EPA Method 540: Selected Organic Contaminants Using Agilent Plexa Cartridges and the Agilent 6460 Triple Quadrupole LC/MS

Application Note

Environmental

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Abstract

EPA Method 540 has been run using the Agilent Plexa SPE cartridge, Agilent Infinity 1290 HPLC System, and the Agilent 6460 Triple Quadrupole LC/MS. Using all 17 compounds that were in the Draft Method 540, recoveries were well within the range specified by the method, with the exception of one compound. Precision easily met the method requirements for all 17 compounds. All LCMRLs were either near or significantly below EPA required levels, and the method run time was less than half that quoted for Method 540.

Agilent Technologies

Introduction

Large sample sizes are required for monitoring water for trace contamination, and solid-phase extraction (SPE) is an ideal tool for concentrating the sample to enable detection of these contaminants. In fact, the United States Environmental Protection Agency (USEPA) has published Method 540, which uses SPE to prepare water samples that are then analyzed using tandem mass spectrometry (MS/MS).

Method 540 calls for liquid chromatography (LC) separation and detection of 12 organic contaminants in finished drinking water using positive ion electrospray ionization (ESI) and MS/MS [1]. Preserved water samples are fortified with surrogates and extracted by SPE using an Oasis HLB or J. T. Baker Speedisk column, followed by LC/MS/MS analysis. The method requires that the precision of Laboratory Fortified Blanks (LFBs, reagent water) must be ≤ 20 % relative standard deviation (RSD), and accuracy must be ± 30 % of the true value. Sample collection, preservation, and extraction parameters may not be changed (Section 1.6). However, the method does allow flexibility in LC columns, LC conditions, and MS conditions, as long as method performance is not affected.

This application note demonstrates the implementation of USEPA Method 540, using Agilent Bond Elut Plexa SPE cartridges for sample extraction, the Agilent 1290 Infinity LC System, and the Agilent 6460 Triple Quadrupole LC/MS. The Agilent Poroshell Phenyl-Hexyl column provides excellent retention and separation of the target compounds. Although Method 540 does not allow use of another SPE cartridge, the Plexa SPE cartridge performs similarly to the Oasis HLB cartridge referenced in EPA Method 540. In addition to the 12 compounds included in the final published Method 540, the Plexa SPE cartridge provided similar or better results for the other five compounds that were included in the draft version of Method 540. Precision was well within the range specified by the method, as were recoveries, with the exception of one compound. The lowest concentration minimum reporting levels (LCMRLs) were either near or significantly below EPA required levels. Importantly, analysis time was less than half that cited for Method 540, enabling the processing of more samples each day. The use of the Plexa SPE cartridges is an attractive alternative for those labs that do not need to strictly follow the EPA method.

Experimental

Reagents and materials

HPLC grade water (Caledon Laboratory Chemicals) and LC/MS grade methanol (EMD Millipore Chemicals) were used for chromatography. The surrogates (Methomyl-$^{13}C_2$, ^{15}N, and Tebucanonazole-d_6) and internal standards (Carbofuran-$^{13}C_6$, Bensulide-d_{14}, and Phorate-d_{10}) were obtained from the EPA. The Agilent Bond Elut Plexa SPE, 200 mg, 6 mL cartridge (p/n 21209206) was used, for comparison with the cartridge specified in Method 540. 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 EPA Method 540 was run using the Agilent 1290 Infinity System coupled to an Agilent 6460A Triple Quadrupole LC/MS with Jet Stream technology. The instrument operating conditions are shown in Table 1.

Table 1. HPLC and MS Conditions

HPLC		
SPE cartridge	Agilent Bond Elut Plexa SPE, 200 mg, 6 mL (p/n 12109206)	
Analytical column	Agilent Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 2.7 µm column (p/n 695975-312)	
Column temperature	40 °C	
Injection volume	3.5 µL	
Mobile phase	A) 0.1 % formic acid + 5 mM ammonium formate in water B) 0.1% formic acid + 5 mM ammonium formate in methanol	
Flow rate	0.4 mL/min (0.6 mL/min at 14.1 minutes to speed flush time)	
Gradient	Time (min)	Mobile phase (% B)
	0	5
	1	5
	3	50
	12	80
	12.1	98
	14	98
	14.1	98
	15	Stop
Post time	3 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	

Sample preparation

Water samples (250 mL) were preserved with Trizma Preset Crystals (7.75 g/L), 2-chloroacetamide (2 g/L), and ascorbic acid (100 mg/L), per Method 540. After the addition of surrogates, the sample was loaded onto the SPE column. The SPE run conditions are shown in Table 2.

Table 2. SPE Cartridge Run Conditions

Step	Procedure
Condition	5 mL methanol followed by 10 mL reagent water
Sample	4-5 mL reagent water followed by sample
Rinse	5 mL reagent water
Dry	5 minutes at 10–15 inches Hg of vacuum
Elution	2 mL methanol (use vacuum to start flow, stop vacuum and wait for 5 minutes). Add 3 mL methanol, continue elution.
Concentration	Add ISTD to extract and concentrate the extract using nitrogen evaporation to ~1 mL. Vortex to rinse walls of tube.
Make up	Transfer extract to an LC vial and add reagent water to the top of the vial label (~1.7 mL total volume).

Analysis parameters

The dynamic multiple reaction monitoring (dMRM) transitions used for the 17 target analytes, surrogates, and internal standards are shown in Table 3.

Results and Discussion

Chromatography and calibration

The Poroshell Phenyl-Hexyl Column provides excellent retention and separation of the target compounds in 18 minutes run time, including equilibration (Figure 1). In contrast, the EPA Method 540 run time is 30 minutes, and 40 minutes including post time. The 17 compounds were analyzed at different concentrations due to differences in sensitivity. 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 4. Figure 2 shows a typical calibration curve using quadratic fit and 1/x weighting, which showed the best fit for most compounds.

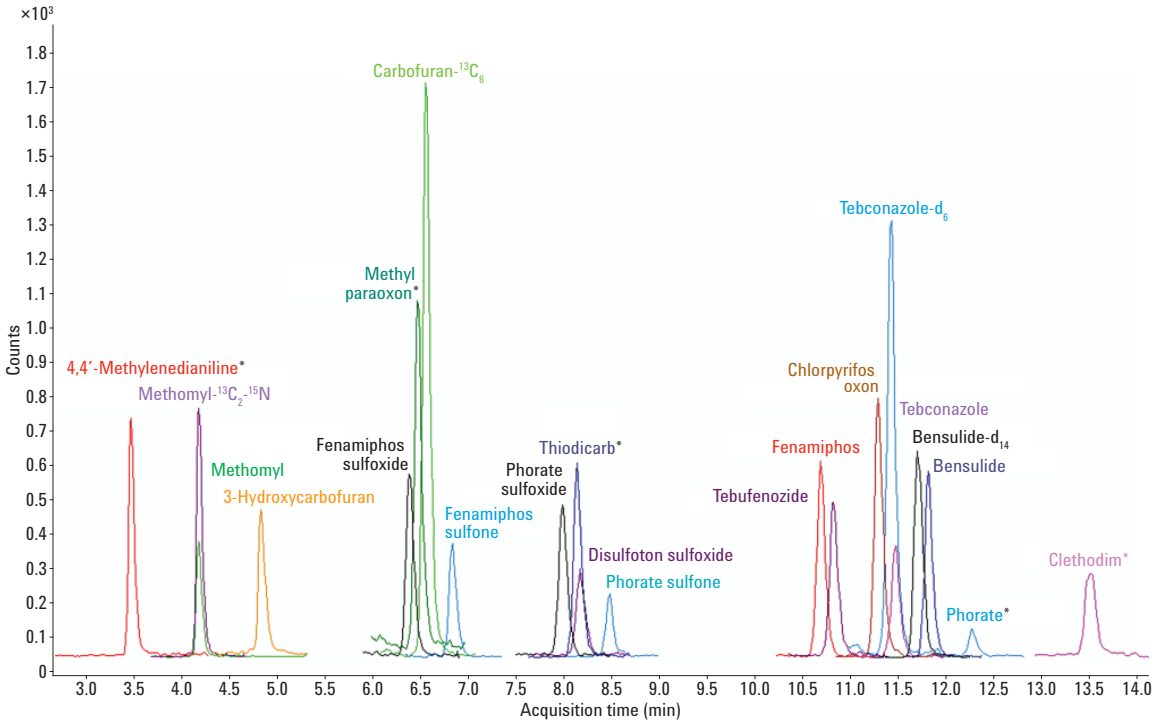

*Figure 1. EIC of quantifier ions for the 12 target compounds in the final EPA Method 540, plus the five compounds that were dropped from the EPA Draft method 540 (marked with an *), as well as two surrogates, and two internal standards.*

Table 3. Dynamic Multiple Reaction Monitoring (dMRM) Analysis Parameters

Compound	Retention time	Precursor ion	Product ion	Fragmentor voltage	Collision energy (V)	Cell acceleration (V)	Delta retention time
4,4′-Methylenedianiline*	3.5	199.1	77.1	118	64	4	1.6
			106.1**	118	28	4	1.6
Methomyl-$^{13}C_2$,^{15}N†	4.19	166.1	109.1	52	4	4	1
			91.1**	52	4	7	1
Methomyl	4.2	163.1	106	50	4	6	1
			88**	50	4	6	1
3-Hydroxycarbofuran	4.84	238.1	220.1	71	0	4	1
			163.1**	71	8	4	1
Fenamiphos sulfoxide	6.41	320.1	233	108	24	4	1
			171.1**	108	20	4	1
			108.1	108	44	4	1
Methyl paraoxon*	6.49	248	202.1**	102	16	4	1
			109	102	28	4	1
Carbofuran-$^{13}C_6$‡	6.59	228.1	171.1**	80	8	4	1
			129.1	80	20	4	1
Fenamiphos sulfone	6.86	336.1	266.1**	105	16	4	1
			188	105	24	4	1
Phorate sulfoxide	8.01	277	199	65	4	6	1
			143**	65	16	4	1
			97	65	32	6	1
Thiodicarb*	8.15	365.1	108	71	8	6	1
			88.1**	71	12	4	1
Disulfoton sulfoxide	8.18	291	185**	74	8	7	1
			157	74	20	4	1
			97	74	32	4	1
Phorate sulfone	8.5	293	171**	56	4	4	1
			115	56	24	4	1
			97	56	40	4	1
Fenamiphos	10.73	304.1	234.1	102	12	4	1
			217**	102	20	4	1
			202	102	36	4	1
Tebufenozide	10.85	353.2	297.1	68	4	4	1
			133.1**	68	12	4	1
Chlorpyrifos oxon	11.34	334	277.9**	84	12	4	1
			198	84	32	7	1
Tebuconazole-d_6†	11.47	314.2	72.1**	161	55	6	1
Tebuconazole	11.51	308.2	125	161	55	6	1
			70**	161	20	4	1
Bensulide-d_{14}‡	11.75	412.2	364.1**	71	0	4	1
			159	71	20	4	
Bensulide	11.86	398.1	356	74	0	4	1
			158**	74	20	4	1
			141	74	32	4	1
Phorate-d_{10}‡	12.21	271.1	75**	56	4	6	1
Phorate*	12.32	261	199	69	0	7	1
			75.1**	69	8	6	1
Clethodim*	13.52	360.1	156.1	96	24	4	1.2
			164.1**	96	16	4	1.2
			136.1	96	32	4	1.2

* Compound not included in the final draft of Method 540
† Surrogate
‡ Internal standard
** Transition used for quantitation

Table 4. Calibration Ranges for the Target Analytes

Compound	Range (ng/mL)
3-Hydroxycarbofuran	0.02–2.9
4,4′-Methylenedianiline	0.008–2.9
Bensulide	0.004–1.5
Chlorpyrifos oxon	0.01–1.5
Disulfoton sulfoxide	0.002–0.36
Fenamiphos	0.004–0.58
Fenamiphos sulfone	0.01–0.73
Fenamiphos sulfoxide	0.002–1.5
Methomyl	0.004–1.5
Methyl paraoxon	0.08–5.8
Phorate	0.08–5.8
Phorate sulfone	0.04–2.9
Phorate sulfoxide	0.01–0.73
Tebuconazole	0.02–2.3
Tebufenozide	0.008–0.29
Thiodicarb	0.001–0.73
Clethodim	0.02–1.5

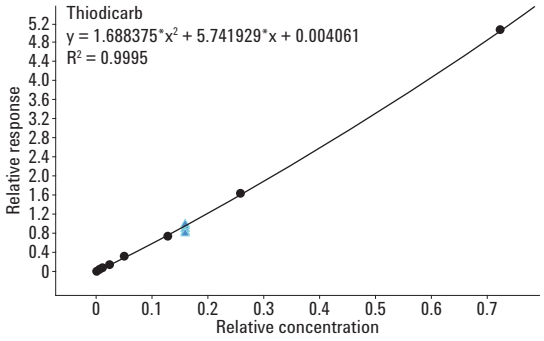

Figure 2. Typical calibration curve for thiodicarb from 0.001–0.73 ng/mL.

Method performance

For accuracy and precision determinations, LFBs (spiked reagent water) and Laboratory Fortified Sample Matrix (LFSM, spiked tap water from a surface water source) were prepared at mid-level concentrations compared to the calibration curve ranges, and seven replicates were analyzed for each analyte. Accuracy is presented as the average recovery of all seven replicates, and precision is presented as the % RSD. The results are shown in Table 5. The range of accuracy was 65 % to 116 %, and the range of precision was 2.6 % to 12 %.

Table 5. Accuracy and Precision Determinations

Compound	Accuracy (% recovery)	Precision (%RSD)
4,4'-Methylenedianiline*	84	4.4
Methomyl-$^{13}C_2$-^{15}N	65	9.1
Methomyl	67	12.0
3-Hydroxycarbofuran	103	4.6
Fenamiphos sulfoxide	116	3.9
Methyl paraoxon*	110	2.7
Fenamiphos sulfone	103	7.7
Phorate sulfoxide	109	3.8
Thiodicarb*	92	8.1
Disulfoton sulfoxide	100	7.5
Phorate sulfone	113	4.6
Fenamiphos	102	2.6
Tebufenozide	108	6.1
Chlorpyrifos oxon	95	4.2
Tebuconazole-d_6	100	8.0
Tebuconazole	93	6.2
Bensulide	111	5.9
Phorate*†	92	8.4
Clethidim*	107	7.1

* Compound included in the Draft EPA Method 540, but not in the Final EPA Method 540.

† The level of Phorate-d_{10} supplied in the combined stock solution from the EPA was too low to be useful, so it was not used for quantitation.

However, only the recoveries for Methomyl-$^{13}C_6$, ^{15}N (surrogate), and Methomyl (65 and 67 % respectively) were slightly below the 70 % limit specified by Method 540. All other compounds, including the five dropped from Draft Method 540, were 84 % or above, and no higher than 116 %. In contrast, the Draft Method 540 gave recoveries lower than 70 % for phorate and 4,4'-methylendianiline. The Agilent Plexa SPE cartridges produced precision results for all 17 target compounds that were well within the requirements of Method 540, which specifies % RSD ≤ 20 %. The Plexa SPE cartridges produce results equivalent to those generated using the Oasis HLB cartridge specified in Method 540.

LCMRL calculations

Method 540 requires the calculation of the LCMRL, which is accomplished by entering values in an EPA-supplied LCMRL calculator [2]. 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). Seven levels were initially run, then a final lower 8th level was required to determine LCMRLs for some target compounds. 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 Plexa SPE cartridges, as well as the values provided in either the Draft or Final Method 540. Calculated values are slightly higher (max factor of 2.5x higher) for some compounds (methyl paraoxon, phorate, and phorate sulfone) than the EPA values given in either draft of Method 540. However, the LCMRL values generated using the Plexa SPE cartridges are much lower (1.7 to 63 times lower) than the EPA values for the other 14 target compounds, including three compounds that were dropped from the draft method. Figure 3 shows the LCMRL plots for two of the compounds that were dropped when the final draft of EPA Method 540 was published.

Table 6. Calculated LCMRL Values (ng/L)

	Agilent Plexa SPE	EPA
4,4'-Methylenedianiline*	0.19	0.86**
Methomyl	0.16	1.2
3-Hydroxycarbofuran	0.65	1.3
Fenamiphos sulfoxide	0.5	0.86
Methyl paraoxon*	1.5	0.87**
Fenamiphos sulfone	0.044	1
Phorate sulfoxide	0.53	2
Thiodicarb*	0.038	2.4**
Disulfoton sulfoxide	0.053	2
Phorate sulfone	1.1	0.99
Fenamiphos	0.061	0.64
Tebufenozide	0.035	0.81
Chlorpyrifos oxon	0.086	2
Tebuconazole	0.12	2
Bensulide	0.14	1.2
Phorate*	2.7	1.1**
Thiodicarb*	0.038	2.4**

* Compound dropped from the Draft EPA Method 540

** Values taken from the Draft EPA Method 540. They do not appear in the final method.

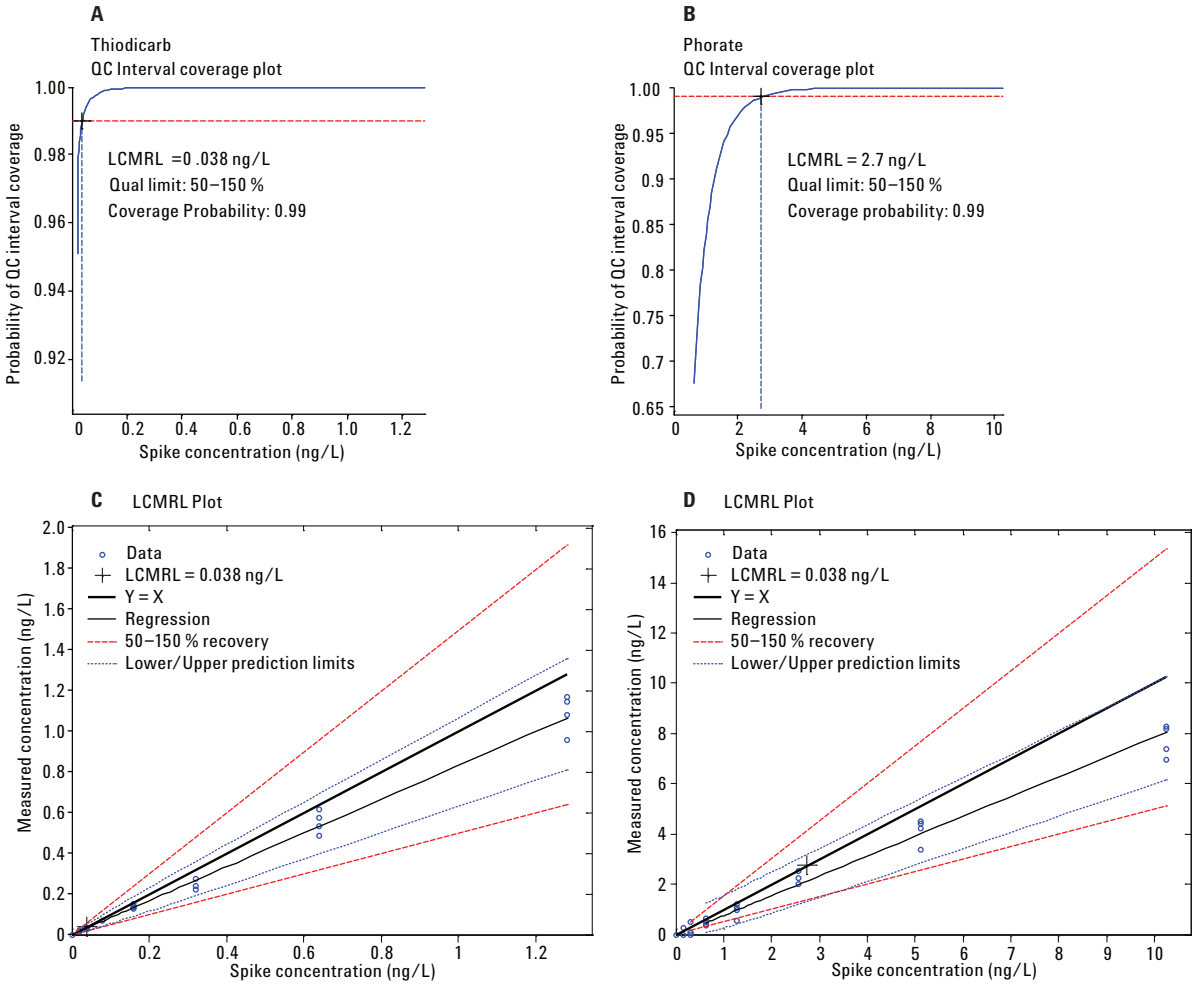

Figure 3. LCMRL Plots for two of the 17 compounds. These two were dropped from EPA Draft Method 540 to generate the final version of the method.

Conclusions

The Agilent Poroshell Phenyl-Hexyl Column provided excellent retention and separation of the target compounds, and the Agilent Bond Elut Plexa SPE cartridges performed similarly to the Oasis cartridges used in the USEPA Method 540. The Agilent 1290 Infinity LC System, coupled with an Agilent 6460 Triple Quadrupole LC/MS is quite suitable for the analysis of pesticides and metabolites in drinking water using this method. In fact, most LCMRLs were significantly below the levels published in Method 540. Importantly, the analysis time of 18 minutes (including equilibration) is significantly shorter than the EPA run time of 40 minutes, enabling more samples to be processed per day. Therefore, this method is a better alternative for the analysis of these compounds for those labs that are not required to adhere to the constraints of EPA Method 540.

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References

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