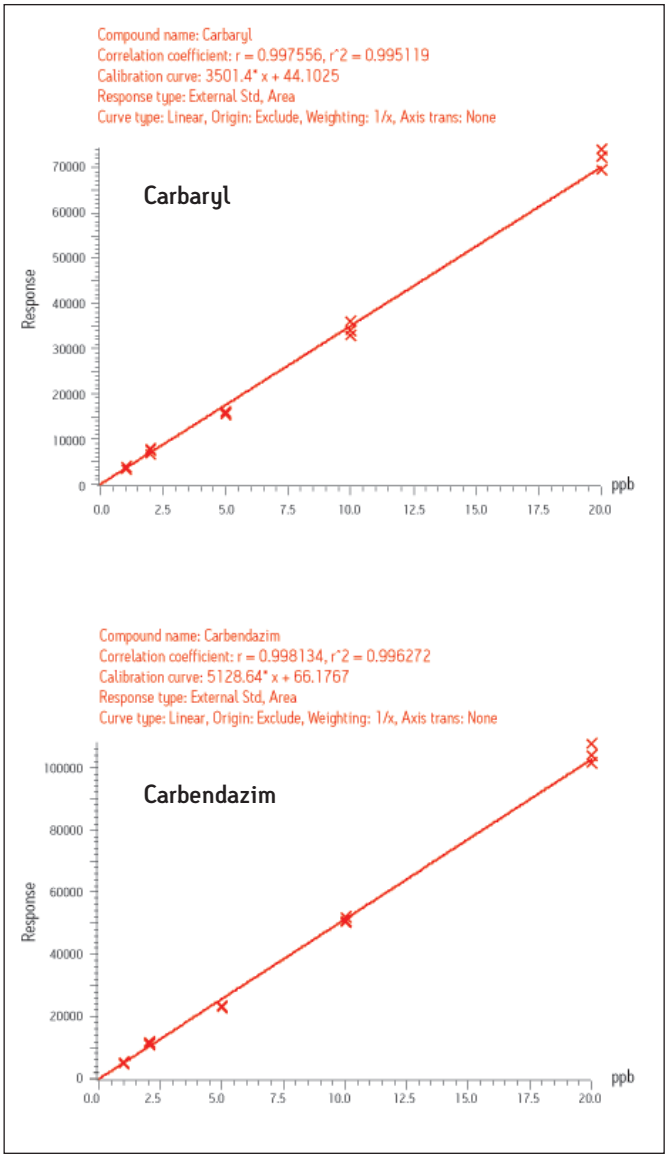

Figure 6. Representative calibration graphs for the analysis of carbaryl (top) and carbendazim (bottom) spiked into blank raisin matrix at a range of concentrations.

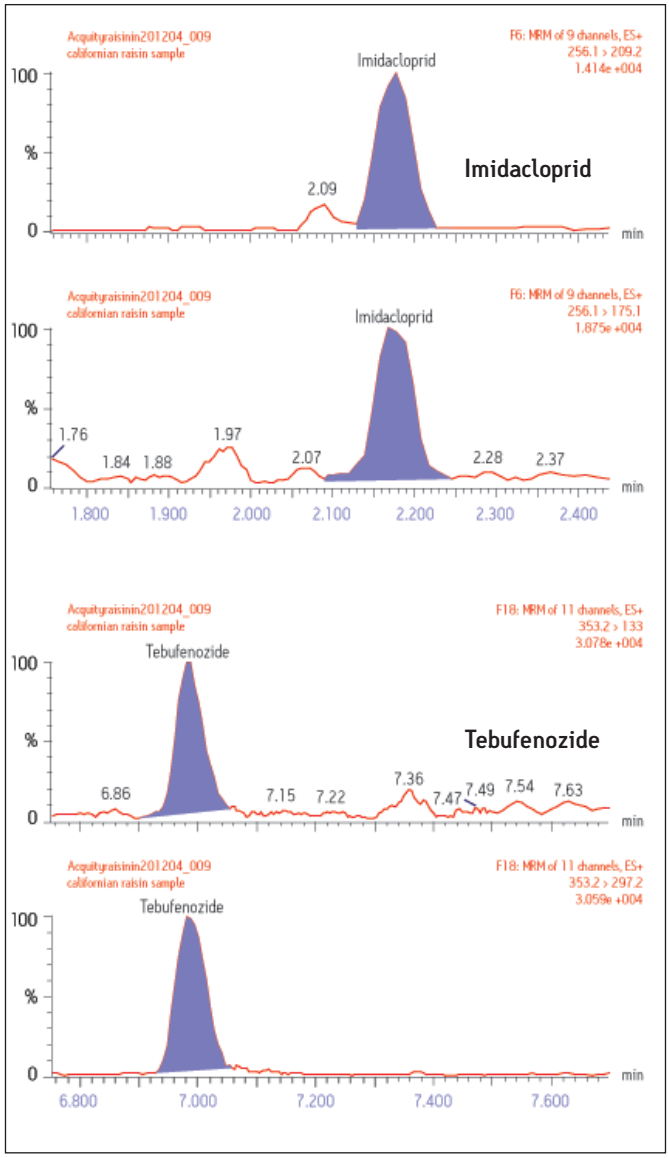

Figure 7. Confirmation that the Californian raisin sample contained imidacloprid (top) and tebufenozide (bottom).

CONCLUSION

- A rapid multi-residue UPLC/MS/MS method has been developed for surveillance monitoring of 100 pesticide residues and has been applied to the analysis of raisins.
- Improved efficiency and increased sample throughput has been realized through the combination of these UPLC and MS technologies which offer:
  - enhanced chromatographic resolution and short analysis times.
  - the ability to group MRM functions into time windows, enabling the incorporation of confirmatory MRM traces.
  - the capability to switch rapidly between MRM channels and between positive and negative ionization modes.
- The sensitivity achieved for the majority of pesticide residues indicates that this UPLC/MS/MS method could be applied to the analysis of pesticides in different matrices over the range analyzed.
- Given the chromatographic improvements afforded by the ACQUITY UPLC system coupled to the advances in data acquisition methods seen with the Quattro Premier mass spectrometer, it is feasible that this method could be extended to over three hundred compounds (provided efficient sample extraction).

References

1. Plumb R., Castro-Perez J., Granger J., Beattie I., Joncour K., Wright A. Rapid Commun. Mass Spectrom. 2004; 18: 2331-7.
2. Waters Application Note 720000686EN: A multi-residue LC/MS/MS method for the determination of 81 pesticide residues in fruit and vegetables: Part 1, Method Overview.
3. Waters Application Note 720000686EN: A multi-residue LC/MS/MS method for the determination of 81 pesticide residues in fruit and vegetables: Part 1, Method Overview.

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Daminozid	0.50	161.1	143.1	18	12	200	0.01
Methamidophos	0.79	141.8	93.8	22	14	80	0.02
			124.9	22	13	80	
Acephate	0.89	184.1	143.0	16	8	40	0.04
Butoxycarboxim-sulfoxide	1.00	207.1	132.1	17	6	30	0.05
Omethoate	1.01	214.0	183.0	20	12	30	0.01
			154.9	20	15	30	
Aldicarb-sulfoxide	1.11	207.1	132.0	16	10	30	0.1
			89.0	16	14	30	
Butoxycarboxim	1.20	240.1	106.1	10	14	30	0.04
Aldoxycarb	1.26	240.1	86.0	15	20	30	0.005
Oxamyl	1.32	237.1	71.9	12	10	30	0.003
Propamocarb	1.36	189.1	102.0	25	17	30	0.01
			144.0	25	12	30	
Oxydemeton-methyl	1.49	247.0	169.0	20	13	10	0.002
Pymetrozin	1.57	218.0	105.0	25	17	10	0.02
6-chloro-4-hydroxy-3-phenyl-pyridazin	1.60	207.1	77.0	35	30	10	0.04
			104.0	35	21	10	
Methomyl	1.60	162.9	87.8	15	8	10	0.01
			105.9	15	10	10	
Demeton-S-methyl-sulfon	1.61	263.1	169.1	28	16	10	0.02
			121.2	28	16	10	

Table 1. MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Table 1. (continued)

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Quinmerac	1.69	222.0	141.0	22	33	10	0.008
Monocrotophos	1.78	224.0	126.9	20	15	10	0.005
Bendiocarb	1.78	224.1	109.0	18	18	10	0.01
			167.1	18	9	10	
Nicosulfuron	1.80	411.0	182.1	22	18	10	0.05
Amidosulfuron	1.84	370.0	261.2	18	14	10	0.02
Metsulfuron - methyl	2.00	382.0	167.0	22	15	10	0.02
Thifensulfuron - methyl	2.00	388.0	167.1	22	15	10	0.02
Ethiofencarbsulfon	2.04	275.1	107.1	10	20	10	0.006
Rimsulfuron	2.05	431.9	182.1	30	22	10	0.02
Ethiofencarbsulfoxide	2.13	242.1	107.0	18	18	10	0.003
Thiofanox - sulfoxide	2.14	252.1	104.0	10	12	10	0.3
Imidacloprid	2.14	256.1	209.2	22	16	10	0.02
			175.1	22	20	10	
Florasulam	2.28	360.1	129.0	30	20	10	0.09
5 Hydroxy - clethodim - sulfon	2.29	408.2	204.2	22	16	10	0.1
Thiofanox - sulfon	2.32	268.1	76.0	10	10	10	0.02
Clethodim -imin - sulfon	2.35	302.2	98.1	35	30	10	0.04
Metamitron	2.37	203.0	175.1	28	16	10	0.02
Cinosulfuron	2.42	414.1	183.1	25	18	10	0.05
Chlorsulfuron	2.43	358.1	141.1	25	16	10	0.08
			167.1	25	16	10	
Bromoxynil *	2.45	273.9	78.9	40	25	30	0.2
Dimethoate	2.48	230.1	125.1	17	20	10	0.03
			199.1	17	10	10	
Clethodim -imin - sulfoxide	2.49	286.2	208.2	25	17	10	0.03
Vamidothion	2.51	288.1	146.1	17	12	10	0.005
Carbofuran -3 - hydroxy	2.56	220.1	163.1	25	10	10	0.007
Flazasulfuron	2.66	408.1	182.1	25	22	10	0.5
Triasulfuron	2.85	402.0	167.1	25	17	10	0.2
			141.0	25	20	10	
Clethodim - sulfon	2.90	392.1	300.2	20	12	10	0.04
Clethodim - sulfoxide	2.95	376.1	206.2	22	15	10	0.05
Carbendazim	2.98	192.1	160.1	25	18	10	0.05
			132.1	25	30	10	
Thiacloprid	3.05	253.0	126.0	28	22	10	0.01
Difenzoquat methylsulfate	3.12	249.2	193.1	45	28	10	0.03
Butocarboxim	3.32	213.1	75.0	20	14	10	0.005
Aldicarb	3.39	208.1	116.0	7	7	10	0.3
Ioxynil *	3.40	369.8	126.9	40	30	20	0.1
Carbofuran	3.41	222.3	165.2	25	15	10	0.1
Iodosulfuron	3.63	508.2	167.2	25	18	30	1
Thiabendazol	3.78	202.0	175.1	40	25	20	0.07
			131.0	40	32	20	
Propoxur	4.17	210.1	111.0	14	15	10	0.01

Table 1. (continued) MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Table 1. (continued)

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Formetanate	4.23	222.1	165.2	20	12	10	0.005
Prosulfuron	4.46	420.0	141.1	25	20	10	0.2
			167.0	25	18	10	
Carbaryl	4.60	202.1	145.0	18	10	10	0.005
Bensulfuron - methyl	4.67	411.1	149.1	25	22	10	0.05
Ethiofencarb	4.76	226.1	107.1	15	15	10	0.01
			164.1	15	8	10	
Primisulfuron - methyl*	4.84	466.9	226.2	20	15	10	1
Triflusulfuron - methyl	4.86	493.0	264.2	28	20	10	0.8
Thiodicarb	4.88	355.1	87.9	15	16	10	0.02
Thiofanox	4.92	219.0	56.9	15	18	10	0.01
Pirimicarb	4.97	239.1	72.0	28	18	10	0.005
			182.1	28	15	10	
Atrazin	5.08	216.1	174.1	30	17	10	0.01
Isoproturon	5.26	207.1	72.1	25	18	10	0.008
Isoxaflutole	5.31	377.1	251.2	15	20	10	0.3
Metalaxyl	5.34	280.1	220.2	20	13	10	0.01
			192.2	20	17	10	
Diuron	5.35	233.1	72.1	25	18	10	0.02
3,4,5 -Trimethacarb	5.41	194.1	137.1	18	10	10	0.01
Clethodim	5.52	360.2	164.1	20	19	10	0.05
Desmedipham	5.56	318.2	182.2	17	12	10	0.01
Phenmedipham	5.69	301.1	168.0	25	10	10	2
Linuron	5.92	249.1	160.0	28	16	10	0.02
			182.1	28	15	10	
Pyrimethanil	5.93	200.1	107.0	42	22	10	0.1
			82.0	42	25	10	
Azoxystrobin	5.97	404.1	372.2	22	15	10	0.02
			329.2	22	30	10	
Methiocarb	6.06	243.1	121.0	10	22	10	0.3
Fludioxonil *	6.20	247.0	180.1	45	28	20	0.1
			126.1	45	35	20	
Promecarb	6.23	208.1	151.0	20	9	10	0.03
			109.0	20	15	10	
Iprovalicarb	6.55	321.2	119.1	15	18	10	0.1
Fenhexamid	6.61	302.1	97.0	35	25	10	0.05
			55.1	35	35	10	
Metolachlor	6.81	284.1	176.1	20	25	10	0.01
			252.1	20	15	10	
Tebufenozide	7.01	353.2	133.0	13	20	10	0.4
			297.2	13	8	10	
Fenoxycarb	7.04	302.1	88.0	20	20	10	0.05
Cyprodinil	7.19	226.2	93.1	45	33	10	0.08
			108.1	45	25	10	
Tebuconazol	7.23	308.1	70.0	30	20	10	0.02

Table 1. (continued) MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Table 1. (continued)

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Imazalil	7.24	297.1	159.0	30	20	10	0.3
			69.1	30	20	10	
Triflumuron	7.49	359.1	156.0	25	18	10	0.2
			139.0	25	37	10	
Haloxyfop - methyl	7.73	376.1	316.2	30	18	10	0.03
Indoxacarb	7.80	527.9	218.1	28	20	10	0.5
Hexaflumuron *	7.85	459.1	276.1	22	22	30	5
Quizalofop - ethyl	8.00	373.1	299.2	30	19	10	0.03
Fluazifop -**P-butyl**	8.07	384.1	282.2	32	22	10	0.02
			328.2	32	16	10	
Haloxyfop - ethoxyethyl	8.07	434.0	316.2	25	20	10	0.05
Spiroxamine	8.11	298.3	144.1	30	20	10	0.03
Furathiocarb	8.12	383.1	195.1	20	16	10	0.04
Diflubenzuron	8.14	311.0	158.1	30	14	10	0.1
Teflubenzuron *	8.31	379.0	196.0	18	25	10	0.8
			339.1	18	15	10	
Flufenoxuron	8.68	488.9	158.1	25	18	10	0.05
Pyridate		379.1	207.1	25	16	120	0.05
Fenpropimorph	9.38	304.2	147.2	45	30	120	0.02

Table 1. MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

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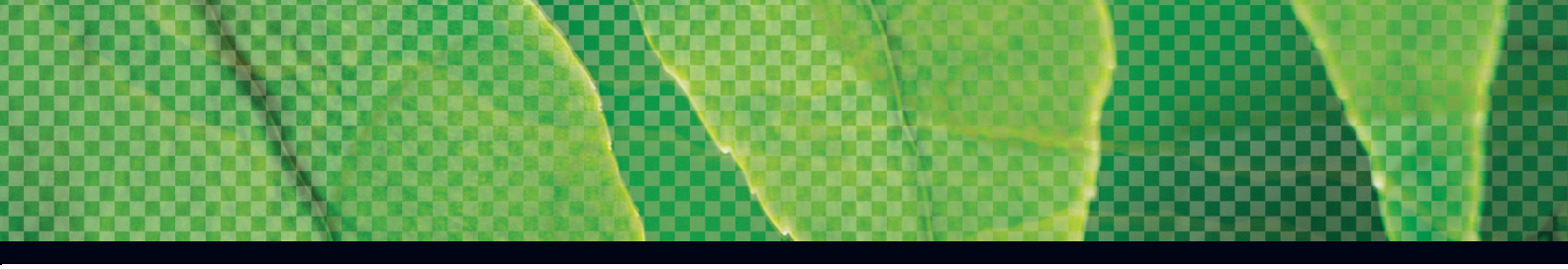

ANALYSIS OF PERFLUORINATED COMPOUNDS (PFC'S) ON THE ACQUITY UPLC SYSTEM & THE QUATTRO PREMIER XE IN ES-MS/MS

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ANALYSIS OF PERFLUORINATED COMPOUNDS (PFCS) ON THE ACQUITY UPLC SYSTEM & THE QUATTRO PREMIER™ XE IN ES-MS/MS

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INTRODUCTION

The worldwide ubiquitous occurrence of perfluorinated compounds (PFCs) in the environment and in human blood has raised the attention of researchers and authorities in recent years[1-7]. PFCs have both hydrophobic and hydrophilic properties and are frequently used for treatment of carpets, fabric, leather, protection of paper and food packaging and also as performance chemicals in plastic production, firefighting foam, polish, cleaners and insecticides[8,9].

Laboratory studies indicate that PFCs may disturb the fatty acid metabolism, affect the reproductive system and/or cause liver damage[10,11]. These effects together with their stability and bio-accumulative properties, suggest that PFCs are potentially harmful to humans and the environment. Therefore, accurate and reproducible determination of PFCs in environmental and human samples is a necessity but can pose many challenges[12]. Contamination from laboratory materials and instrumental parts are common problems.

The commercialization of high performance liquid chromatography-mass spectrometry (HPLC/MS) has facilitated the selective and sensitive analysis of PFC acids. This note describes a method using ultra performance liquid chromatography (UPLC®) and tandem mass spectrometry (MS/MS).

The Waters® ACQUITY UPLC®, launched in 2004, uses columns with 1.7 µm particle size that can operate at high pressure (maximum pressure of 15,000 psi). The combination of smaller particle size and higher pressure provide a fast, high resolution separation that increase sensitivity and minimize matrix interference.

In this study, 13 PFC's normally run using HPLC have been analyzed by UPLC. The HPLC method requires 22 minutes compared to a run-time of less than five minutes for UPLC. Faster run times not only increase the throughput of the instrument but also reduce method development time.

ACQUITY UPLC and Quattro Premier XE mass spectrometer.

This method includes an extraction procedure involving a solid-phase extraction (SPE) step using Waters Oasis® WAX columns followed by analysis on the ACQUITY UPLC and the Waters Quattro Premier™ XE in negative electro spray (UPLC/ES-MS/MS).

EXPERIMENTAL

Compounds

PFBuS tetrabutylammonium-salt (≥98%), PFOS potassium-salt (≥98%), PFDA (>97%), PFHxA (≥97%) were purchased from Fluka (Steinheim, Germany). PFHpA (99%), PFNA (97%), PFOA (96%), PFUnDA (95%), were purchased from Aldrich (Steinheim, Germany and Milwaukee, WI, USA). 7HPFHpA (98%) was purchased from ABCR (Karlsruhe, Germany). PFHxS (98%) was purchased from Interchim (Montlucon, France). $^{13}C_4$PFOS, $^{13}C_4$PFOA, $^{13}C_5$PFNA were from Wellington Laboratories (Ontario, Canada). HPLC grade solvents were used (Fisher Scientific).

Extraction Procedure (based on Taniyasu et al.[13])

Waters Oasis WAX SPE Column

Conditioning parameters: 2 mL methanol, 2 mL water

Wash: 2 mL 40% methanol in water (vacuum) until dry.

Elute: 1 mL 2% ammonium hydroxide in methanol.

Evaporate extract under a gentle nitrogen stream to 0.5 mL

filter using a 0.2 µm polypropylene filter into a vial.

Add recovery standards ($^{13}C_5$-PFNA and 7H-PFHpA) 0.5 mL plasma or whole blood internal standards ($^{13}C_4$-PFOS and $^{13}C_4$-PFOA).

Mix well and add 2 mL 50 v/v% formic acid/water.

Sonicate for 15 minutes.

Centrifuge at 10,000 x g for 30 minutes.

Take the supernatant and extract using a Waters Oasis WAX SPE column (200 mg/2 mL).

Mobile Phase Residue Trap (MPRT)

Figure 1. Modification of UPLC system to reduce the interference of mobile phase PFC presence.

UPLC Method

Waters ACQUITY UPLC

Mobile phase A: 2 mM Aq. ammonium acetate

Mobile phase B: Methanol + 2 mM ammonium acetate Mobile phase residue trap (MPRT) (see Figure 1)

Column: ACQUITY BEH C_{18} 2.1 x 50 mm, 1.7 µm, (P/N 186002350)

Column temp.: 50 °C

Flow rate: 0.4 mL/min

Injection volume: 10 µL

UPLC Gradient

0.00 min:	70% A	30% B
0.50 min:	70% A	30% B
5.00 min:	10% A	90% B
5.10 min:	0% A	100% B
6.00 min:	0% A	100% B
7.00 min:	70% A	30% B
10.00 min:	70% A	30% B

MS Method

Waters Quattro Premier XE

Electrospray mode with negative polarity

The MRM transitions along with the cone voltages and collision energies are listed in Table 1.

Function 1 10.00-3.10						
RT	PFC	Parent	Daughter	Dwell	Cone E	Coll E
2.09	PFBuS	299.00	80.00	0.20	45.00	29.00
2.50	7H-PFHpA	345.00	281.00	0.20	16.00	10.00
2.81	PFHxA	313.00	269.00	0.20	15.00	10.00
Function 2 3.10-3.85						
RT	PFC	Parent	Daughter	Dwell	Cone E	Coll E
3.45	PFHpA	363.00	319.00	0.05	16.00	10.00
3.51	PFHxS	399.00	80.00	0.05	45.00	35.00
Function 3 3.75-4.10						
RT	PFC	Parent	Daughter	Dwell	Cone E	Coll E
3.88	THPFOS	427.00	80.00	0.05	45.00	40.00
3.90	PFOA	413.00	369.00	0.05	17.00	11.00
3.90	13C-PFOA	417.00	372.00	0.05	17.00	11.00
Function 4 3.80-5.00						
RT	PFC	Parent	Daughter	Dwell	Cone E	Coll E
4.26	PFNA	463.00	419.00	0.05	16.00	11.00
4.26	13C-PFNA	468.00	423.00	0.05	16.00	11.00
4.28	PFOS	499.00	80.00	0.05	45.00	40.00
4.28	13C-PFOS	503.00	80.00	0.05	45.00	40.00
Function 5 4.40-5.20						
RT	PFC	Parent	Daughter	Dwell	Cone E	Coll E
4.56	PFDA	513.00	469.00	0.05	17.00	12.00
4.83	PFUnDA	563.00	519.00	0.05	18.00	12.00

Table 1. MRM transition parameters for PFCs in ES-.

RESULTS AND DISCUSSION

Figure 2 shows the full names and the abbreviations for the PFCs used in this report. Two internal standards were used in the quantification, ^{13}C-PFOA and ^{13}CPFOS Recovery standards were also included and these were 7HPFHpA and ^{13}CPFNA. Initial experiments used water and methanol without buffer, but it was found that although the compounds were retained on the column, in order to sustain good peak shape, the addition of 2 mM ammonium acetate was required in the mobile phases.

Perfluorinated compound	Abbreviation	Formula
perfluorobutanesulfonate	PFBuS	$C_4F_9SO_3$
perfluorohexanesulfonate	PFHxS	$C_6F_{13}SO_3$
perfluorooctanesulfonate	PFOS	$C_8F_{17}SO_3$
perfluorohexanoic acid	PFHxA	$C^5F_{11}CO_2H$
perfluoroheptanoic acid	PFHpA	$C_6F_{13}CO_2H$
perfluorooctanoic acid	PFOA	$C_7F_{15}CO_2H$
perfluorononanoic acid	PFNA	$C_8F_{17}CO_2H$
perfluorodecanoic acid	PFDA	$C_9F_{19}CO_2H$
perfluoroundecanoic acid	PFUnDA	$C_{10}F_{21}CO_2H$
Internal & Recovery Standards		
7H-perfluoroheptanoic acid	7H-PFHpA	$HC_6F_{12}CO_2H$
perfluorooctanesulfonate	13C-PFOS	$^{13}C_8F_{17}SO^3$
perfluorooctanoic acid	13C-PFOA	$^{13}C_7F_{15}CO_2H$

Figure 2. List of PFCs used in the experiment.

TargetLynx™, an application manager available with Waters MassLynx™ software, was used to process the data and quantify the amount of compounds present in the blank plasma and whole blood samples. Good linearity (r^2>0.99) is observed for all compounds. The PFOS calibration curve has been included in this report (Figure 3). A chromatogram from the PFC run using the ACQUITY UPLC/Quattro Premier XE is shown in Figure 4.

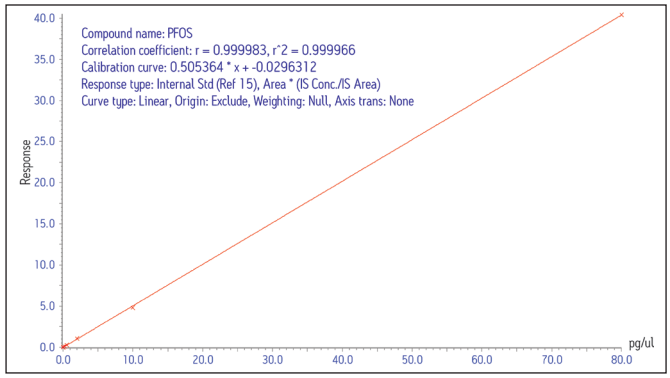

Figure 3. Calibration curve for PFOS covering the concentration range 0.01-80 ng/ml.

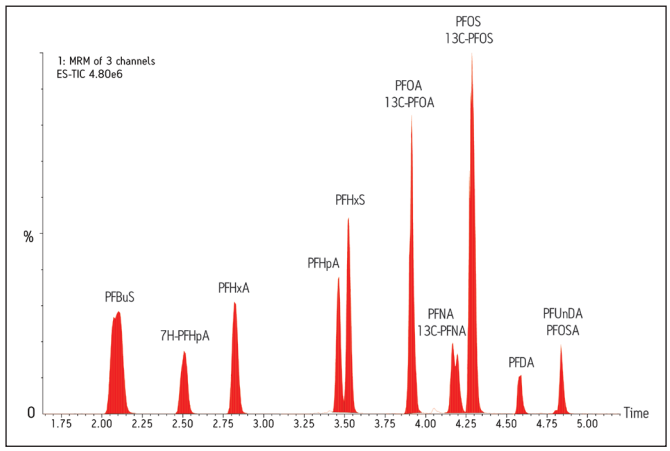

Figure 4. Chromatogram of a standard solution containing 13 PFCs using UPLC/MS/MS.

Performance

Figure 5 illustrates the improved chromatography with respect to run-time and peak width using UPLC compared to traditional HPLC (where the HPLC data was obtained using LC/MS and the UPLC data employs LC/MS/MS). The run time for these 13 compounds is 22 minutes with HPLC, but this is shortened to five minutes using UPLC.

A number of advantages result from the faster run times afforded by UPLC, including reduced method development times and smaller peak widths. In Figure 4, the peak width for PFOS has been reduced from 20.3 s to 4.2 s. This reduces the opportunity for co-elution, increases the chance that isomers will be separated and may improve sensitivity.

Recoveries

Table 2 shows the instrumental and the method detection limits. The instrumental detection limit was defined as the concentration required to produce a signal to noise ratio of 3:1. The method detection limit for 0.5 ml blood was estimated from blood samples spiked at low concentrations and was defined as the concentration with an S/N ratio of 3.

Recovery and reproducibility of the extraction procedure were evaluated by adding known amounts of PFCs to whole blood and plasma samples in 5 replicates (Table 3: PART A). Recovery was calculated by comparing the obtained area in the sample extracts, corrected for volume, with the corresponding area, corrected for volume, in a standard solvent solution. Acceptable values are regarded as being between 70-130%. (For some of the longer chain PFCs, the values are slightly lower for the plasma samples. The standard deviation is also higher for these compounds, but they are generally more difficult to quantify than the shorter chain PFCs).

Possible suppression or enhancement of the signal in the electrospray ionization were evaluated (Table 3: PART B) by extracting unspiked whole blood and plasma according to the method and adding known amounts of PFCs to the extracts prior to injection (and post- SPE).

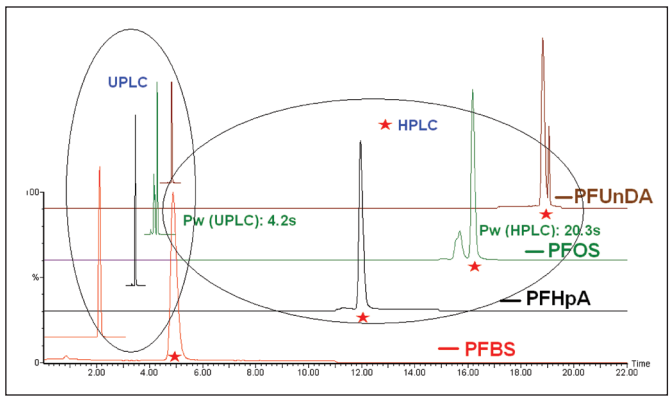

Figure 5. Comparison of run times and peak widths obtained using UPLC and traditional HPLC.

pg/ul	Detection limits		
	Instrument	Method - whole blood	Method - Plasma
PFBuS	0.0003	0.001-0.002	0.002
PFHxS	0.0006	0.002	0.003-0.005
PFOS	0.0035	0.035	0.018-0.025
PFHxA	0.0045	0.028-0.034	0.016-0.023
PFHpA	0.0016	0.008	0.009-0.011
PFOA	0.0031	0.038-0.044	0.017-0.023
PFNA	0.0021	0.013	0.018-0.035
PFDA	0.0110	0.026-0.032	0.067-0.083
PFUnDA	0.0022	0.018-0.024	0.032-0.042

Table 2. Detection limits for the experiment, in pg/µL.

The obtained areas, corrected for volume, were then divided by corresponding areas in a standard solvent solution. A ratio >1 indicates that the sample matrix enhanced the signal and a ratio <1 indicates that the signal is suppressed by the sample matrix. These results are relatively close to 1 (ideal), except for PFUnDA in whole blood, which is high at 2.47.

PART A: Recovery and reproducibility							PART B: Matrix effects		
	Whole blood (n=5)			Plasma (n=5)				Whole blood (n=1)	Plasma (n=3)
	Average	Stddev	RSD%	Average	Stddev	RSD%		Average	Average
PFBuS	77%	0.045	6%	77%	0.031	4%	PFBuS	0.86	0.89
PFHxA	92%	0.035	4%	83%	0.027	3%	PFHxA	1.11	0.97
PFHpA	82%	0.039	5%	79%	0.011	1%	PFHpA	0.98	0.92
PFHxS	70%	0.045	6%	77%	0.037	5%	PFHxS	0.79	0.88
PFOA	82%	0.063	8%	94%	0.083	9%	PFOA	0.94	1.04
PFNA	86%	0.037	4%	99%	0.048	5%	PFNA	1.07	1.09
PFOS	78%	0.032	4%	65%	0.061	9%	PFOS	0.91	0.77
PFDA	92%	0.130	14%	70%	0.246	35%	PFDA	1.37	0.93
PFUnDA	124%	0.307	25%	64%	0.212	33%	PFUnDA	2.47	0.95

Table 3. Recovery and reproducibility of whole blood and plasma samples.

Contamination

Contamination is a known problem when analyzing for PFCs, and was prevalent for a number of compounds (Table 4). A set of experiments was designed to determine sources of contamination: 1) blank air injection + gradient run; 2) gradient run without injection; 3) methanol injection (solvent blank) and 4) procedure blank. Contamination for compounds PFOA and PFNA was found from the instrument pre-injector (either from the solvent used or instrument parts), and the injector; contamination eluted as a peak as the amount of methanol increased. To reduce contamination, a column was inserted post-pump and pre-injector, which allowed the contaminating peaks to be separated from the analytical peak.

Accumulation of these compounds can occur at the head of the column if the flow is stopped (where the source may be the methanol or components in the UPLC system). To prevent this, solvent flow was left at 0.050 ml/min once the sequence had been run and until the next sequence was run.

Two procedure blanks prepared at different times were monitored. The procedure blanks showed more contamination than the solvent blanks, and in some cases the contamination observed may have come from the internal standards. A secondary explanation for incidents of slightly higher contamination may be glassware or other sources that had contact with the solvents.

Contamination from the extraction procedure was evaluated by including one MilliQ water sample (blank) for each set of 12 blood samples extracted.

Contamination from the instrument was evaluated by multiple methanol injections during the sample sequence.

CONCLUSION

A sensitive and rapid method for the analysis of PFCs using UPLC/MS/MS has been described. This method reduces the typical HPLC run-time of 22 minutes to less than five minutes. The detection limits using 0.5 mL blood and plasma were between 0.002-0.04 pg/µL and 0.002-0.08 pg/µL, respectively.

Further investigation is required to extend the complete method to include additional longer chain PFCs. The initial results for these compounds were not successful and have been omitted from this application note.

Contamination of blanks (pg/ul) – solvent and procedure				
	Solvent blank	Procedure blanks		
	Water: Methanol (65:35)	60103	20051027	Comments
PFBuS	✘	✔		
PFHxA	✘	~ 0.002	~ 0.002	
PFHpA	✘	~ 0.001	~ 0.001	
PFHxS	✘	➜	✘	
PFOA	~ 0.0065	~ 0.1	~ 0.4	
PFNA	~ 0.023	~ 0.05		About 50:50 from both the instrument: ISTD
PFOS	✘	✔		Could be contamination from the ISTD
PFDA	➜	✘	~ 0.05	
PFUnDA	✘	✘	✘	

✘ = not detected

✔ = found but not quantified

➜ = maybe traces

Table 4. Contamination of monitored PFCs 1) from the solvent/injector, and 2) from the procedure.

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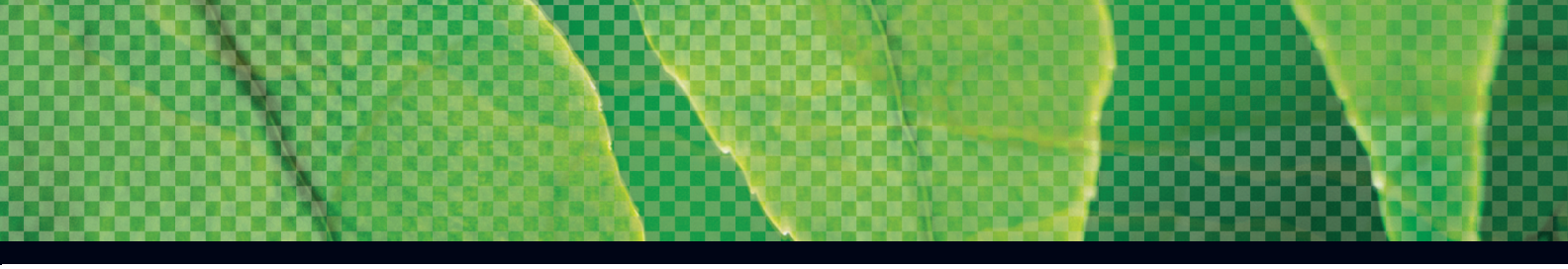

MULTI RESIDUE ANALYSIS OF PRIORITY POLLUTANTS IN DRINKING AND SURFACE WATER USING SOLID PHASE EXTRACTIONAND GC TANDEM QUADRUPOLE MS/MS

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MULTI RESIDUE ANALYSIS OF PRIORITY POLLUTANTS IN DRINKING AND SURFACE WATER USING SOLID PHASE EXTRACTION AND GC TANDEM QUADRUPOLE MS/MS

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INTRODUCTION

EU council directive 76/464/EC[1] lists 132 compounds that have restricted levels in drinking and surface waters. Of these compounds, 109 are amenable to gas chromatographic analysis. Currently published methods[2] involve the use of two injections, one using selected ion recording as a screen, followed by a full scan injection for confirmation. The use of tandem quadrupole GC/MS/MS allows the analyst to combine the screening and confirmatory injections into one run, while also allowing a reduction of the chromatographic separation required for confirmation of some of the target compounds. The EU list has many similarities with the target compound lists of U.S. EPA water quality methods such as 625[3] and 8270[4] (it should be noted that the list analyzed in this method is by no means an exhaustive one). The compound groups represent a wide range of polarities and compound types, and include benzidines, chloronitrotoluenes, organochloro pesticides, organophosphorus pesticides, chloroanilines, chlorophenols, chloronitrobenzenes, chlorotoluidines, phenylurea pesticides, PCBs, semi-volatile halogenated compounds, PAHs (Polynuclear Aromatic Hydrocarbons), triazines and volatile amines.

Many of these compound groups will typically have their own dedicated analysis method that requires specific extraction/clean-up and final analysis.

Combining these groups into a single method would allow the laboratory to significantly increase sample throughput. The high selectivity and specificity of multiple reaction monitoring (MRM) acquisitions also help to shorten the time required for data processing by reducing the possibility of false positives and time spent confirming the presence of target compounds. The method presented is intended as an example of what is possible by implementing techniques such as GC tandem quadrupole MS/MS and solid phase extraction.

METHODS AND MATERIALS

All chemicals were obtained from Sigma-Aldrich, with all compounds having >99.5% purity. All analyses were performed using an Agilent 6890 GC oven fitted with a CTC Combi PAL autosampler.

The GC was directly interfaced to a Waters® Quattro micro GC™ tandem quadrupole mass spectrometer that was operated in the EI+ ion mode. The instrument ion source was operated at 70 eV electron energy, with a source temperature of 180 °C. Three GC columns were evaluated, J&W DB17-ms 30 m 0.25 mm ID, 0.25 µm df, Restek RTX-5, 40 m 0.18 mm ID, 0.2 µm df and Varian factor four vf5-ms 30 m 0.25 mm ID, 0.25 µm df. Injections were made using both pulsed splitless and cool on column (COC) injections, with a 2 m 0.53 mm ID retention gap fitted for COC injections. All compounds were acquired in full scan and daughter scanning acquisition modes, with the results used to optimize at least two MRM transitions per compound. Internal and recovery standards had one MRM transition optimized. MRM analysis was performed using a single transition per compound, where confirmation is based upon one MRM transition plus the retention time, and also using two MRM transitions per compound, where the strictest EU confirmatory criteria are satisfied. The difference in sensitivity between the two approaches was compared. The three GC columns were assessed for chromatographic resolution of critical pairs of co-eluting peaks, overall run time, and sensitivity of active components. All standards were prepared from >99.5% purity solids dissolved in dichloromethane (DCM), with a mixed standard being prepared at a concentration of 5 ng/L in DCM, and also acetone (for spiking purposes).

Calibration curves were acquired over the concentration range of 0.05 to 5 µg/L. Extraction and clean-up were performed using Waters Oasis® HLB 3cc, 60 mg SPE cartridges. 200 mL of each filtered water sample was spiked with an internal standard mixture containing d_5-nitrophenol, 2-fluorobiphenyl and p-terphenyl-d_{14} at a level of 500 ng for each component. The water was adjusted to

pH4 using 1 N HCl solution. The SPE cartridges were conditioned with 6 mL DCM, 6 mL acetonitrile and 6 mL of water at a flow rate of 3 mL/min. The water samples were then loaded at a flow rate of *ca* 6 mL/min. After sample loading was completed, the cartridges were washed with 1 mL water. The cartridges were then dried under a flow of nitrogen (*ca* 1 mL/min) for 20 mins, followed by final elution with either A. 2.5 mL DCM/ACN (4:1), 5 mL DCM; or B. 5 mL DCM. After elution, the extract was adjusted to a volume of *ca* 0.5 ml under a stream of dry nitrogen at ambient temperature, followed by the addition of 500 ng of d_{10}-anthracene as a recovery standard. Drinking and canal water samples were spiked with the analytes at concentrations of 0.5 µg/L and 5 µg/L prior to extraction for recovery tests.

The GC temperature ramps employed were:

30 m DB17-ms
40 °C/1 min, 3 °C/min to 160 °C, 7 °C/min to 240 °C,
15 °C/min to 305 °C, hold 15 mins. 1 mL/min He flow

40m RTX5
40 ° C/1 min, 3 °C/min to 160 °C, 7 °C/min to 240 °C,
15 °C/min to 310 °C, hold 15 mins. 0.7 ml/min He flow

30 m vf5-ms
40 °C/0.8 min, 6 °C/min to 160 °C, 8 °C/min to 310 °C,
hold 2 mins. 0.9 mL/min He flow

All injections in pulsed splitless mode were made with an injection temperature of 250 °C, using a double gooseneck 4 mm ID liner and 1 µL injection volume. The injections were made with a 1 min 110 kPa pulse, a purge time of 1 minute and a purge flow of 70 mL/min.

Cool on column injections were made in track oven mode.

Data were acquired with Waters MassLynx™ software and processed with Waters TargetLynx™ Application Manager.

RESULTS AND DISCUSSION

The optimized MRM transitions for the compounds analyzed are presented in Table 1. The transitions given in the MRM 1 column were used as the quantification transition for the confirmatory method, and as the analytical transition for the screening method. The three GC columns were evaluated for both sensitivity and chromatographic separation. The optimum conditions for separation were obtained using the DB17-ms column with COC injection. However, these conditions resulted in a 70 minute run time, with a 22 function MRM experiment required. Figure 1 shows the reconstructed TIC chromatogram from a 1 ng/µL (5 µg/L) injection in MRM mode. Figure 2 shows the separation obtained for the two main critical pairs (E/Z Mevinphos and o,p'-DDT and p,p'-DDD). The DB17-ms column showed excellent selectivity for these compounds, as well as achieving baseline separation of 3-chlorophenol and 4-chlorophenol. The COC injection technique was found to be less robust when compared with pulsed splitless injection, and was not deemed suitable for a high throughput screening method. However, due to the possibility of larger volume injection, it would be suitable for maximizing sensitivity within a high sensitivity confirmatory method.

Analysis using the vf5-ms column, combined with pulsed splitless injection afforded the best overall compromise of separation: sensitivity and robustness. This analysis was the most suitable option studied for a robust, high throughput screening/confirmatory method. The vf5-ms resulted in a total run time of <43 minutes, requiring 19 MRM time windows to be employed for confirmatory analysis. Due to the distribution of eluting peaks, it also afforded the opportunity for overlapping time windows in some areas of the elution range. This gives more flexibility if retention times were to change for any reason (typically as the GC column is shortened during its lifetime). The separation of the previously mentioned critical pairs (Mevinphos, DDD/DDT) was also adequate. Figure 3 shows the reconstructed TIC from a 1 ng/µL (5 µg/L) injection acquired in MRM mode. Figure 4 shows the separation of the critical pairs (E/Z Mevinphos and o,p'-DDT and p,p'-DDD). The RTX5 column resulted in comparable separation but a longer run time when compared with the vf5-ms. The pulsed splitless injection combined with vf5-ms separation was adopted for all further analyses.

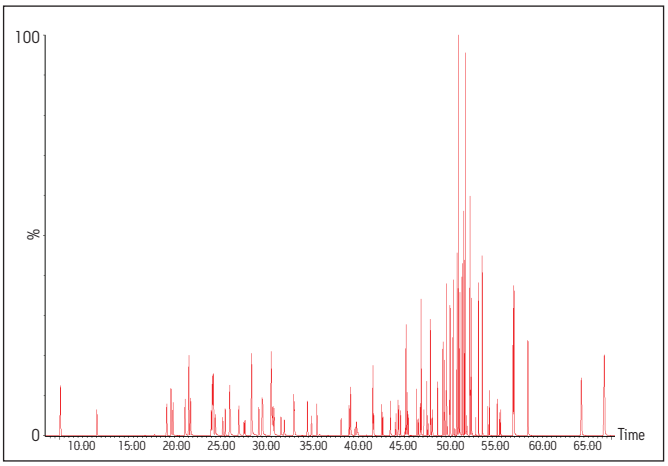

Figure 1. Reconstructed TIC for all compounds analyzed using DB17-ms column with COC injection.

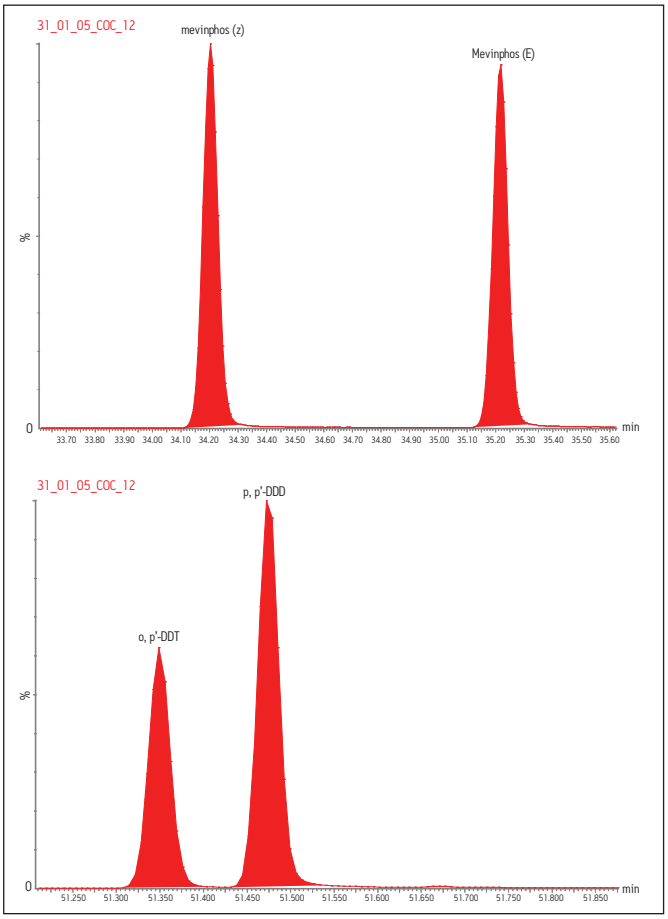

Figure 2. Critical pairs separation when analyzed using the DB17-ms column with COC injection.

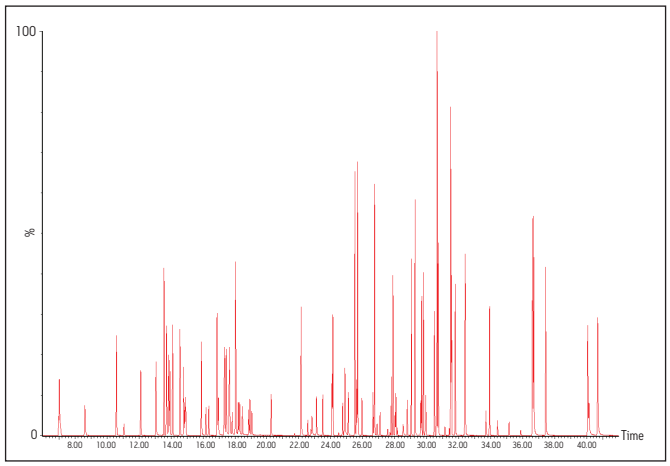

Figure 3. Reconstructed TIC for all compounds analyzed using the vf5-ms column with pulsed splitless injection.

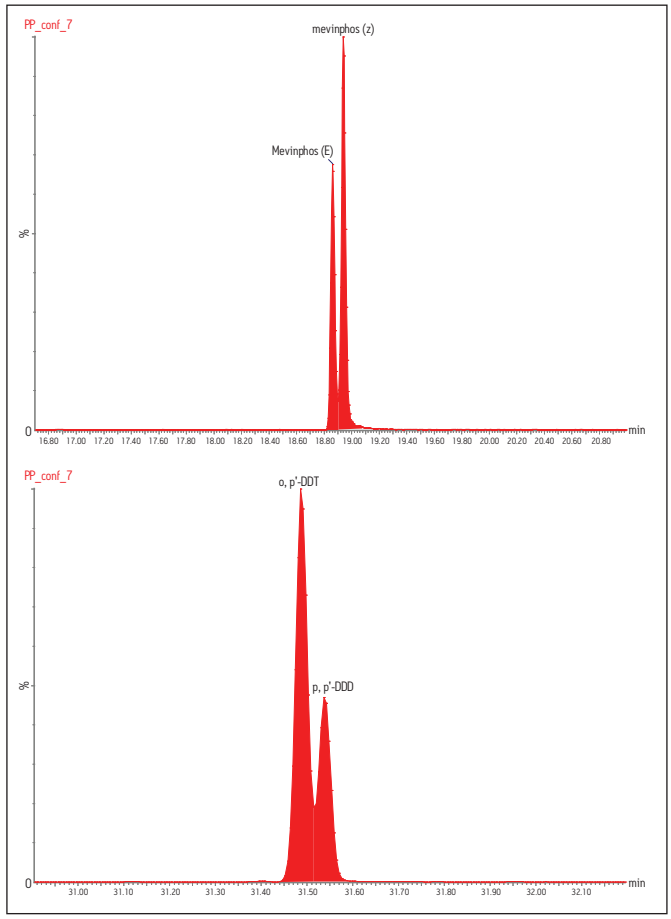

Figure 4. Critical pairs separation when analyzed using the vf5-ms column with pulsed splitless injection.

Compound	MRM 1	CE	MRM 2	CE
1,2-Dichloronaphthalene	196 > 126	25	196 > 161	15
1-Chloro-2,4-dinitrobenzene	202 > 107	10	202 > 79	10
1-Chloro-3-nitrobenzene	157 > 111	10	157 > 75	25
1-Chloro-4-nitrobenzene	157 > 99	10	157 > 75	25
2,3,4-Trichlorophenol	196 > 97	25	198 > 97	25
2,3,5-Trichlorophenol	196 > 97	25	198 > 97	25
2,3,6-Trichlorophenol	196 > 97	25	198 > 97	25
2,3-Dichloroaniline	161 > 90	15	161 > 125	10
2,3-Dichloronitrobenzene	145 > 109	10	191 > 109	27
2,4,5-Trichlorophenol	196 > 97	25	198 > 97	25
2,4,6-Trichlorophenol	196 > 97	25	198 > 97	25
2,4-Dichloroaniline	161 > 90	15	161 > 125	10
2,4-Dichloronitrobenzene	145 > 109	10	191 > 109	27
2,4-Dichlorophenol	162 > 63	20	164 > 63	20
2,5-Dichloroaniline	161 > 90	15	161 > 125	10
2,5-Dichloronitrobenzene	145 > 109	10	191 > 109	27
2,6-Dichloroaniline	161 > 90	15	161 > 125	10
2-Chloro-3-nitrotoluene	171 > 77	12	171 > 113	10
2-Chloro-4-toluidine	141 > 106	12	141 > 77	30
2-Chloro-6-nitrotoluene	171 > 154	7	154 > 126	7
2-Chloroaniline	127 > 65	15	127 > 100	10
2-Chlorophenol	128 > 64	15	128 > 100	10
2-Fluorobiphenyl [Internal STD]	172 > 151	20		
3,3'-Dichlorobenzidine	252 > 154	25	252 > 127	45
3,4,5-Trichlorophenol	196 > 133	15	198 > 135	10
3,4-Dichloroaniline	161 > 90	15	161 > 125	10
3,4-Dichloronitrobenzene	145 > 109	10	191 > 109	27
3,5-Dichloroaniline	161 > 90	15	161 > 125	10
3,5-Dichloronitrobenzene	145 > 109	10	191 > 109	27
3-Chloroaniline	127 > 65	15	127 > 100	10
3-Chlorophenol	128 > 65	15	128 > 100	5
4-Chloro-2-nitrotoluene	171 > 154	7	154 > 126	7
4-Chloro-3-methylphenol	142 > 107	10	142 > 77	25
4-Chloro-3-nitroltoluene	171 > 77	12	171 > 113	10
4-Chloroaniline	127 > 65	15	127 > 100	10
4-Chlorophenol	128 > 65	15	128 > 100	5

Table 1. Optimized MRM transitions for analytes and internal/recovery standards.

Table 1. (continued)

Compound	MRM 1	CE	MRM 2	CE
Aldrin	263 > 193	25	293 > 186	30
Alpha-chlordane	372.9 > 265.9	20	372.9 > 300.9	7
Alpha-endosulfan	241 > 206	10	241 > 170	20
Alpha-hexachlorocyclohexane	219 > 183	8	181 > 145	10
Anthracene	178 > 152	15	178 > 151	40
Atrazine	200 > 122	10	200 > 94	15
Azinphos-ethyl	160 > 132	5	160 > 77	15
Azinphos-methyl	160 > 132	5	160 > 77	15
Bentazone	198 > 119	10	198 > 92	25
Benzidine	184 > 156	18	184 > 139	32
Benzo[a]pyrene	252 > 250	30	252 > 224	47
Benzo[b]fluoranthene	252 > 250	30	252 > 224	47
Benzo[ghi]perylene	276 > 274	40	276 > 272	55
Benzo[k]fluoranthene	252 > 250	30	252 > 224	47
Beta endosulfan	241 > 206	10	241 > 170	20
Beta-hexachlorocyclohexane	219 > 183	8	181 > 145	10
Biphenyl	154 > 152	20	154 > 102	30
Coumaphos	362 > 109	15	362 > 334	5
Cumene	120 > 105	7	120 > 77	25
d10-anthracene [recovery STD]	188.1 > 160	20		
d5-nitrobenzene [Internal STD]	128 > 82	10		
Delta-hexachlorocyclohexane	219 > 183	8	181 > 145	10
Demeton-O	171 > 115	10	171 > 143	5
Demton-S-methyl	142 > 112	6	230 > 88	6
Dibenz(a,h)anthracene	278 > 276	40	278 > 274	55
Dichlorvos	185 > 93	10	220 > 185	5
Dieldrin	344.9 > 263	15	279 > 243	10
Dimethoate	229 > 87	7	229 > 86	20
Disulfoton	274 > 88	5	186 > 142	5
Endrin	263 > 193	30	263 > 191	30
Fenitrothion	277 > 109	15	277 > 127	15
Fenthion	278 > 109	15	278 > 79	30
Fluoranthene	202 > 200	30	202 > 150	45
Gamma-chlordane	372.9 > 265.9	20	372.9 > 300.9	10
Heptachlor	272 > 237	10	272 > 142.9	30
Hexachlorobenzene	283.8 > 248.9	15	285.8 > 213.8	25

Table 1. (continued) Optimized MRM transitions for analytes and internal/recovery standards.

Table 1. (continued)

Compound	MRM 1	CE	MRM 2	CE
Hexachlorobutadiene	225 > 190	13	260 > 225	10
Indeno(1,2,3-cd)pyrene	276 > 274	40	276 > 272	55
Isodrin	193 > 123	25	263 > 193	25
Lindane	219 > 183	8	181 > 145	10
Linuron	248 > 61	10	250 > 61	8
Malathion	173 > 99	10	173 > 127	5
Mevinphos(E)	192 > 127	10	192 > 164	5
mevinphos(Z)	192 > 127	10	192 > 164	5
Monolinuron	126 > 99	10	214 > 61	10
Naphthalene	128 > 102	15	128 > 78	15
o,o'-DDE	246 > 176	21	318 > 248	18
o,o'-DDE	246 > 176	21	318 > 248	18
o,p-DDD	235 > 165	20	237 > 165	20
o,p'-DDT	235 > 165	20	237 > 165	20
Omethoate	156 > 110	7	156 > 79	20
p,p'-DDD	235 > 165	20	237 > 165	20
p,p'-DDE	246 > 176	21	318 > 248	18
p,p'-DDT	235 > 165	20	237 > 165	20
Parathion-ethyl	291 > 109	12	291 > 81	35
Parathion-methyl	263 > 109	10	263 > 127	10
PCB#101	325.9 > 255.9	25	327.9 > 255.9	25
PCB#118	325.9 > 255.9	25	327.9 > 255.9	25
PCB#126	325.9 > 255.9	25	327.9 > 255.9	25
PCB#138	359.8 > 289.9	25	361.8 > 289.9	25
PCB#153	359.8 > 289.9	25	361.8 > 289.9	25
PCB#169	359.8 > 289.9	25	361.8 > 289.9	25
PCB#180	393.8 > 323.9	22	395.8 > 323.9	22
PCB#28	256 > 186	15	258 > 186	15
PCB#52	289.9 > 220	23	291.9 > 220	23
PCB#77	289.9 > 220	23	291.9 > 220	23
Pentachlorophenol	265.8 > 166.9	20	267.8 > 166.9	20
Phenanthrene	178 > 152	15	178 > 151	40
Propanil	217 > 161	10	161 > 126	15
p-Terphenyl-d14 [Internal STD]	244.1 > 226	20		
Pyrazon	221 > 77	15	221 > 105	10
Simazine	201 > 173	6	201 > 138	10

Table 1. (continued) Optimized MRM transitions for analytes and internal/recovery standards.

Table 1. (continued)

Compound	MRM 1	CE	MRM 2	CE
Tetrachloronaphthalene	265.9 > 196	25	265.9 > 194	25
Triazophos	257 > 162	7	257 > 119	22
Tributyl Phosphate	155 > 99	5	211 > 99	10
Trifluralin	306 > 264	10	306 > 160	20

Table 1. (continued) Optimized MRM transitions for analytes and internal/recovery standards.

The 0.5 µg/L spiked water samples were analyzed and quantified to determine the specific recoveries for >100 compounds using the single SPE sorbent, with a single extraction procedure. Table 2 summarizes the recoveries achieved for the compounds, using both elution methods (A. 2.5 mL DCM/ACN [4:1], 5 mL DCM; B. 5 mL DCM), showing the percentage of compounds that fit within each recovery range.

Recovery range	70-120%	50-70%	<50%	>120%
Elution A	36%	27%	14%	24%
Elution B	72%	8%	13%	7%

Table 2. Summary of extraction recoveries, expressed as percentage of total number of compounds within each range. Based upon average of five replicates.

Elution method B was found to give the best overall performance with 72% of compounds recovered within the range 70-120%. The compounds recovered <50% included compounds such as disulfoton, which undergoes rapid degradation[5] in aqueous solution.

Other compounds within this range were the benzidines and bentazone, compounds which are either more suitable for LC/MS/MS determination, or require derivatization prior to GC based analysis. Elution method B also gave poorer recoveries for 4-chloroaniline and 3,4,5-trichlorophenol (average recoveries; n=10; 32%, 14% respectively).

However, the use of elution method A resulted in a number of difficulties, with degradation of chromatographic performance due to residual ACN in the extracts, and drastically reduced recovery of lower boiling compounds, such as cumene and hexachlorobutadiene. As a result, elution method B was adopted for the final method. The chart shown in figure 5 depicts the average recoveries (based upon 5 replicates) for all of the compounds analyzed. Some of the recoveries >100% can be explained by reduced internal standard recoveries given that all blanks were residue free. Overall, the distribution of recoveries for such a wide range of polarities, boiling points, pKa's and water octanol partition coefficients (K_{ow}) using a single SPE sorbent is excellent.

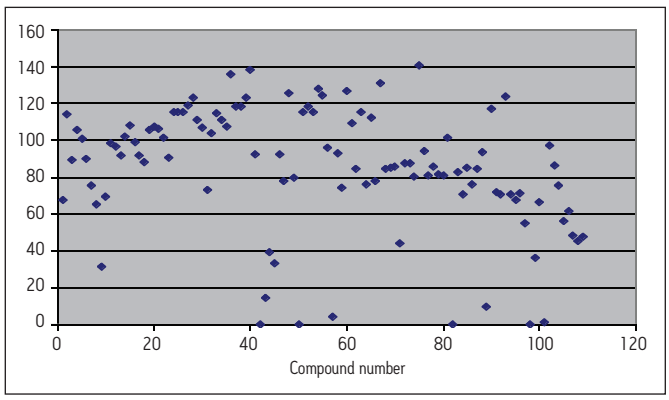

Figure 5. Distribution of average recoveries (n=5) for elution method B (5 mL DCM).

	<0.1 µg/L	>0.1 µg/L	Avg
Instrument LOD confirmatory	96	10	0.03
Instrument LOD screen	100	6	0.01
Method LOD confirmatory	77	29	0.3
Method LOD screen	94	12	0.1

Table 3. Summary of instrumental and method LODs, based upon average of 5 replicates for method LOD calculations, showing number of compounds within each range.

The method LODs were assessed, both for the confirmatory (two MRM transitions per compound) and screen (single MRM transition per compound. All LODs are based upon a signal to noise ratio of 3:1, using the confirmatory transition (where applicable). The instrumental LODs are based upon the lowest concentration standard injection where possible. The method LODs are based upon the average LOD obtained from 5 replicate 0.5 µg/L spiked water samples, extracted using elution method B. Table 3 summarizes the LOD's achieved. Figure 6 gives a graphical representation of the LODs for all compounds determined, showing the distribution of LOD across the complete range of compounds analyzed. The LODs reported are excellent for such a wide range of compounds with a single generic extraction, with many method confirmatory LODs in the low ppt (ng/L) range.

The overall linearity of the method is excellent with >95% of the compounds having coefficients of determination (r^2) >0.99. Coupled with this is the excellent agreement of detected ion ratios, compared with theoretical ratios. Figure 7 shows the chromatograms for both MRM transitions for dichlorvos, detected at a concentration of 0.05 µg/L using the confirmatory method. The chromatograms show excellent signal to noise, and the presence of the compound is confirmed by an actual ion ratio of 2.62 (-3.2%) compared with a theoretical ratio of 2.70.

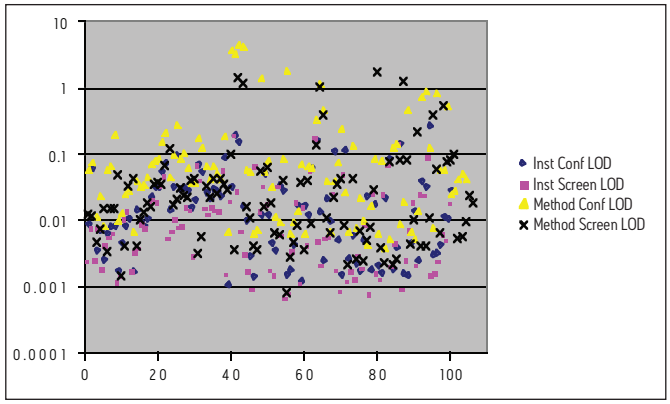

Figure 6. Distribution of instrumental and method LODs for all compounds.

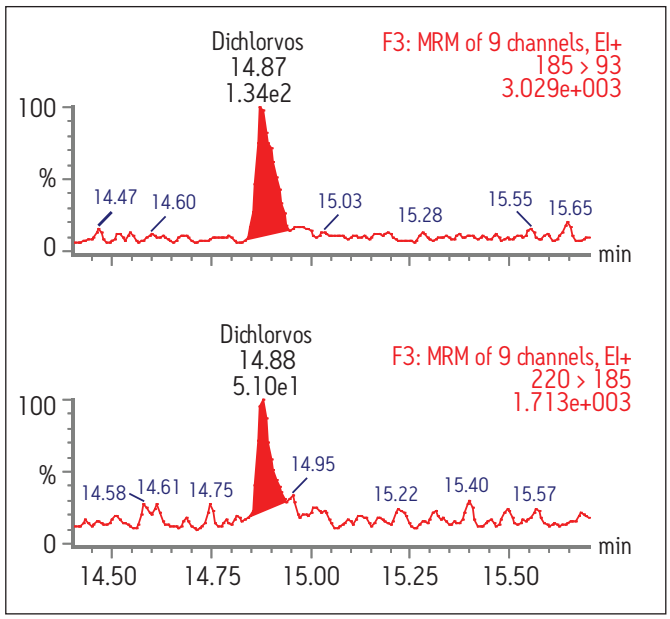

Figure 7. Dichlorvos at a concentration of 0.05 µg/L, demonstrating detection and confirmation at low concentration.

The same concentration acquired using the single MRM transition screening approach is shown in Figure 8, demonstrating the excellent sensitivity that can be achieved. Figure 9 shows the linearity that can be achieved, showing an excellent coefficient of determination (r2) of 0.998 for dichlorvos of the concentration range 0.05 to 5 µg/L.

The reconstructed TIC for a canal water extract is shown in Figure 10, with Figure 11 showing the reconstructed TIC a portion of the same sample spiked at a level of 0.1 µg/L prior to extraction and analysis. No target peaks were detected above the LOD in the unspiked sample.

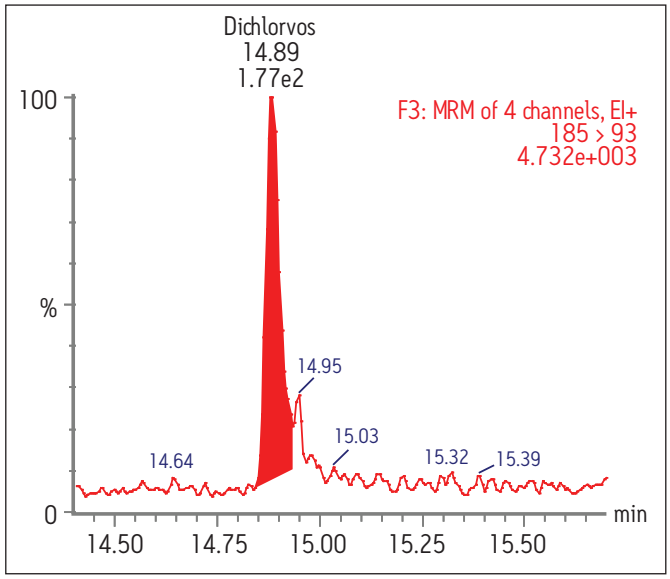

Figure 8. Dichlorvos at a concentration of 0.05 µg/L, demonstrating detection and confirmation at low concentration.

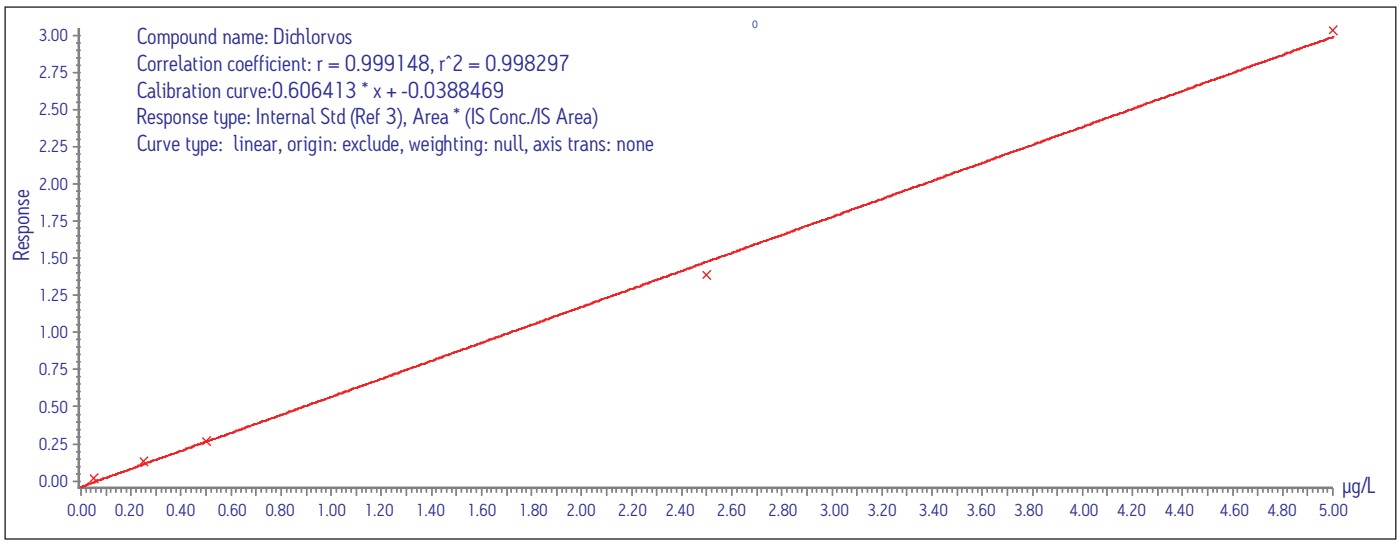

Figure 9. Dichlorvos linearity over the concentration range 0.05 to 5 µg/L.

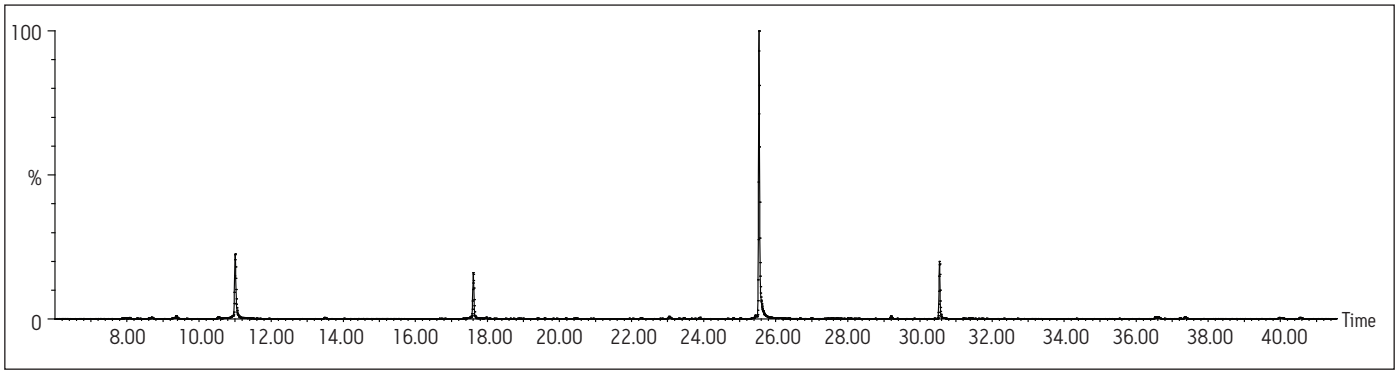

Figure 10. Reconstructed TIC for a canal water extract.

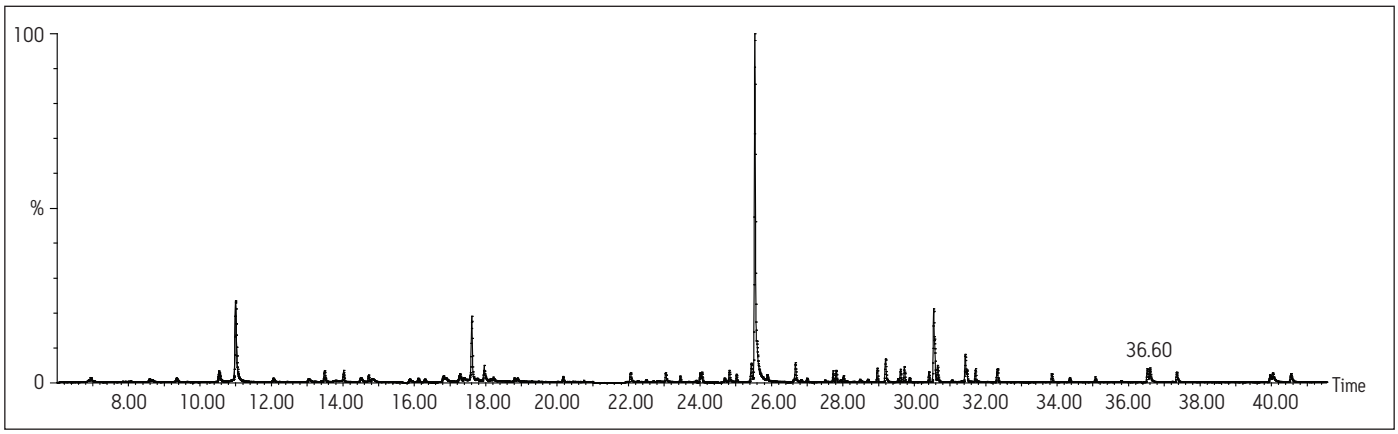

Figure 11. Reconstructed TIC for a 0.1 µg/L spiked canal water extract.

CONCLUSION

The analysis of pollutants in water requires the laboratory to analyze a large number of samples for a wide range of compounds. The analysis can be time consuming requiring the application of a number of different methods for different compound groups. The method described here presents the laboratory with the opportunity to combine a number of these class specific analyses into a single method that can result in the reduction of sample turnaround times. The use of solid phase extraction, combined with GC/MS/MS detection allows the laboratory to achieve much greater confidence in results obtained. Additionally, the laboratory can reduce solvent usage and improve analyte recovery during sample preparation when compared with traditional liquid-liquid techniques.

References

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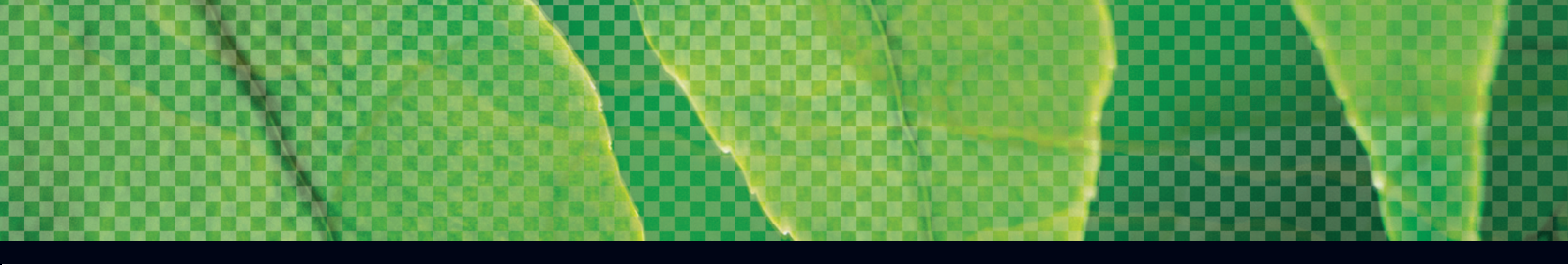

THE DETERMINATION OF PERCHLORATE IN WATER USING LC/MS/MS

THE DETERMINATION OF PERCHLORATE IN WATER USING LC/MS/MS

Jim Krol, Waters Corporation, Milford, MA, USA

INTRODUCTION

Perchlorate is both a naturally occurring and man-made chemical. Naturally occurring perchlorate is a contaminant in fertilizers.

Man-made perchlorate is used in many applications, including tanning and leather finishing, rubber manufacture and paint and enamel production. Perchlorate is also used as an additive in lubricating oils, and is a primary ingredient of solid rocket propellant. Usage and improper disposal of the highly water soluble perchlorate migrates through the underground aquifers and surface water sources, contaminating soil, drinking water and irrigation water.

Thirty-five states have detected perchlorate in drinking water at higher levels than expected. The same water is used for crop irrigation. As a result, there is concern for the food safety of fruits, vegetables and grain, and their use as animal feed in the southwestern U.S.

Today, Maryland, Massachusetts and New Mexico have established a one part per billion (ppb) perchlorate action limit. California and Texas have established 4 ppb. Action limits are higher in Nevada and Arizona. In February 2005, the EPA established an official reference dose (RfD) of 0.0007 mg/kg per day of perchlorate. However, the creation of sub-ppb detection limits is desirable.

METHOD

US EPA method 314.1, "The Determination of Perchlorate in Drinking Water Using Ion Chromatography," 2004, is the current approved method for detecting perchlorate in drinking water. This ion chromatography method is capable of detecting sub-ppb ClO_4, but is limited by sample ionic strength, mainly chloride and sulfate concentrations, thus adversely limiting detection. Other common anions (thiosulfate, thiocyanate and iodide) elute in the same region. Analyte identification, an alternative, simpler method is required.

Chromatographic Conditions

System:	Alliance® 2695 system with the Waters conductivity detector and Waters Micromass® Quattro micro™ mass spectrometer
Column:	IC-Pak™ Anion HR, 4.6 x 75 mm, 6 µm (Waters part no. WAT026765)
Eluent:	25 mM NH_4HCO_3, pH 10 with NH_4OH in 50% AcCN
Flow rate:	0.5 mL/min
Col temp:	30 °C
BackPress:	<1000 psi
Back cond:	~1600 µS
Inj volume:	100 µL

Mass spectrometer tune conditions:

Ionization	-ESP	LM 1 Resolution:	15.0
Capillary (V):	.58	HM 1 Resolution:	15.0
Cone (V):	40	IonEnergy 1:	0.6
Extractor (V):	3	Entrance (V):	1
RF Lens (V):	0.3	Collision Energy:	30
Source Temp °C:	125	Exit:	1
Desolvation Temp:	400	LM 2 Resolution:	14.0
Cone Gas (L/hr):	50	HM 2 Resolution:	14.0
Desolvation Gas:	500	Ion Energy 2:	1.0
Gas Cell Pressure:	2 x 10-2 mbar	Multiplier:	650

The key to this application is the resolution of <0.5 ppb perchlorate from mid-ppm levels of chloride and sulfate. Sulfate is the main interferent with method 314.1, and the ^{34}S isotope as $H^{34}SO_4^-$, at 4.2% abundance, has the same MS/MS transition as perchlorate. It is critical to remove SO_4 or provide baseline resolution from perchlorate.

By combining chromatographic selectivity and mass spectrometry selectivity and sensitivity, perchlorate can be determined in High Total Dissolved Solids (HTDS) water at the sub-ppb levels.

HTDS is a synthetic solution defined as 1000 mg/L each of bicarbonate, chloride and sulfate prepared in drinking water.

Using increased concentrations of organic solvent, acetonitrile (AcCN), in this case because of low back pressure, perchlorate can be chromatographically positioned after high chloride and before high sulfate. Use of ammonium bicarbonate eluent allows for the sub-ppb determination of perchlorate within 15 minutes without the need for sample prep. (See Figure 1.)

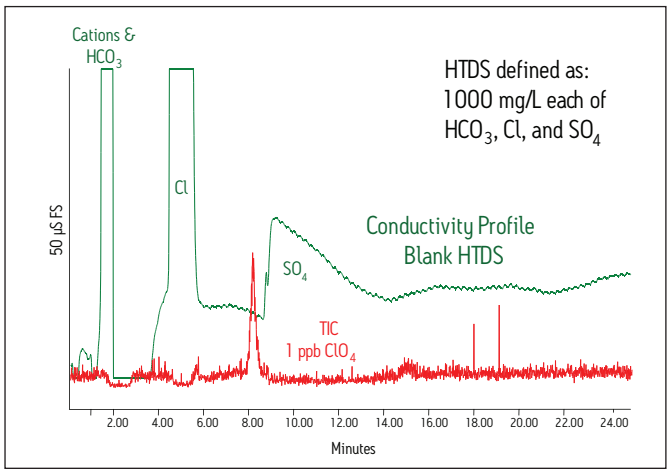

Figure 1. 1 ppb perchlorate in HTDS.

Ammonium bicarbonate is also a volatile buffer conducive for use with mass spectrometry. Any sodium (Na) or potassium (K) based eluent requires chemical suppression prior to MS. Chemical suppression converts KOH to H_2O. Chemical suppression is not needed for this application.

To quantitatively measure sub-ppb perchlorate requires a method detection limit of less than 0.5 ppb perchlorate, using either 3x signal to noise (S/N) or EPA protocols. Figure 2 shows a 0.5 ppb perchlorate standard prepared in reagent water, drinking water and HTDS solutions. Detection limit is approximately <0.1 ppb, defined as 3x S/N, and limit of quantification is 0.2 ppb, at 10 times S/N.

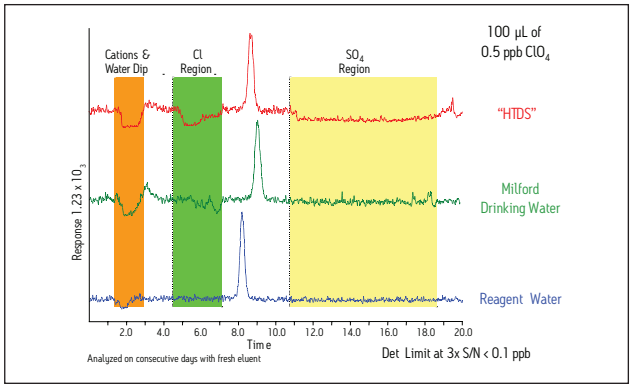

Figure 2. 0.5 ppb perchlorate detection in three different sample matrices by LC/MS/MS.

This LC/MS/MS method meets the requirements. Lower detection may be achieved by using a larger injection volume.

The use of multiple reaction monitoring (MRM) mass spectrometry to monitor the transition of perchlorate to chlorate, 99.1>82.7 (loss of 1 oxygen) is used for perchlorate quantification. Positive perchlorate confirmation is achieved using a second transition of 101.1>84.7 for the ^{37}Cl isotope and the ion ratio of the ^{35}Cl and ^{37}Cl isotopes. Although $H^{34}SO_4^-$ sulfate may give a 99>83 response, it will not yield the required Cl isotope ratio (See Figure 3).

The linearity of this method was evaluated between 0.25 and 100 ppb perchlorate containing 10 ppb internal standard using a 100 µL injection. The calibration curve, using 1/x weighting, Figure 4 shows good linearity between 0.25 and 10 ppb. Similar data is observed with calibration standards prepared in HTDS solution and drinking water.

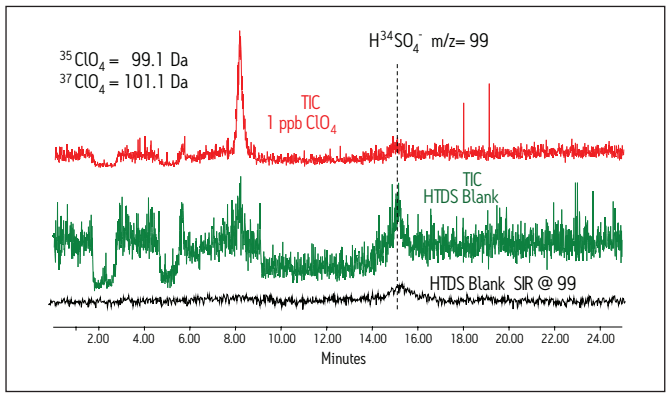

Figure 3. Sulfate interference.

Figure 5 shows the LOD and LOQ for perchlorate quantification. LOD = 0.02 ppb, LOQ = 0.05 ppb by EPA definition of method detection limit (MDL).

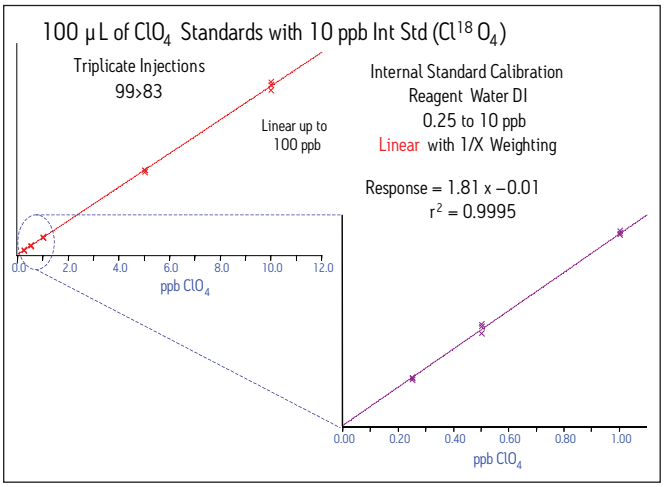

Figure 4. Perchlorate linearity.

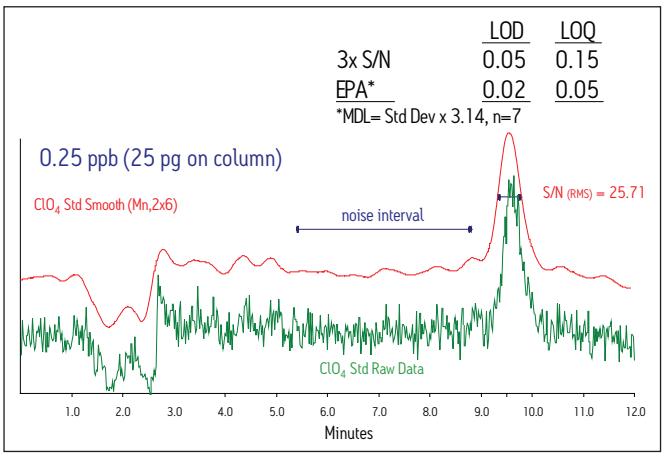

Figure 5. Perchlorate LOD and LOQ after chromatographic smoothing.

CONCLUSION

This work demonstrates the feasibility of using Waters IC-Pak Anion/HR chemistry with the Waters Alliance® HPLC system and the Quattro micro™ API mass spectrometer for the selective analysis of perchlorate in environmental waters.

Chromatographic selectivity of the polymethacrylate-based IC-Pak A/HR column using ammonium bicarbonate and AcCN, eliminates the interference from sulfate and the need for sample prep to remove chloride and sulfate.

LC/MS/MS is capable of detecting <100 ppt in a high totally dissolved solids water, such as 0.3% brackish water or soils leachate, within 15 minutes injection to injection.

The 50% AcCN eliminates any build-up neutral TOC effecting column performance, normally observed with all aqueous eluents.

This chromatographic method has not been fully validated and represents feasibility. It is the responsibility of the user of this method to provide an initial demonstration of performance on the expected matrices before quantitative work can begin. However, draft EPA method 331.0 (2005) has validated the MS/MS acquisition and processing for perchlorate in drinking and waste water using a similar column and eluent.

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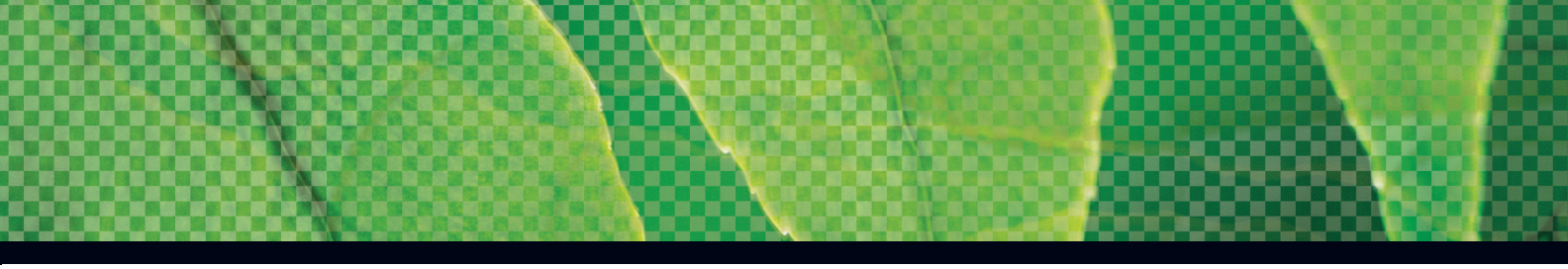

THE SCIENCE OF ACQUITY UPLC APPLIED TO ENVIRONMENTAL ANALYSIS OF PAH'S AND EXPLOSIVES IN WATER

THE SCIENCE OF ACQUITY UPLC APPLIED TO ENVIRONMENTAL ANALYSES OF PAHs AND EXPLOSIVES IN WATER

Mark E. Benvenuti, Waters Corporation, Milford, MA, USA

INTRODUCTION

Polynuclear Aromatic Hydrocarbons (PAHs) and explosive residues are ubiquitous contaminants in our environment; the former as a result of many industrial processes, the latter due to military activity. Standard and official methods for the analysis of PAHs and explosives are found in compendia for air, water, solid waste and food analysis[1]. These methods specify HPLC with run times in excess of 30 minutes. The Waters® ACQUITY UltraPerformance LC® (UPLC®) system can perform these same analyses in less than 10 minutes, a reduction of over 60 percent. Additionally, the lower flow rates of the ACQUITY UPLC system decrease the amounts of solvent consumed and waste generated, providing a cost savings for the laboratory.

APPLICATIONS

Polynuclear aromatic hydrocarbons

The analysis of PAHs is a high priority environmental application. Using HPLC, injection-to-injection cycle times exceeding 45 minutes are common, resulting in the completion of only 10 to 11 analyses over the course of a typical eight-hour working shift. Figure 1 illustrates the UPLC separation of 21 PAH analytes with a run time of only 7 minutes. The shorter run time allows for the analysis of over 50 samples in the same eight-hour shift. Higher sensitivity and superior peak shape result in more accurate quantitation.

Explosives residues

Explosives residues in soil or water are of both forensic and environmental concern. Military sites around the world have produced, stockpiled, expended and disposed of explosives for many years. These munitions contain nitroaromatic and nitramine compounds, which can pose a significant human health risk.

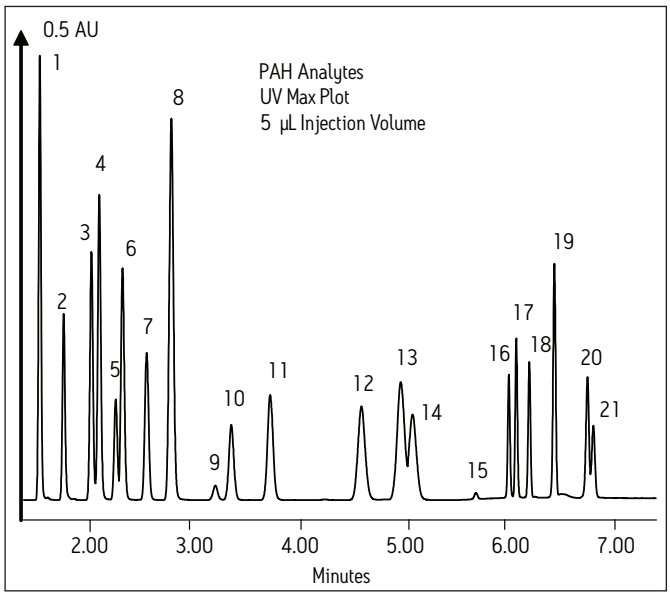

Figure 1. PAH analysis using an ACQUITY UPLC column, 2.1 x 100 mm, 1.7 micron BEH C18. A water:acetonitrile gradient from 66 to 90% acetonitrile at a flow rate of 0.4 mL/min was used. Detection was UV Maxplot mode. Sample was a 5 µL injection. 10 parts per million (ppm) analyte mixture as follows:

1: *Naphthalene*
2: *Acenaphthalene*
3: *1- Methylnaphthalene*
4: *2-methyl-naphthalene*
5: *Fluorene*
6: *Acenaphthene*
7: *Phenanthrene*
8: *Anthracene*
9: *Decafluorobiphenyl*
10: *Fluoranthene*
11: *Pyrene*
12: *p-Terphenal-d-14*
13: *Chrysene*
14: *Benzo(a)anthracene*
15: *Decachlorobiphenyl*
16: *Benzo(b)fluoranthene*
17: *Benzo(k)fluoranthene*
18: *Benzo(a)pyrene*
19: *Dibenzo(a,h)anthracene*
20: *Indeno(1,2,3-cd)pyrene*
21: *Benzo(g,h,i)perylene.*

HPLC-based assays of these compounds have proven to be challenging due to the selectivity needed to resolve positional isomers. In addition, HPLC methods (e.g. IPA in water) require viscous buffered mobile phases operated at high temperatures (40 °C) and analysis times exceeding 30 minutes.[2]

Figure 2 shows the separation of a complex mixture of explosive compounds and its degradates using the ACQUITY UPLC system. The analysis is completed in less than 7 minutes with a much simpler, more robust water:methanol mobile phase.

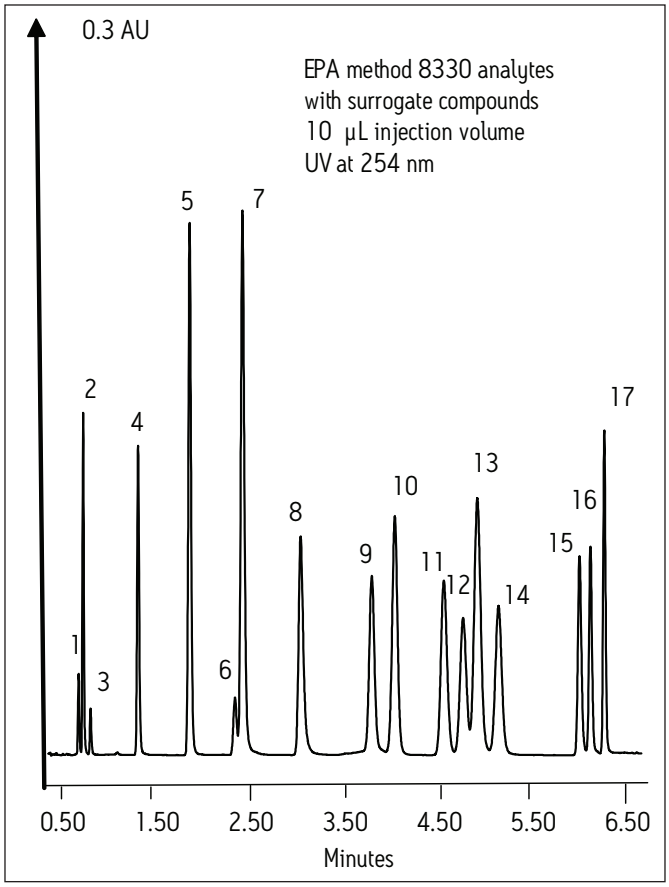

Figure 2. Explosives analysis using the ACQUITY UPLC column, 2.1 x 100 mm, 1.7 micron BEH C_{18}. A water:methanol gradient from 31 to 60% methanol at a flow rate of 0.5 mL/min was used. Detection was UV @ 254 nm. Sample was a 5 µL injection 10 ppm analyte mixture as follows:

*1: 2,6 Diamino - 4 nitrotoluene**
2: HMX
*3: 2,4 Diamino - 6 nitrotoluene**
4: RDX
5: 1,3,5 - Trinitrotoluene
*6: 1,2 - Dinitrobenzene**
7: 1,3 - Dinitrobenzene
8: Nitrobenzene
9: Tetryl
10: 2,4,6 - Trinitrotoluene
11: 2 - Amino - 4,6 - Dinitrotoluene
12: 4 - Amino - 2,6 - Dinitrotoluene
13: 2,4 - Dinitrotoluene
14: 2,6 - Dinitrotoluene
15: 2 - Nitrotoluene
16: 4 - Nitrotoluene
17: 3 - Nitrotoluene

** Surrogate compounds.*

CONCLUSION

The ACQUITY UPLC system significantly decreases run times while improving selectivity and sensitivity in the analysis of PAHs and explosives. At a time when scientists have reached barriers pushing the limits of conventional HPLC, UPLC provides the technology to extend and expand the utility of separation sciences.

References

1. AOAC 973.30; Deutsche DIN TVO; UK ISBN 0 11 & 752032 2; U.S EPA Methods TO-13, 550 & 550.1, 610, 8310 & 8330.
2. Oasis® Applications Notebook, page 82, Waters Corporation.

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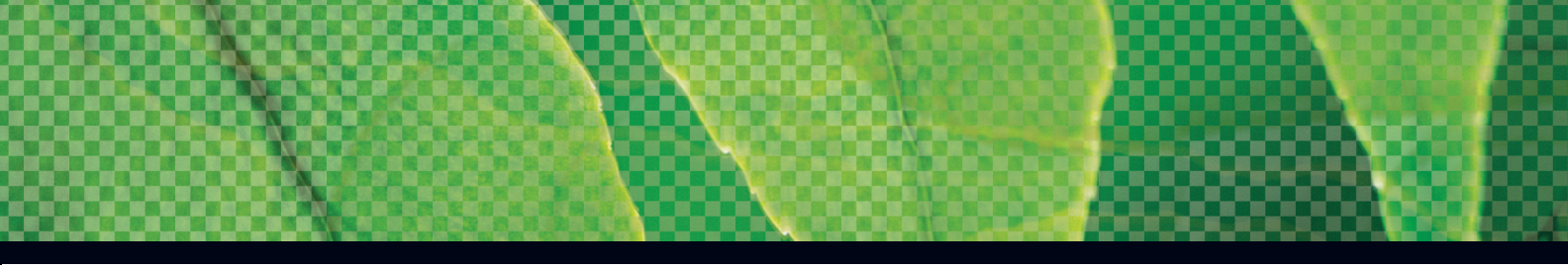

FAST ANALYSIS OF ALDEHYDES AND KETONES BY ACQUITY UPLC

FAST ANALYSIS OF ALDEHYDES AND KETONES BY ACQUITY UPLC

Mark E. Benvenuti
Waters Corporation, Milford, MA, USA

INTRODUCTION

Aldehydes and ketones are products of combustion that permeate the environment. A number of these compounds are known carcinogens and as a result, several US EPA and state methods have been developed for their analysis. These methods, US EPA TO5 (air), 554 (drinking water), 8315 A, Options 1 and 2, (waste water, soil, and air), along with California method 1004 (carbonyl compounds as alcohol oxidants in auto exhaust), describe the derivatization of these compounds using Dinitrophenylhydrazine (DNPH) followed by HPLC separation and ultraviolet (UV) detection at 360 nm. Target analytes vary slightly by method. Present methodologies describe run times of over 40 minutes and multiple column setups to achieve acceptable analyte resolution.

This application note demonstrates that with Waters® ACQUITY UPLC®, analysis times can be reduced by as much as 75 percent, thereby increasing throughput and productivity in the lab. For example, in a typical 8-hour day, one could run 12 40-minute, traditional HPLC analyses. Using the ACQUITY UPLC, this becomes 48 ten-minute analyses or 36 more runs per day.

In addition to the increased throughput, excellent chromatographic resolution is achieved for the indoor air method (particularly for acetone, acrolein and propanal), and the auto exhaust method, particularly for methacrolein and methyl ethyl ketone). For air sampling, Waters offers DNPH coated cartridges including Sep-Pak® DNPH-Silica (WAT 037500) for outdoor air and XPoSure™ Sampler (WAT 047205) for indoor air. To eliminate ozone interferences, Scrubber cartridges are also available from Waters (WAT 054220).

ACQUITY UPLC system.

METHOD

Chromatographic conditions:

System:	Waters ACQUITY UPLC
Injection mode:	Full loop
Loop size:	5 µL (5 µL injection volume)
	Use 15 µL needle
Weak wash:	5% acqueous acetonitrile - 800 µL
Strong wash:	50% acqueous acetonitrile - 500 µL
Sample temp.:	25 °C
Detection:	UV @ 360 nm

Allow 2 minute equilibration between injections.

Column:	Waters ACQUITY UPLC BEH Phenyl
	2.1 x 100 mm, 1.7 µm @ 35 °C
Eluent:	A - 90:10 water - THF (stabilized)*
	B - acetonitrile

*Mix 900 mL water and 100 mL stabilized tetrahydrofuran (THF), filter and degas.

Flow rate:	0.5 mL/min

Software

Data were acquired and processed with Waters Empower™ chromatography software.

RESULTS AND DISCUSSION

The chromatographic gradient profiles are unique per the method used due to the differences in the selectivity of target analytes. EPA method 554 (drinking water), and 8315 A Option 1 (collected by method 0011), target the same 12 analytes, including the strongly retained C5-C10 compounds (pentanal-decanal). The gradient profile (Table 1) and the UPLC chromatogram (Figure 1) are shown.

	Time	Flow	%A	%B	Curve
1	Initial	0.5	70.0	30.0	Initial
2	9.0	0.5	36.0	64.0	6
3	9.5	0.5	70.0	30.0	11

Table 1. Gradient profile for EPA method 554.

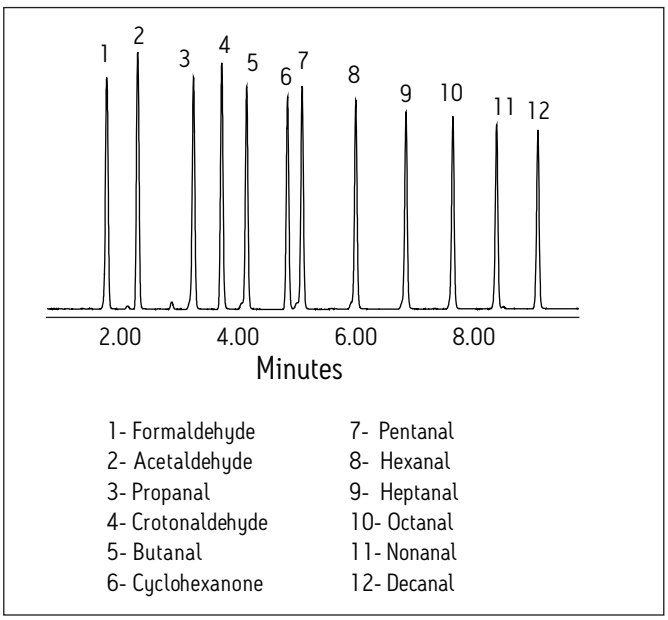

Figure 1. EPA method 554,8315 A-Opt. 1 analytes 20 ppm as DNPH derivatives.

EPA method 8315 A Option 2 targets 15 analytes including the difficult baseline triad of acetone, acrolein and proponal, along with the three tolualdehye isomers (o, p, m). This is an indoor air method using collection protocol 0100. The gradient profile (table 2) and UPLC chromatogram (Figure 2) are shown.

	Time	Flow	%A	%B	Curve
1	Initial	0.5	70.0	30.0	Initial
2	6.5	0.5	53.0	47.0	6
3	9.5	0.5	70.0	30.0	11

Table 2. Gradient profile for EPA method 8315.

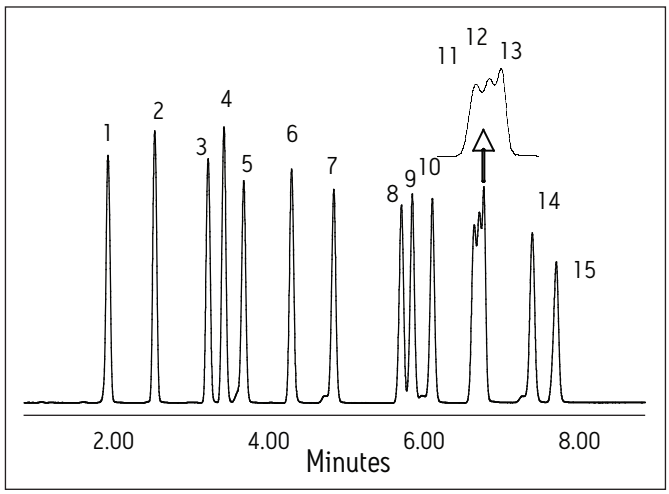

Figure 2. EPA method 8315 A-Opt. 2 analytes 20 ppm as DNPH derivatives.

California method 1004 targets 14 analytes contained in the alcohol oxidation products emitted from auto engines exhausts. Here, the critical pair is methyl ethyl ketone and methacrolein. The gradient profile (Table 3) and UPLC chromatogram (Figure 3) are shown.

	Time	Flow	%A	%B	Curve
1	Initial	0.5	70.0	30.0	Initial
2	8.0	0.5	70.0	30.0	6
3	9.0	0.5	50.0	50.0	6
4	11.0	0.5	70.0	30.0	11

Table 3. Gradient profile for California method 1004.

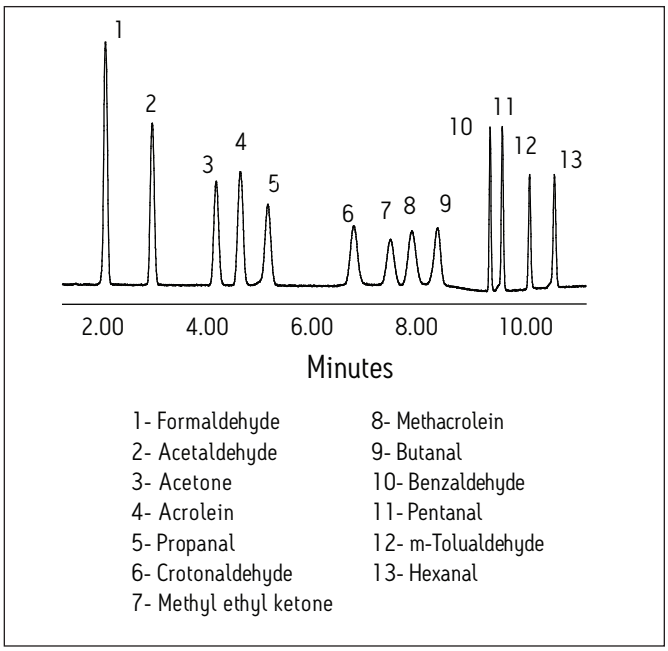

Figure 3. California method 1004 analytes, 0.75 ppm as parent compounds.

As in any separation, retention time and area reproducibility are essential. Figure 4 is an overlay of 5 injections of the EPA 554 standard. As demonstrated in Table 4, RT and area reproducibility are less than 0.2% and 0.5% for all analytes respectively.

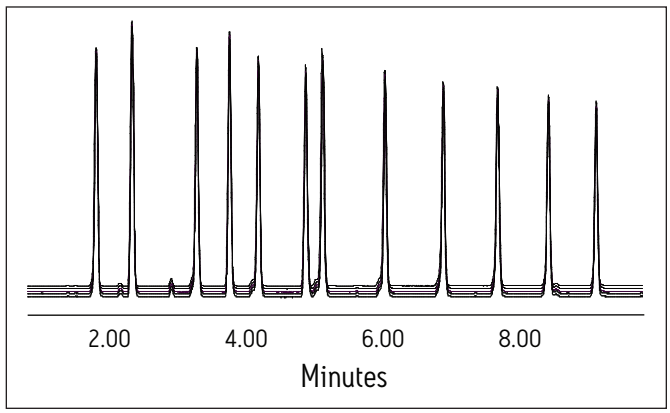

Figure 4. Overlay of 5 injections EPA 554 analytes.

Analyte	% RSD RT	% RSD Area
Formaldehyde	0.163	0.398
Acetaldehyde	0.119	0.408
Propanal	0.078	0.410
Crotonaldehyde	0.071	0.377
Butanal	0.061	0.336
Cyclohexanone	0.053	0.308
Pentanal	0.103	0.471
Hexanal	0.075	0.296
Heptanal	0.049	0.288
Octanal	0.039	0.267
Nonanal	0.026	0.333
Decanal	0.024	0.338

Table 4. Reproducibility data for 5 injections EPA 554 analytes.

CONCLUSION

Aldehydes and ketones can be analyzed as DNPH derivatives rapidly and efficiency using ACQUITY UPLC. Results can be obtained several times faster than conventional HPLC technologies with corresponding cost savings, which allows more samples to be processed in a given day. By a simple variation of gradient conditions, the separation requirements of the major EPA methods 554 and 8315 A, Options 1 and 2, along with California method 1004 can be achieved.

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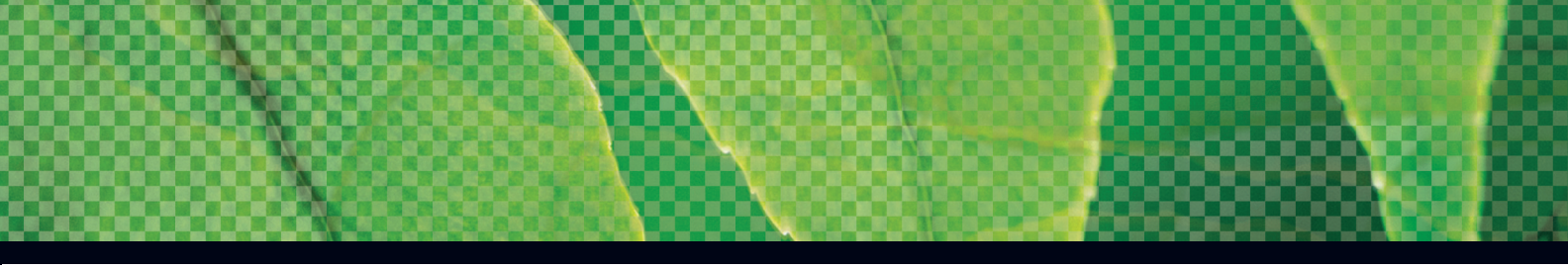

FAST GC/MS/MS ANALYSIS OF POLYAROMATIC HYDROCARBONS (PAH'S) USING WATERS QUATTRO MICRO GC

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FAST GC/MS/MS ANALYSIS OF POLYAROMATIC HYDROCARBONS USING WATERS QUATTRO MICRO GC

Keith Worrall , Waters Corporation, Manchester UK

INTRODUCTION

Fast GC

GC/MS analysis of polyaromatic hydrocarbons (PAHs) is recognized as one of the most sensitive methods for the analysis of these persistent organic pollutants (POPs). PAHs are easily resolved using standard GC columns without a requirement for derivatization. Most separations can be achieved in less than 30 minutes using columns such as 30 m 0.25 mm ID 0.25 µm df 5% phenyl polysiloxane type phases. The use of a narrow bore, thin film column allows an increase in chromatographic resolving power, coupled with a reduction in analysis time.

As the column ID is reduced, the column efficiency increases. For example, a column of 0.25 mm ID has 2,500 plates per meter, whereas a column of 0.1 mm ID has 6,700 plates per meter. The injection volume is critical to avoid overloading of the GC column. Figure 1 shows that injecting half the volume of a standard onto the column can give greater intensity and better chromatography. The figure shows benz(a)anthracene and chrysene acquired under fast GC conditions, using 0.5 µL and 1 µL injection volume onto a 0.18 mm ID GC column.

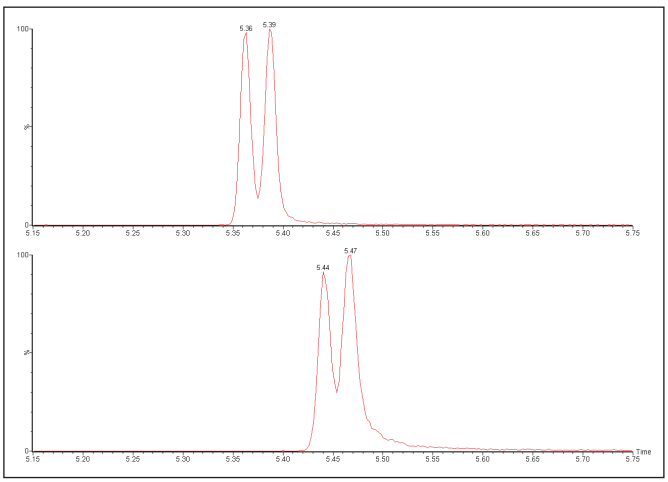

Figure 1. Separation and intensity for 0.5 µL injection (top trace) and 1 µL injection (bottom trace) for benz(a)anthracene and chrysene under fast GC conditions.

INSTRUMENTAL

Fast GC/MS/MS

The column used for this analysis was a DB5-ms 20 m 0.18 mm ID 0.18 µm df column with a constant He flow rate of 0.7 ml min^{-1}, installed in an Agilent 6890 GC oven, directly interfaced to a Waters® Quattro micro GC™ mass spectrometer operated in EI+ mode. The GC oven was fitted with a fast GC oven insert, and was configured in the fast oven ramping mode. All injections were made in pulsed splitless mode with an injection volume of 0.5 µL, using a purge time of 0.4 minute, 0.4 min pulse (280 kPa). The GC temperature ramp employed was: - 50 °C/0.4 min, 100 °C min^{-1} to 90 °C, 65 °C min^{-1} to 190 °C, 50 °C min^{-1} to 265 °C, 40 °C min^{-1} to 310 °C, hold 4 minutes. The GC ramp and column combination employed results in peak widths of between 0.9 and 3 seconds, with a run time of less than 9 minutes. All critical pairs were suitably resolved under these conditions. Figure 2 shows the separation for phenanthrene and anthracene (top trace) and benzo(b)fluoranthene, benzo(k)fluoranthene and benzo[a]pyrene (bottom trace).

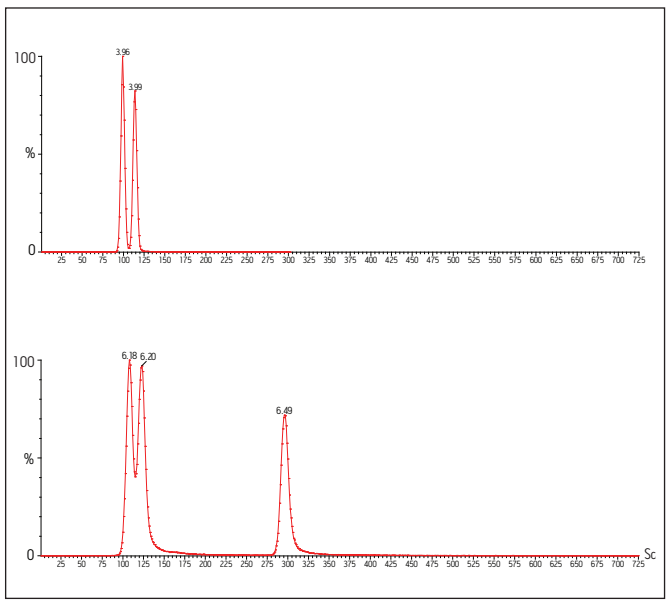

Figure 2. Critical pairs separation showing number of data points (scans) across each peak.

Figure 2 also shows the number of scans across each peak with a minimum of 8 data points per peak. This is possible due to the ability of the Quattro micro GC to acquire with dwell times and interchannel delay times of 10 ms. The top chromatogram shows 12 data points across a GC peak 1.2 seconds wide; while acquiring 3 MRM channels simultaneously. This exceeds the quantitative requirement for a minimum of 8 data points.

All US EPA 16 PAHs were separated and determined with the last eluting peak eluting at 8.21 minutes. Excellent linearity was observed over a concentration range from 0.25 pg to 250 pg on column, with all LODs (based upon peak to peak signal to noise of 3:1) being 0.25 pg or less. Table 1 presents the r^2 values, retention times and LODs for the US EPA 16 PAHs. The linear dynamic range is affected when acquiring fast GC because the loading capacity of the column is exceeded at lower concentrations due to the narrow peak widths. Comparing the LODs determined with those acquired under 'normal' chromatographic conditions, fast GC operation can be seen to give a significant sensitivity improvement. The calibration curves acquired were linear up to 250 pg, but above this concentration, the dynamic range of the column is exceeded when acquiring under these conditions.

The MRM transitions optimized for the PAHs are given in Table 1. All transitions were optimized with a collision cell gas pressure of $3e^{-3}$ mbar (Argon). In some cases, parent to parent MRM transitions are quoted. In these cases, a third transition has been optimized, because parent to parent transitions may not always offer adequate selectivity in the presence of a matrix, especially the hydrocarbon matrix often encountered during PAH analysis.

The comparative sensitivity for injections of a spiked 0.1 μgL^{-1} canal water extract is shown in Figures 3 and 4. Figure 3 shows a 1 µL injection of the sample extract acquired under standard chromatographic conditions. Figure 4 shows a 0.5 µL injection of the same extract acquired using the fast GC conditions described above. It can be seen that the sensitivity has more than doubled, while maintaining good chromatographic resolution.

Name	RT	Coeff. of Determination	LOD (pg)	MRM1	eV	MRM2	eV	MRM3	eV
Naphthalene	2.59	0.999	0.1	128>128	15	128>102	20	128>78	20
Acenaphthene	3.22	0.996	0.2	152>151	20	152>150	25	n/a	n/a
Acenaphthylene	3.29	0.997	0.1	154>153	20	154>152	30	n/a	n/a
Fluorene	3.52	0.100	0.1	166>165	20	166>164	35	n/a	n/a
Phenanthrene	3.97	0.999	0.12	178>151	40	178>152	15	n/a	n/a
d10-Anthracene	3.98	n/a	n/a	188>160	20	n/a	n/a	n/a	n/a
Anthracene	3.99	0.999	0.17	178>151	40	178>152	15	n/a	n/a
Fluoranthene	4.56	0.998	0.1	202>202	20	202>200	35	202>150	45
Pyrene	4.68	0.999	0.1	202>202	20	202>200	35	202>150	45
Benz(a)anthracene	5.36	0.999	0.1	228>226	30	228>228	25	228>202	30
Chrysene	5.39	0.999	0.1	228>226	30	228>228	25	228>202	30
Benzo[b]fluoranthene	6.18	0.999	0.12	252>250	30	252>252	25	252>224	47
Benzo[k]fluoranthene	6.21	0.999	0.2	252>250	30	252>252	25	252>224	47
Benzo[a]pyrene	6.49	0.998	0.2	252>250	30	252>252	25	252>224	47
Indeno(1,2,3-cd)pyrene	7.84	0.997	0.25	276>274	40	276>276	25	276>248	50
Dibenz(a,h)anthracene	7.87	0.996	0.2	278>276	35	278>278	25	278>274	55
Benzo[ghi]perylene	8.21	0.997	0.2	276>274	40	276>276	25	276>248	50

Table 1. Retention time, coefficient of determination, LOD and MRM transitions for each of the PAHs analyzed.

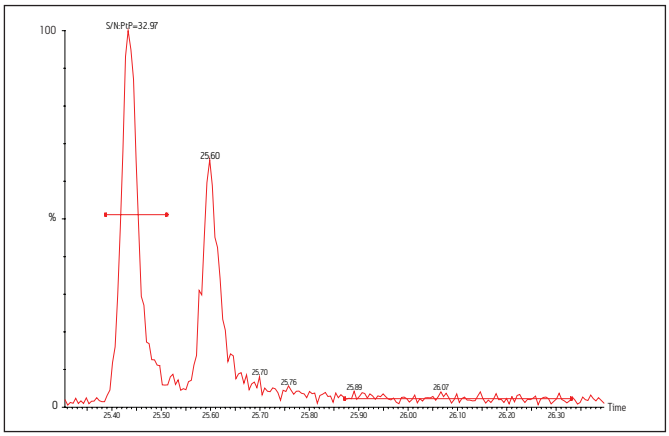

Figure 3. 1 µL injection of 0.1 µgL⁻¹ canal water extract acquired using standard chromatographic conditions.

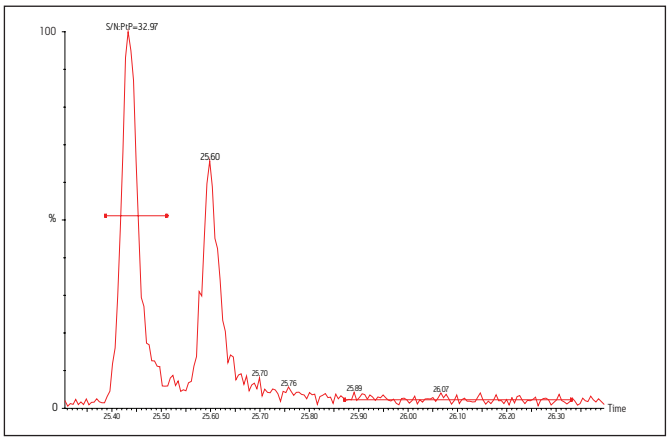

Figure 4. 0.5 µL injection of 0.1 µgL⁻¹ canal water extract acquired using fast GC conditions.

CONCLUSION

This example of polyaromatic hydrocarbon analysis using fast GC chromatographic conditions clearly demonstrates the ability of the Quattro micro GC to acquire data using very short dwell times. All data was acquired using 10 ms dwell and inter-channel delay times when using fast chromatographic conditions, with the exception of the last eluting peaks, where longer dwell times could be utilized due to the broader chromatographic peaks. This is enabled by the ability of Waters MassLynx™ software control to allow flexible dwell times within an acquisition function. This allows the user to maximize the time spent monitoring transitions when necessary. Fast GC conditions can be used to enhance the sensitivity of a method. However, this enhancement comes at the cost of reduced dynamic range due to column loading capacities.

The data presented here shows that it is possible to determine all US EPA 16 PAHs within a 9-minute analytical run time, without compromising chromatographic resolution.

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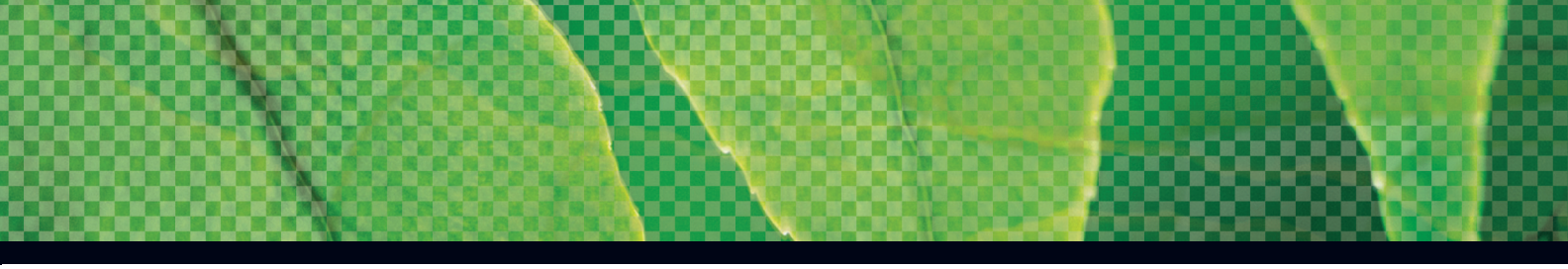

A SENSITIVE METHOD FOR THE DETERMINATION OF ENDOCRINE-DISRUPTING COMPOUNDS IN RIVER WATER BY LC/MS/MS

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A SENSITIVE METHOD FOR THE DETERMINATION OF ENDOCRINE-DISRUPTING COMPOUNDS IN RIVER WATER BY LC/MS/MS

Patricia Revilla-Ruiz[1], Gordon Kearney[2], Daniel McMillan[2], and Encarnación Rodriguez-Gonzalo[1]
[1]Universidad de Salamanca, Salamanca, Spain [2]Waters Corporation, Manchester, UK

INTRODUCTION

Emerging evidence from wildlife and laboratory studies indicates that some chemicals may interfere with the endocrine system. Compounds identified as endocrine-disrupting chemicals (EDCs) include pesticides, polychlorinated biphenyls (PCBs), dioxins, furans, alkylphenols, and steroid hormones (natural and synthetic). The steroid hormones are of special concern due to their potency. The natural sex hormone estradiol and its metabolites (estrone and estriol) and the synthetic steroid ethinylestradiol are excreted in the urine of mammals and can be found in surface and ground waters. Other EDCs, such as the alkylphenols—nonylphenol, bisphenol A- and pentachlorophenol are derived from industrial and domestic activities and also occur in environmental waters. Bisphenol A is used in the production of epoxy resins and polycarbonate plastics which are utilized extensively in the manufacture of food and drink packaging materials. Nonylphenol, which is produced as a derivative of non-ionic surfactants, is used extensively as a plasticizer. Pentachlorophenol is still used in some countries as a heavy-duty wood preservative.

The environmental presence of these compounds highlights the need to develop highly sensitive and specific analytical methods. Liquid chromatography-tandem mass spectrometry (LC/MS/MS) is a technology applicable to a wide range of molecules and matrices, which provides the sensitivity required for trace analysis. Electrospray ionization (ESI) in negative ion (NI) mode is generally the method of choice for determination of estrogens, alkylphenols and chlorophenols[1,2]. These chemicals have been shown in laboratory and field studies to provoke endocrine disruption at sub-ng/L levels. Thus, analyte enrichment is sometimes necessary in order to achieve the required detection limits. For pre-concentration of EDCs from aqueous samples, solid-phase extraction (SPE) is considered to be the most appropriate technique[3]. This application note describes a sensitive method for the simultaneous determination of eight endocrine-disrupting compounds in river water samples based on SPE followed by LC/MS/MS.

Alliance 2690 HPLC and Micromass Quattro micro mass spectrometer.

METHOD

Solid-phase extraction

The following extraction method was used[4]:

Cartridge:	Waters® Oasis® HLB (60 mg)
Conditioning:	3 mL Methyl *t*-butyl ether (MTBE)
	3 mL Methanol (MeOH)
	3 mL ultra-high quality water
Load:	500 mL acidified river water
Sample:	(10 mM formate buffer pH=3.0)
Wash:	3 mL 40% MeOH in UHQ water
	3 mL UHQ water
	3 mL 10% MeOH/2% NH_4OH in water
Elute:	6 mL 10% MeOH/MTBE
Evaporate:	to dryness by gentle stream of nitrogen at 50 °C
Re-dissolution:	500 µL (50:50, v/v, acetonitrile/ ammonium formate buffer pH 3.0)

LC Conditions

Waters Alliance 2690 HPLC system

Mobile Phase A:	Methanol
Mobile Phase B:	Water
Column:	Waters SunFire™ C_{18}, 2.1 x 50 mm with 3.5 µm particle size
Flow rate:	0.2 mL/min
Injection volume:	20 µL

Gradient

Time 0,	60% A; 40% B
Time 10 min,	100% A
Time 18 min,	100% A
Time 20 min,	60% A; 40% B
Time 23 min,	60% A; 40% B

MS Conditions

A Micromass® Quattro micro® triple quadrupole mass spectrometer was operated in the negative ion electrospray mode. Nitrogen gas, at a flow rate of 450 L/hr and a temperature of 250 °C, was used for spray desolvation. The source temperature was maintained at 120 °C and the capillary voltage was 3.2 kV.

The MRM transitions, along with the optimum cone voltage and collision energy for the individual compound, are listed in Table 1. The ion used for quantitative determinations has been highlighted in bold font. In the case of pentachlorophenol, no significant fragmentation could be obtained even at high collision energy, but the full scan acquisition mode revealed that enough structural information is obtained due to the characteristic isotope ratio signals of the halide. Thus, for this compound, a SIR of four of its characteristic ions have been monitored.

Data was acquired using Waters MassLynx™ software and processed using the TargetLynx™ Application Manager.

RESULTS AND DISCUSSION

The nature of all eight compounds studied makes them amenable to analysis by electrospray injection (ESI-) MS/MS. Methanol and acetonitrile, two organic solvents commonly used in reversed-phase LC, were evaluated. The MS signals for all the compounds assayed were higher for methanol than for acetonitrile, probably due to the more favorable ionization properties of the former solvent. Calibration models for the LC/(ESI-) MS/MS method were constructed by injecting standard solutions and obtaining a good linear relationship

Compound	Fw	Precursor ion m/z	Product ions m/z	Corresponding structure of product ions	Cone Voltage (V)	Collision Energy (eV)
Estriol	288.4	287.3	**144.9**; 171.1	**$[M - C_8H_{14}O_2]^-$**; $[M - C_6H_{12}O_2]$	50	40
Bisphenol A	228.3	227.2	**133.0**; 210.8	**$[M - C_6H_6O]^-$**	37	33
17α-Ethinylestradiol	296.4	295.5	**145.1**; 199.5	**$[M - C_{10}H_{14}O]^-$**; $[M - C_6H_9O]^-$	37	49
17α-Estradiol	272.4	271.3	**145.2**; 183.1	**$[M - C_{10}H_{14}O]^-$**; $[M - C_5H_{12}O]^-$	37	45
17β-Estradiol	272.4	271.3	**145.2**; 183.1	**$[M - C_8H_{14}O]^-$**; $[M - C_5H_{12}O]^-$	37	45
Estrone	270.4	269.2	**144.8**; 143	**$[M - C_8H_{12}O]^-$**; $[M - C_8H_{14}O]^-$	37	33
4-n-Nonylphenol	220.4	219.3	**106.0**; 118.7	**$[M - C_8H_{17}]^-$**; $[M - C_7H_{17}]^-$	40	34
Pentachlorophenol	266.3	SIR of **264.9**; 263.0; 267.1; 269.0			40	-

Table 1. MRM parameters.

between the analytical signal and analyte concentration for all compounds. Figure 1 shows a representative calibration curve for estrone generated in the concentration range of 0.5-100 ng/mL. This calibration curve was generated using solvent standards and the concentrations stated refer to the analyte concentration in the sample vial.

Figure 2 shows the chromatograms for the compounds at the lowest calibrated level. The instrument LoDs in solvent standard (S/N ratio = 3 to 1) are estimated to be 0.6 ng/mL for estrone, 0.5 ng/mL for pentachlorophenol, 3 ng/mL for 17α-estradiol, 5 ng/mL for l17β-estradiol, 10 ng/mL for bisphenolA, 7 ng/mL for 4-n-nonylphenol, 5 ng/mL for estriol, and 30 ng/mL for 17α-ethinylestradiol.

In order to obtain a more sensitive method for the quantification of these compounds in river water, a solid-phase extraction step with Oasis HLB cartridges was performed prior to chromatographic determination. A study with different volumes of river water samples was carried out first to evaluate if effects of matrix suppression or breakthrough appear when the volume of water to be pre-concentrated was increased. The results show that the signals were practically independent of the pre-concentrated sample volume. Accordingly, a 500 mL river water sample volume was selected. With the proposed SPE procedure, satisfactory percentage recoveries in river water were obtained ranging from 74% for 4-n-nonylphenol to 105% for 17α-ethinylestradiol.

The whole procedure was applied to river water samples. The analysis of unspiked river water samples revealed that two of the compounds studied, bisphenol A and estrone were present, as can be seen in Figure 3.

To estimate the concentration of these two compounds in the river water sample, a standard addition method was performed. For the standard addition experiments, addition of known amounts of target analytes into the sample at four concentrations levels (1, 2, 4 and 8 ng/L for estrone and 0.25, 5, 10 and 20 ng/L for bisphenol A) was carried out. From the calibration curves obtained, the concentration found in river water is estimated to be 2 ±1 ng/L for estrone and 6 ±1 ng/L for bisphenol A.

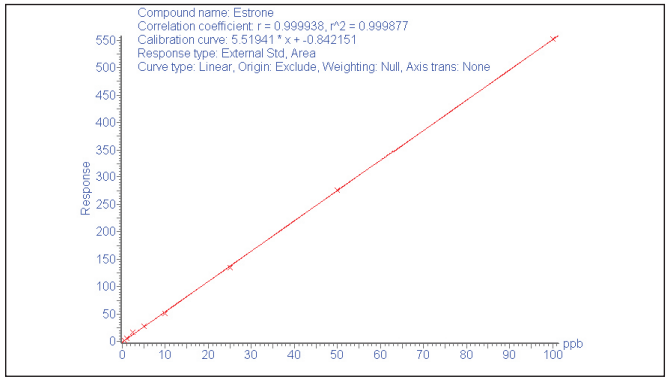

Figure 1. Calibration graph for estrone.

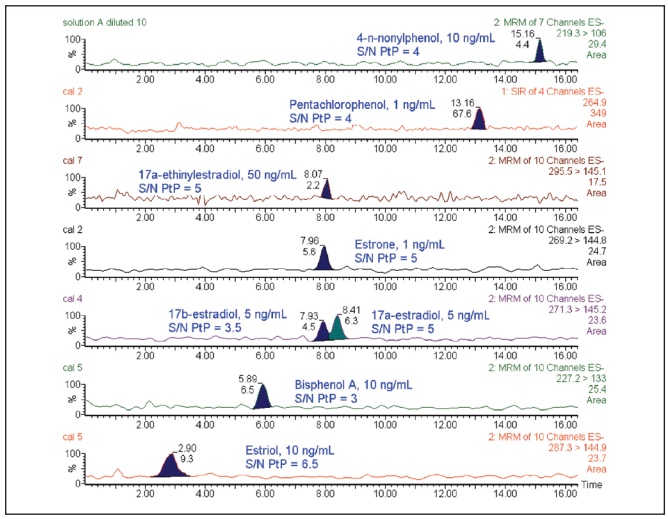

Figure 2. Chromatograms showing peaks close to the instrument LoDs.

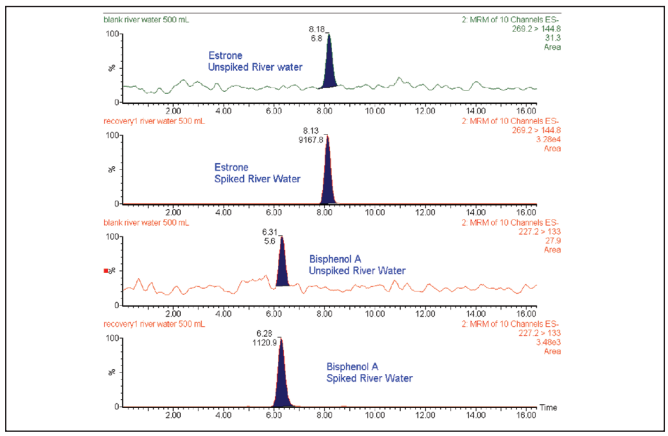

Figure 3. The presence of bisphenol A and estron in unspiked river water samples.

Figure 4 shows the chromatograms obtained after the SPE extraction, for river water samples spiked with the compounds at the lowest concentration level tested (except for bisphenol A and estrone for which chromatograms in unspiked river water are presented). From Figure 4, the method LoDs in river water (S/N ratio = 3 to 1) are estimated to be 1 ng/L for estrone, 0.6 ng/L for pentachlorophenol, 2.5 ng/L for 17α-estradiol, 5 ng/L for 17β-estradiol, 6 ng/L for bisphenol A, 9 ng/L for 4-n-nonylphenol, 12 ng/L for estriol, and 50 ng/L for 17α-ethinylestradiol. These estimates refer to the concentration of analyte spiked into the river water prior to extraction.

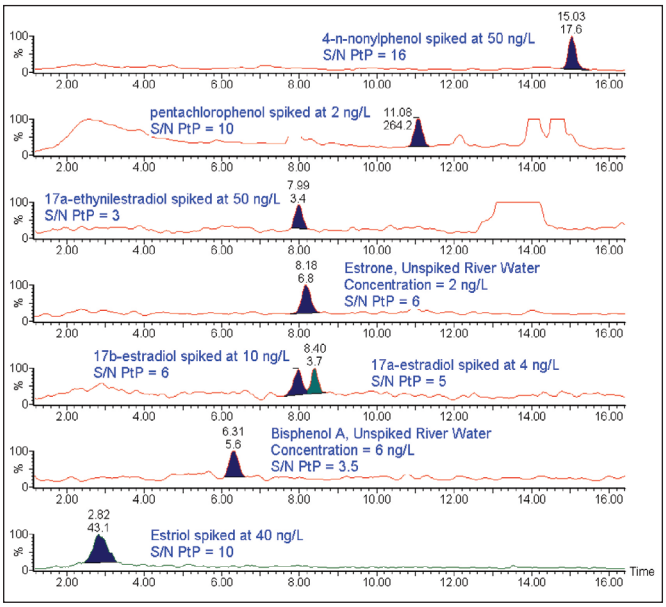

Figure 4. Chromatograms of spiked river water samples.

CONCLUSION

A sensitive and selective LC/MS/MS method has been developed for the determination of eight endocrine-disrupting compounds in river water samples at low-nanogram per liter level. The 1000-fold concentration step in the sample preparation method improves the sensitivity of the analysis by 500 to 1000 fold, depending on analyte. This indicates that the SPE method used gives excellent analyte enrichment and cleanup, with minimal matrix effects.

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2. A. Laganá, A. Bacaloni, I. De Leva, A. Faberi, G. Fago and A. Marino; Anal. Chim. Acta, 501 (2004) 79.
3. M.J. López de Alda and D. Barceló; J. Chromatogr. A, 938 (2001) 145.
4. Oasis Applications Notebook, Page 80, Waters Corporation.

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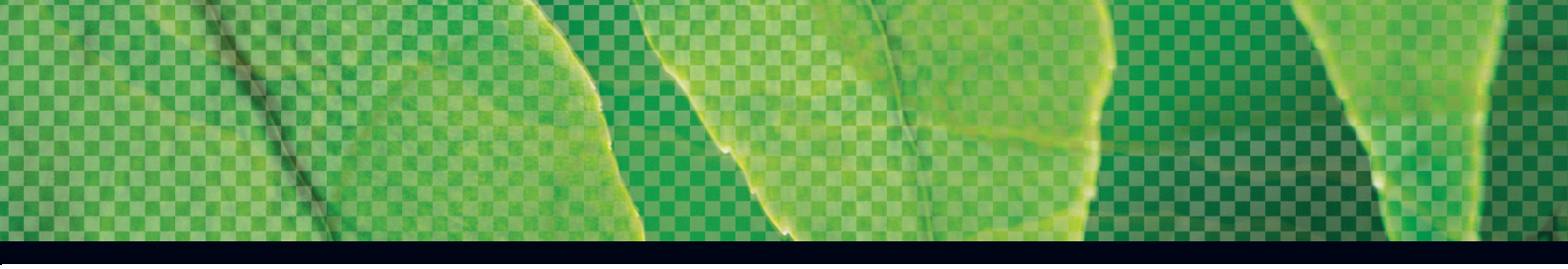

THE ANALYSIS OF DIOXINS AND FURANS USING HRGC-HIGH RESOLUTION MS WITH THE AUTOSPEC-ULTIMA NT

THE ANALYSIS OF DIOXINS AND FURANS USING HRGC-HIGH RESOLUTION MS WITH THE AUTOSPEC-*ULTIMA* NT

Keith Worrall, Anthony Newton and Ramesh Rao, Waters Corporation, Manchester, UK

INTRODUCTION

'Dioxins' refers to a group of chemical compounds that share certain similar chemical structures and biological characteristics. Several hundred of these toxic compounds exist and are members of three closely related families: the polychlorinated dibenzop-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and certain polychlorinated biphenyls (PCBs).

Sometimes the term dioxin is also used to refer to the best studied and one of the most toxic dioxins, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). PCDDs and PCDFs are not created intentionally, but are produced inadvertently by a number of human activities. Natural processes also produce PCDDs and PCDFs.

Over the past decade, regulatory environmental agencies and industries have worked together to dramatically reduce dioxin emissions. Because dioxins are extremely persistent compounds, levels of dioxins still exist in the environment from both man-made and natural sources and will take years to decline.

The detection and quantification of dioxins is a particularly demanding analysis due to the low level of regulatory exposure limits and the variety of complex sample matrices encountered. High Resolution Gas Chromatography (HRGC), coupled with High Resolution Mass Spectrometry (HRMS) offers the high sensitivity, selectivity and quantitative dynamic range for this application and as such, is the technique of choice.

This application note gives examples of the performance of the Waters® Micromass® AutoSpec *Ultima*™ NT and describes QuanLynx™ Application Manager, the most advanced quantification software for dioxin and furan analysis, illustrating why this combination is the market leader in the field.

Regulatory Considerations

Across the world, there are many different legislative methods for dioxin and furan analysis, including US EPA method 1613, European method EN1948, Canadian EPS1/RM/19 and other variants of these.

In the UK and across much of Europe, there is no specific legislative method for the analysis of dioxins and furans. In UK-based laboratories, for example, methods using the extraction and clean-up processes of US EPA method 1613 and the labeled internal standard mixtures of US EPA method 23 are employed for the analysis of all environmental samples other than those obtained by air emission sampling.

The general common factor is the use of labeled internal standards for quantification and determination of recoveries, the use of resolutions in excess of 10,000 resolving power (5% height, 10% valley definition) coupled with a 60 m GC column, either DB5, SP2331 or similar.

While the procedures and results described in this application note are intended to show the performance of the AutoSpec *Ultima* NT for dioxin and furan analysis, this could be as easily applied following any legislative method explicitly.

EXPERIMENTAL

In preparation for the analyses, the AutoSpec Ultima NT was tuned to in excess of 10,000 resolutions at electron energy of 30 eV, before calibration over the mass range for the experiment was acquired. The experiment used was a standard EPA1613 five-function voltage selected ion recording (VSIR) acquisition system. The calibration is performed on a daily basis and by keeping a hardcopy record of the per-fluorokerosene (PFK) peaks during calibration, a permanent record of instrument resolution is maintained which is essential for the level of auditing in a modern accredited laboratory.

First, a single function survey injection was performed to determine the acquisition time windows for the multifunction analysis.

Next, a sample list was set up to include a solvent blank injection of nonane, a CS1 to CS5, five-point calibration using standard EPA1613 standard sets. After the calibration, more nonane solvent blank injections were included, before the sample extract injections were to be performed. The sample list is shown in Figure 1.

The samples were spiked with labeled internal standards similar to those used for US EPA method 23, having one labeled standard for each level of chlorination for each group of congeners i.e. 13C-2,3,7,8-TCDF and 13C-TCDD for the tetra dioxins and tetra furans. 13C-1,2,3,4-TCDD and 13C-1,2,3,7,8,9-HxCDD were added as recovery standards.

The sample list was then started and the data acquired and processed automatically using QuanLynx 4.0.

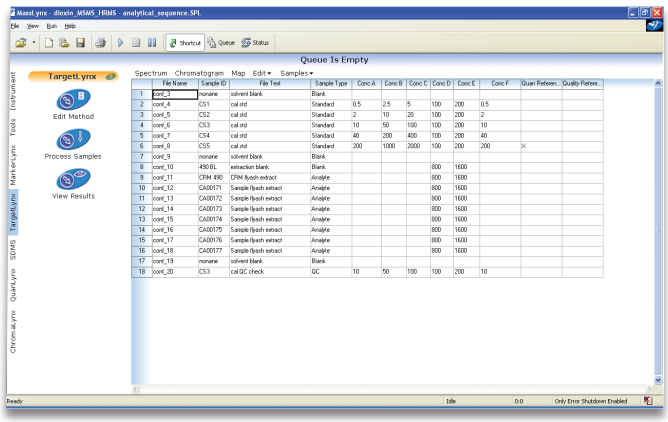

Figure 1. Sample list for dioxin and furan analysis.

RESULTS

Figures 2 and 3 show the calibration curves for 2,3,7,8-TCDD and OCDD illustrating excellent quantitative linearity.

Table 1 shows a summary of the calibration curves. It can be seen that the RRF % relative standard deviations are well within the regulatory limits of <15% (US EPA method 1613 and most European methods) and <10% (Japanese and some other methods).

GC conditions

Column:	J&W DB5-ms, 60 m,0.25 mm ID, 0.25 µm
Flow rate (He):	1 mL/min constant flow
Oven program:	140 °C, hold 4 min, 9 °C/min to 220 °C, 1.4 °C/min to 260 °C, 4 °C/min to 310 °C hold 6 min
Injection volume:	1 µL splitless
Injector temperature:	280 °C
Purge time:	4 mins
Purge flow:	30 mL/min

MS Conditions

Ionization mode:	Electron impact (EI+)
Acquisition mode:	Voltage SIR (quantitative analysis)
Resolution:	10,000 (5% height, 10% valley definition)
Electron energy:	30 eV

All acquisition and data processing was performed using MassLynx™ 4.0 and QuanLynx 4.0 software.

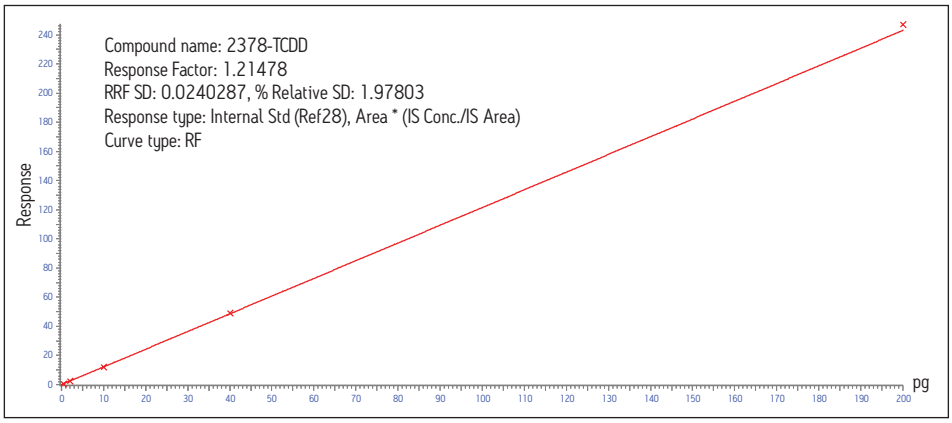

Figure 2. Calibration curve for 2, 3, 7, 8-TCDD.

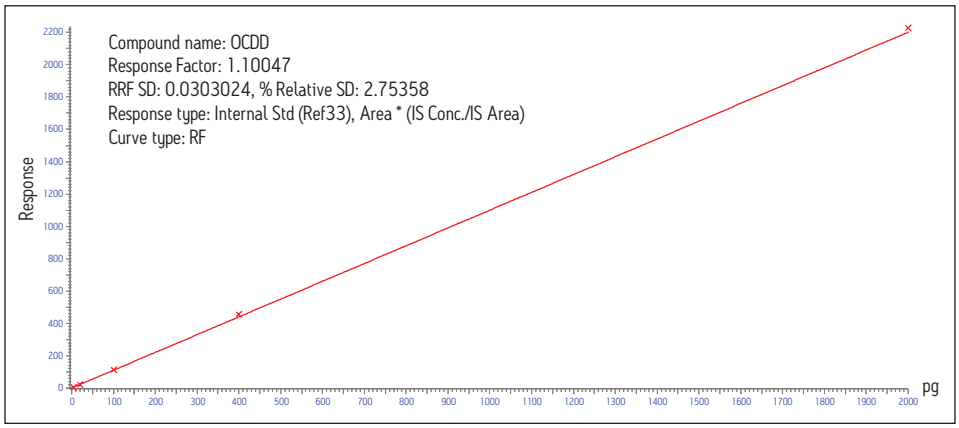

Figure 3. Calibration curve for OCDD.

60m DB5-ms calibration curve summary

Congener	RRF Mean	RRF %Rel SD	LOD (pg)	LOD (pg/g-WHO-TEQ)	WHO-TEF
2378-TCDF	1.017	3.457	0.011	0.00011	0.1
12378-PeCDF	0.956	2.905	0.015	0.000075	0.05
23478-PeCDF	0.934	5.707	0.015	0.00075	0.5
123478-HxCDF	1.196	2.769	0.02	0.0002	0.1
123678-HxCDF	1.218	1.762	0.019	0.00019	0.1
234678-HxCDF	1.068	4.224	0.022	0.00022	0.1
123789-HxCDF	0.929	5.873	0.026	0.00026	0.1
1234678-HpCDF	1.459	4.447	0.021	0.000021	0.01
1234789-HpCDF	1.124	3.992	0.027	0.000027	0.01
OCDF	1.186	3.92	0.035	0.00000035	0.0001
2378-TCDD	1.077	1.267	0.009	0.0009	1
12378-PeCDD	1.006	3.851	0.021	0.0021	1
123478-HxCDD	1.043	3.559	0.039	0.00039	0.1
123678-HxCDD	0.992	2.405	0.041	0.00041	0.1
123789-HxCDD	1.025	4.312	0.039	0.00039	0.1
1234678-HpCDD	0.987	0.837	0.033	0.000033	0.01
OCDD	1.05	4.643	0.03	0.0000003	0.0001
		Total WHO-TEQ		0.006	

Total TEQ detection limit based upon 10g of sample.

Table 1. Summary of calibration curve results.

Similarly, the Total WHO-TEQ LOD falls well below the regulatory level required for dioxin analysis illustrating the unmatched sensitivity of the AutoSpec *Ultima* NT.

The results from the quantitative data processing are stored and displayed for ease of review in the QuanLynx browser.

Figures 4 and 5 show views of the QuanLynx browser for the hexa-furans in a calibration curve standard and in a real sample respectively.

Figures 6 and 7 show data for the tetrafurans and hexa-dioxins in the real sample.

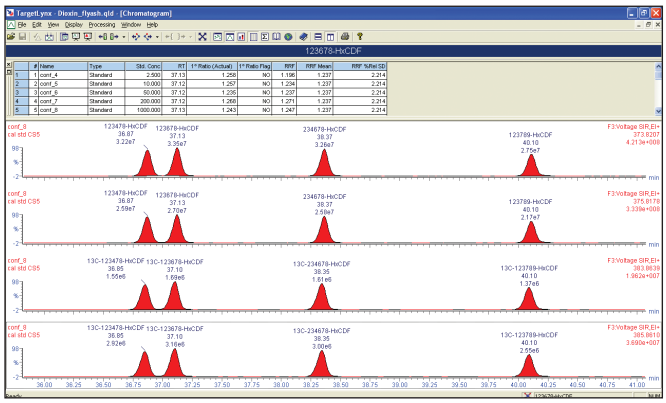

Figure 4. QuanLynx browser display of hexa-furans in calibration standard.

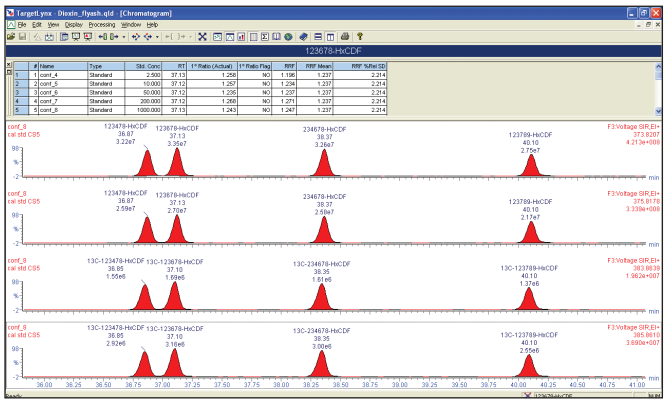

Figure 5. QuanLynx browser display of hexa-furans in sample.

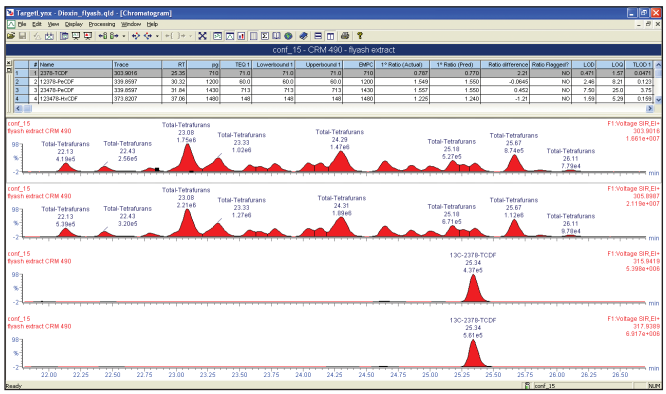

Figure 6. Tetra-furan results from sample.

Figure 7. Hexa-dioxin results from sample.

Location and review of sample data is made easier using the numerous dioxin dedicated features included in QuanLynx. Figure 8 shows 'Congener Select', a drop-down menu provided to allow the user to quickly locate and view quantitative results for a particular congener.

After data acquisition and processing various options are available, including export of results in various forms for reporting purposes, storage of calibration for future use and/or acceptance and locking of the data set to prevent modification of processing results (see Figure 9).

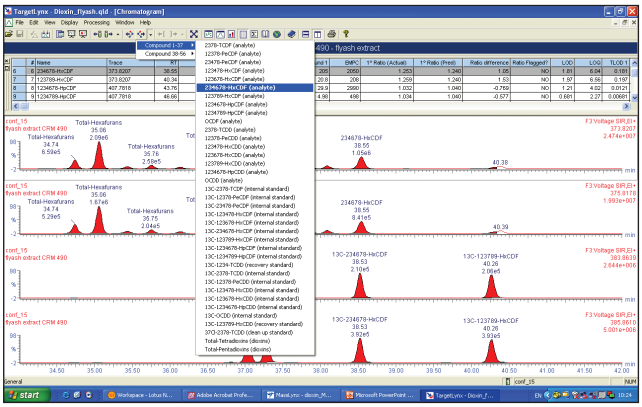

Figure 8. Congener Select functionality of QuanLynx browser.

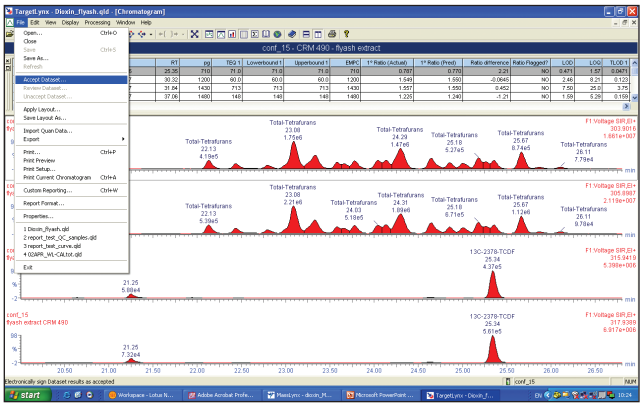

Figure 9. Acceptance and locking of quantification results.

CONCLUSION

High Resolution Gas Chromatography (HRGC) coupled with High Resolution Mass Spectrometry (HRMS) is the analytical technique of choice for analysis of dioxins and furans. The Micromass AutoSpec *Ultima* NT is the market-leading instrument of choice, offering the ultimate sensitivity, quantitative linearity, reproducibility necessary for regulatory dioxin and furan monitoring. In addition, MassLynx 4.0 and QuanLynx 4.0 give unprecedented automation, ease-of-use, data acquisition, and processing functionality with numerous features dedicated to dioxin and furan analysis.

Please see the Micromass website at www.micromass.co.uk for details of the latest technological developments and applications of the Micromass AutoSpec *Ultima* NT.

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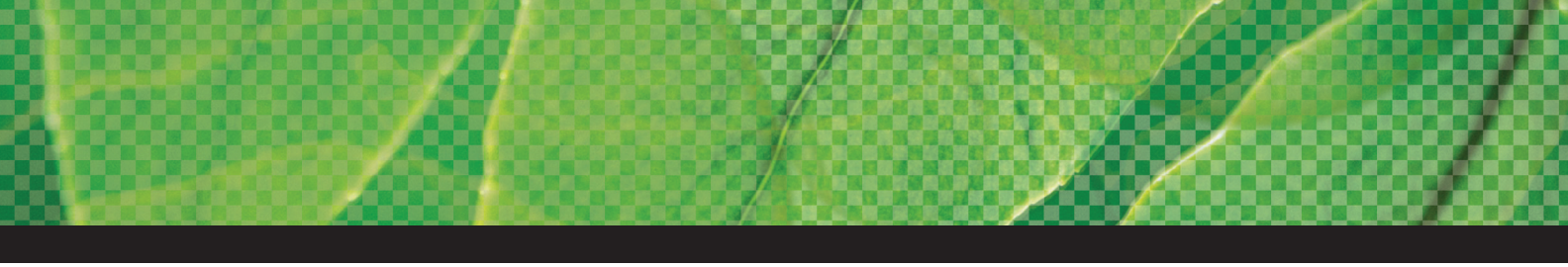

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