EPA Method 543: Selected Organic Contaminants by Online SPE LC/MS/MS Using the Agilent Flexible Cube

Application Note

Environmental

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Abstract

The novel Agilent Flexible Cube LC module for online SPE, in combination with the Agilent PLRP-S SPE cartridge and the Agilent 6460A Triple Quadrupole LC/MS, enables similar accuracy, precision, and sensitivity in less than half the run time of the system used in the published EPA Method 543.

Introduction

One of the challenges to monitoring water sources for trace contamination is the size of the sample, and the sample preparation required to adequately detect the contaminants. Conventional analytical approaches often require large volumes of water, solid-phase extraction, and extract concentration before instrumental analysis can be applied. These methods require transportation of large sample volumes, are labor intensive, and consume high volumes of organic solvents.

Online solid-phase extraction (SPE), with the SPE column placed in front of the analytical column, offers the ability to use much smaller sample volumes by capturing all of the analytes of interest for chromatographic analysis. For example, an injection of 1.8 mL of sample by online SPE is equivalent to processing a 360 mL sample by off-line SPE, concentrating the eluant to 1 mL and injecting 5 µL, in terms of the amount of target compound on-column.

In addition, online SPE enables reduced labor costs and a high degree of precision through automation, as each sample is processed by the instrument and not a person.

Sample turnaround is faster, due to vastly reduced sample preparation time, and materials costs are reduced, because the online SPE cartridges can be used for many samples (likely hundreds) rather than one cartridge per sample as with offline SPE.

Given the advantages of online SPE for water analysis, the United States Environmental Protection Agency (US EPA) has developed Method 543 for selected organic chemicals in drinking water using online SPE with liquid chromatography and tandem mass spectrometry (LC/MS/MS). The method allows flexibility in LC column, LC gradient, and MS conditions, but no other method parameters [1].

While online SPE has several demonstrated advantages over offline SPE, setup can be complicated, requiring multiple pumps for sample loading and analysis, as well as multiple valves for controlling the flow from each pump. This complexity can add to the cost of online SPE, provide a technical barrier, and present potential technology transfer problems.

This application note describes the use of the Agilent Flexible Cube to implement Method 543. The Flexible Cube is a novel, integrated valving and liquid-metering system for online SPE that uses a built-in single piston pump with solvent selection capability and user-selectable valves. Very low detection levels are possible without prior sample preparation, and the Flexible Cube significantly reduces the complexity, cost, and footprint of the HPLC system. Implementing Method 543 using the Flexible Cube and the Agilent PLRP-S SPE cartridge resulted in Lowest Concentration Minimum Reporting Levels (LCMRLs) near or below required EPA levels, with similar accuracy and precision.

Experimental

Reagents and materials

Type 1 Ultrapure water and Sigma-Aldrich Chromasolve V acetonitrile were used for chromatography. The SPE cartridge specified in Method 543 was also used. An Agilent Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 2.7 µm column (p/n 695975-312) was used for the HPLC separations.

Instruments

The system was built using Agilent 1200 Infinity Series LC modules coupled to an Agilent 6460A Triple Quadrupole LC/MS with Agilent Jet Stream technology. The online enrichment system used the Agilent 1290 Infinity Binary Pump (G4220A), Agilent 1260 Infinity Standard Autosampler (G1329B), Agilent 1290 Infinity Column Compartment (G1316C), and the Agilent 1290 Infinity Flexible Cube (G4227A) configured with two 10-port/2-position valves and two SPE cartridges to maximize sample throughput. The system operating conditions are shown in Table 1.

Sample preparation

Water samples (10 mL) were preserved with Trizma Preset Crystals (7.75 g/L), 2-chloroacetamide (2 g/L), and ascorbic acid (100 mg/L) per Method 543.

Table 1. Online SPE, HPLC, and MS Conditions

Online SPE (Agilent Flexible Cube)		
Injection volume	1.8 mL	
Flow rate	2 mL/min	
Solvents	A1 = 20 mM Ammonium acetate A2 = Acetonitrile	
Program	Time (min)	Function
	0	Pump 7.6 mL A1
	4	Increase valve position
	4.5	Pump 6 mL A2
	8	Pump 6 mL A1
HPLC		
Analytical column	Agilent Poroshell 120 PhenylHexyl, 3.0 × 100 mm, 2.7 µm column (p/n 695975-312)	
Column temperature	40 °C	
Injection volume	1,800 µL	
Mobile phase	A = 20 mM Ammonium acetate B = Acetonitrile	
Flow rate	0.4 mL/min (0.6 mL/min at end of run to speed flush time)	
Gradient	Time (min)	Mobile phase
	0	10% B
	4.1	10% B
	10	98% B
	11	Stop
Post time	0 minutes	
Run time	18 minutes, injection to injection	
MS		
Acquisition parameters	ESI mode, positive ionization; Dynamic MRM	
Sheath gas temperature	375 °C	
Sheath gas flow rate	12 L/min	
Drying gas temperature	300 °C	
Drying gas flow rate	7 L/min	
Nebulizer pressure	35 psig	
Nozzle voltage	0 V	
Vcap	4,500 V positive	

Analysis parameters

The dynamic multiple reaction monitoring (dMRM) transitions used for the seven target analytes are shown in Table 2.

Table 2. Dynamic Multiple Reaction Monitoring (dMRM) Analysis Parameters

Compound	Retention time	Precursor ion	Product ion	Fragmentor voltage	Collision energy (V)	Cell acceleration (V)	Retention window	ESI mode
Methomyl-13C2-15N†	6.95	166.1	91.1*	65	4	7	1.2	Positive
3-Hydroxycarbofuran	7.3	238.1	181.1*	100	5	4	1.2	Positive
			163.1	100	8	4	1.2	Positive
Fenamiphos sulfoxide	7.65	320.1	233	120	24	4	1.2	Positive
			171.1*	120	20	4	1.2	Positive
Fenamiphos sulfone	8.2	336.1	266.1	120	16	4	1.2	Positive
			188	120	24	4	1.2	Positive
Carbofuran-13C6†	8.45	228.1	171.1*	90	8	4	1.2	Positive
Fenamiphos	9.1	304.1	234.1*	115	12	4	1.2	Positive
			217	115	20	4	1.2	Positive
Tebuconazole	9.25	308.2	70*	130	20	4	1.2	Positive
Tebufenozide	9.55	353.2	297.1*	68	4	4	1.2	Positive
			133.1	68	12	4	1.2	Positive
Bensulide-d14†	9.85	412.2	364.1*	80	0	4	1.2	Positive
Bensulide	9.85	398.1	356	85	0	4	1.2	Positive
			158*	85	20	4	1.2	Positive

†Internal standard

*Denotes transition used for quantitation

Results and Discussion

Flexible cube operation

The 1290 Infinity Flexible Cube LC module (Figure 1) has a single piston pump with a three-solvent selection valve, and can also have one or two quick-change valves [2]. In the configuration used in this application, the left valve controls sample introduction to one of the SPE cartridges for online enrichment. The autosampler is connected to the piston pump, which loads the sample directly onto one of the trapping columns (SPE1) while the other trapping column (SPE2) has previously been loaded and is now in front of the analytical column and connected to the binary pump to initiate chromatographic separation. When the first trapping column is loaded with sample, the 2-position/10-port valve is switched to change the positions of the trapping columns, and the binary pump then delivers gradient to backflush the sample from SPE1 onto the analytical column.

On the other side of the valve, the trapping column used in the previous run (SPE2) is cleaned and re-equilibrated. Using the solvent selection valve in the 1290 Infinity Flexible Cube, the operator can clean and re-equilibrate the trapping columns with up to three solvents [3].

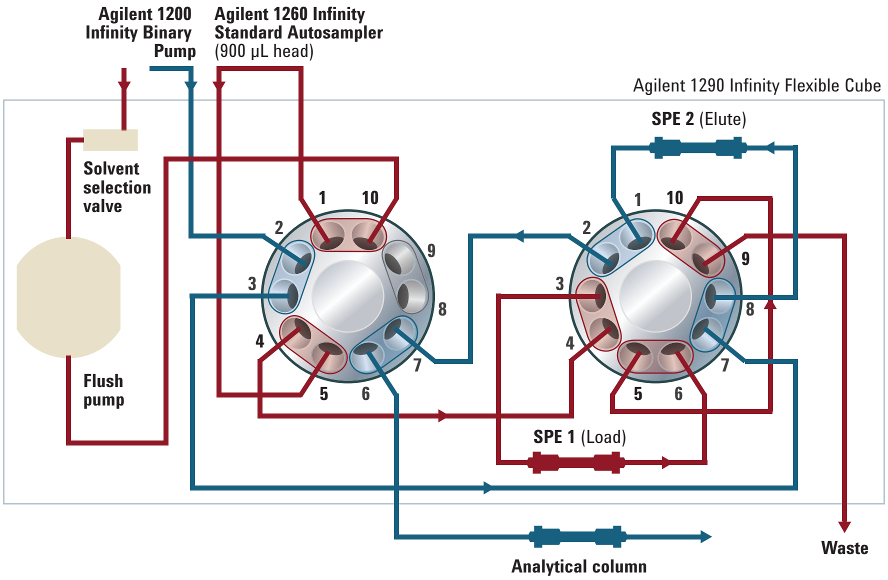

Figure 1. Flexible Cube Online SPE configuration depicting the beginning of an analysis, in which the Agilent 1200 Infinity Binary Pump moves sample through the left valve to the SPE2 trapping column, towards the analytical column (blue flow path). At the same time, the Agilent 1290 Infinity Flexible Cube piston pump (Flush pump) delivers sample to the other SPE cartridge by backflushing from the autosampler through SPE1, then out to waste to clean SPE1 of any unbound substances (red flow path). When the analysis is complete from SPE2, the valving configuration changes to place SPE1 in front of the analytical column and reload SPE2 with the next sample.

Method performance

An extracted ion chromatogram (EIC) for the analysis of the seven organic chemicals included in Method 543 illustrates almost baseline resolution for all seven, and good peak shape after backflushing the compounds from the SPE cartridge onto the analytical column (Figure 2). The seven compounds were analyzed at different concentrations due to differences in sensitivity, and calibration standards were prepared down to levels where the compounds were no longer detected. The range of concentrations used for the calibration curve for each compound is shown in Table 3. Figure 3 shows a typical calibration curve using quadratic fit and 1/x weighting.

One significant advantage of the method developed here is its speed. The total run time is 18 minutes, from injection to injection. In contrast, Method 543 run time is 28 minutes, and 40 minutes including post time.

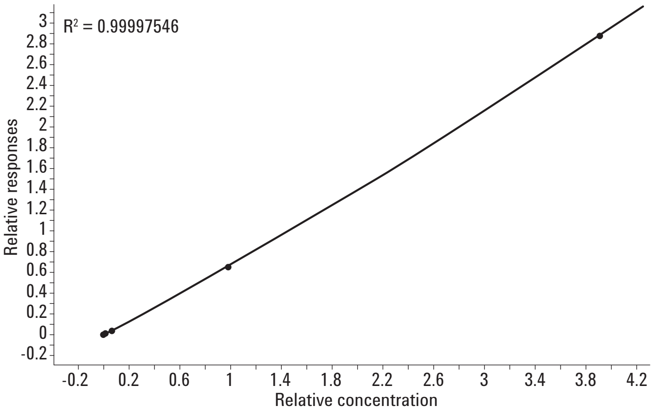

Figure 2. Extracted ion chromatogram (EIC) of quantifier ions for the seven target compounds and three internal standards.

Table 3. Calibration Ranges for the Target Analytes

Compound	Range (ng/L)
3-Hydroxycarbofuran	0.20–50
Bensulide	0.05–50
Fenamiphos	0.02–20
Fenamiphos sulfone	0.05–50
Fenamiphos sulfoxide	0.05–50
Tebuconazole	0.02–20
Tebufenozide	0.02–20

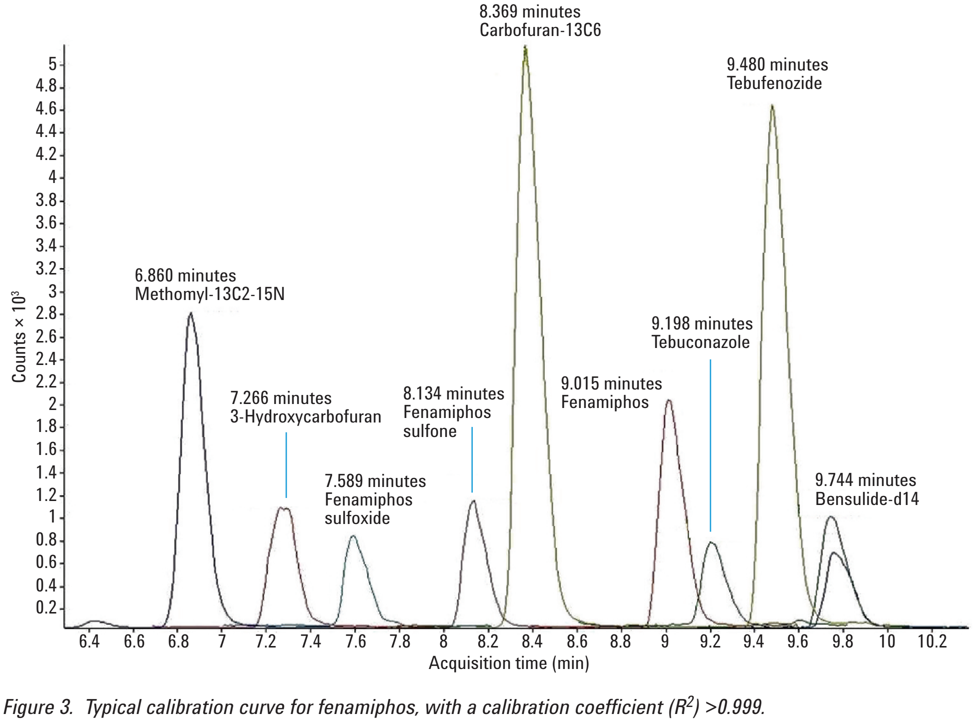

Figure 3. Typical calibration curve for fenamiphos, with a calibration coefficient (R^2) >0.999.

For accuracy and precision determinations, Laboratory Fortified Blanks (LFBs, spiked reagent water) and Laboratory Fortified Sample Matrix (LFSM, spiked tap water from a surface water source) were prepared at midlevel concentrations compared to the calibration curve ranges, and seven replicates were analyzed for each analyte. The determinations were made using the SPE cartridge specified in Method 543. Accuracy is presented as the average recovery of all seven replicates, and precision is presented as the percent Relative Standard Deviation (%RSD). The results are shown in Tables 4 and 5.

It can be seen from the data that the accuracy of this online SPE method using the Flexible Cube is comparable to that provided in Method 543. The data in Table 5 also demonstrate that the precision of this online SPE method using the Flexible Cube is comparable to that provided in Method 543. The precision values obtained using the Flexible Cube method were most often lower than those provided in the EPA method.

Table 4. Accuracy Determinations (%)

	LFB		LFSM	
Compound	**Flexible Cube**	**EPA***	**Flexible Cube**	**EPA***
3-Hydroxycarbofuran	107	100	93.1	108
Fenamiphos sulfoxide	104	102	100	95.4
Fenamiphos sulfone	100	101	102	94.1
Fenamiphos	101	105	104	91.9
Tebuconazole	87.7	112	101	87.6
Tebufenozide	96.7	101	100	97.5
Bensulide	101	95.2	101	89.8

* EPA data from Method 543 using fortifications at similar concentration levels, and from a surface water source for LFSM.

Table 5. Precision Determinations (%)

	LFB		LFSM	
Compound	**Flexible Cube**	**EPA***	**Flexible Cube**	**EPA***
3-Hydroxycarbofuran	1.6	4.1	4.8	14
Fenamiphos sulfoxide	1.3	4.0	3.5	2.1
Fenamiphos sulfone	0.8	4.4	0.9	6.7
Fenamiphos	2.0	3.7	2.8	3.8
Tebuconazole	4.2	5.4	6.5	4.3
Tebufenozide	1.4	3.6	5.1	2.2
Bensulide	2.7	2.9	1.2	1.7

* EPA data from Method 543 using fortifications at similar concentration levels, and from a surface water source for LFSM.

LCMRL calculations

Method 543 requires the calculation of the Lowest Calculated Minimum Reporting Level (LCMRL), which is accomplished by entering values in an EPA-supplied LCMRL Calculator [4]. The LCMRL is defined as the lowest spiking concentration at which recovery of between 50 and 150% is expected 99% of the time by a single analyst

It requires a minimum of four replicates at each of seven fortification levels, plus four Laboratory Reagent Blanks (LRBs). The LCMRL Calculator constructs mean and variance models of measurement as a function of spiking level, taking into account both precision and accuracy. Table 6 shows the calculated LCMRL values using the Flexible Cube method, as well as the values provided in Method 543. The method developed here using the Flexible Cube provides LCMRL values that are mostly lower than the EPA values.

Table 6. Calculated LCMRL Values (ng/L)

	Flexible Cube	**EPA**
3-Hydroxycarbofuran	2.1	1.7
Fenamiphos sulfoxide	0.33	1.2
Fenamiphos sulfone	0.25	1.4
Fenamiphos	0.072	0.27
Tebuconazole	0.21	1.3
Tebufenozide	0.13	0.47
Bensulide	0.73	1.2

Conclusions

The Agilent Flexible Cube removes the complexity that can increase the cost of online SPE and raise barriers to its implementation. As a result, it is a perfect match for Method 543 for online solid phase extraction and detection of selected organic contaminants. The method developed here using the Agilent Flexible Cube, an Agilent 1200 Infinity Series LC, an Agilent Poroshell Phenyl-Hexyl column, and an Agilent 6460 Triple Quadrupole LC/MS provides LCMRLs that are near or below EPA levels and accuracy and precision values similar to the EPA values, in less than half the run time of the system described in the EPA method. In addition, when each is used in the Flexible Cube method, the Agilent Bond Elut Online SPE PLRP-S cartridge provides similar performance to the SPE cartridge specified in the EPA method.

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