

Development of a U.S. EPA Method for the Analysis of Selected CCL 3 Drinking Water Contaminants By Solid Phase Extraction and LC/MS/MS

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Introduction

U.S. EPA's Office of Ground Water and Drinking Water (OGWDW) uses Unregulated Contaminant Monitoring Regulations (UCMRs) to collect nationwide occurrence data on drinking water contaminants that may be candidates for future regulation. These contaminants may be selected from a Contaminant Candidate List (CCL), or may be emerging contaminants that are being considered for inclusion on future CCLs. When a UCMR is proposed in the Federal Register, the analytical method to be used for the monitoring is also proposed. However, standardized methods that will produce data of sufficient quality for UCMR monitoring are not available for all CCL or emerging chemicals. A group of 19 potential contaminants have been evaluated for inclusion in a new drinking water method using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) for quantitation.

Objectives

- Develop an SPE-LC/MS/MS method which meets data quality objectives (DQOs) of 70-130% recovery and <30% relative standard deviation (RSD)
- Determine appropriate surrogates and internal standards (ISs)
- Determine appropriate preservatives (antimicrobial and dechlorination agent)
- Establish sample holding times
- Determine single laboratory lowest concentration minimum reporting levels (LCMRLs) – below health reference levels (HRLs)
- Develop a rugged, selective and sensitive method that can be implemented by many laboratories

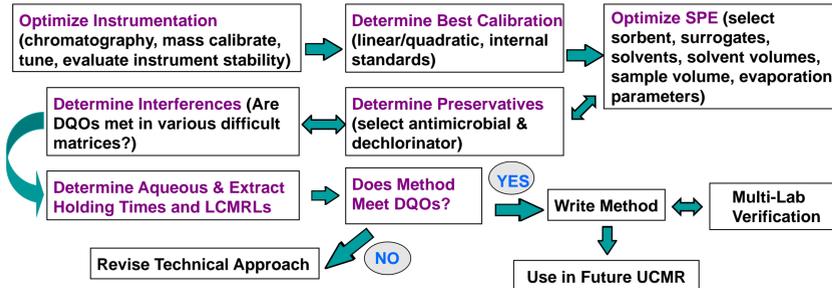
Chemicals Being Evaluated

Table 1. Chemicals Being Evaluated for Inclusion in a SPE-LC/MS/MS Method

Chemicals	CCL 3/NHSRC	Degradate of CCL 3 parent
3-hydroxycarbofuran	X	
4,4'-methyleneedianiline	X	
bensulide	X	
chlorpyrifos		X
chlorpyrifos oxon		X
clethodim total (Z and E isomers)	X	X
disulfoton		X
disulfoton sulfoxide		X
fenamiphos	X	X
fenamiphos sulfone		X (fenamiphos)
fenamiphos sulfoxide		X (fenamiphos)
methomyl		X (thiodicarb)
methyl paraoxon		X
phorate		X
phorate sulfone		X
phorate sulfoxide		X
tebuconazole	X	
tebufenozide	X	
thiodicarb	X	

Table 1. Eight of the 19 chemicals being evaluated in this method are listed on the final CCL 3¹ and three are degradates of the CCL 3 chemicals. Ten chemicals, including two of these CCL 3 chemicals, are being evaluated for inclusion in this method by request of EPA's National Homeland Security Research Center (NHSRC). The validated method may then be included in the next version of the NHSRC's Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events (SAM)² document.

Method Development Process



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Approach

Table 2. LC Operating Conditions

Time (min)	% Aqueous 10 mM ammonium formate with 0.05% formic acid	% Methanol
Initial	90.0	10.0
8.0	60.0	40.0
9.0	50.0	50.0
28.0	17.7	82.3
28.1	10.0	90.0
30.0	10.0	90.0
30.1	90.0	10.0
40.0	90.0	10.0

Restek, Ultra Aqueous, 2.1 x 100 mm packed with 5.0 µm C₁₈
Flow rate of 0.3 mL/min, 10 µL injection

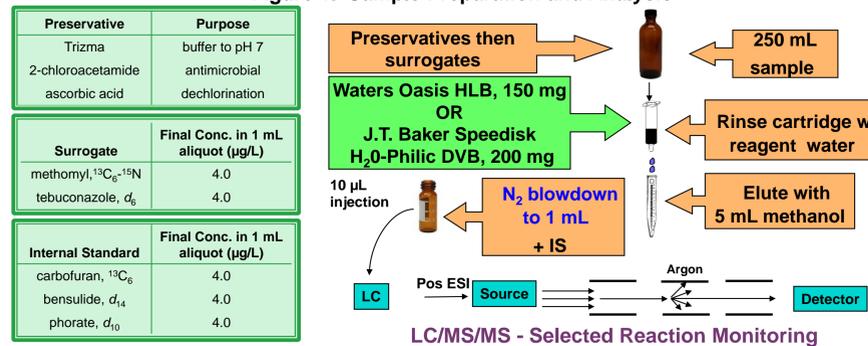
Table 2 contains the conditions used on a Waters Acquity LC to achieve separation of the analytes. The MS/MS data were obtained using the electrospray (ESI) conditions in Table 3 on a Waters Micromass Quattro Premier triple quadrupole mass spectrometer.

Table 3. ESI Operating Conditions

ESI Conditions	
Polarity	Positive ion
Capillary needle voltage	+4 kV
Cone gas flow	100 L/hr
N ₂ desolvation gas flow	1000 L/hr
Desolvation gas temp.	350°C

All samples were preserved, extracted and concentrated by SPE, then analyzed by LC/MS/MS as depicted in Figure 1 (samples in Table 4 were not preserved to demonstrate need for dechlorination). Preservation agents consisted of a dechlorinating agent and an antimicrobial. The dechlorinating agent was added to prevent reaction of the free chlorine with the method analytes during the aqueous holding time. The antimicrobial prevented microbial growth and, therefore, potential degradation of the target analytes. Quantitation was performed using selected reaction monitoring (SRM) MS/MS where the [M+H]⁺ ion was selected with the first quadrupole mass analyzer and the third quadrupole mass analyzer scanned the predominant product ion. Ionization and collision cell parameters were optimized for each analyte. Deuterated internal standards (ISs) were used to minimize variability and matrix effects.

Figure 1. Sample Preparation and Analysis



Results

Table 4. Recovery and Precision in Unpreserved Chlorinated Tap Water (n=4)

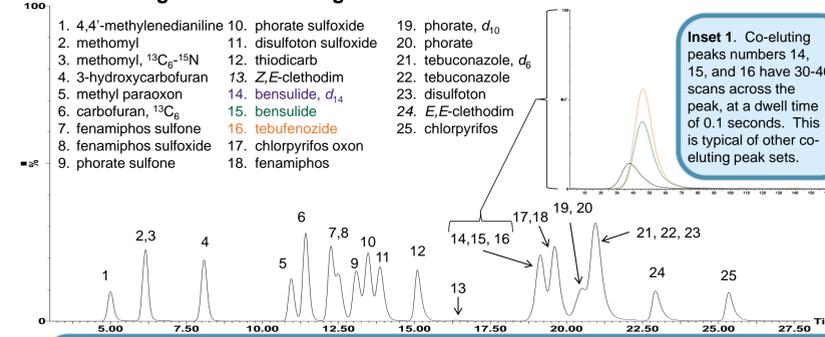
Chemical	Fortified Conc. ng/L	Mean Recovery %	RSD %
**Not possible to calculate %RSD			
4,4'-methyleneedianiline	32.0	0.0	**
methomyl	32.0	109	2.2
3-hydroxycarbofuran	32.0	97	5.5
methyl paraoxon	32.0	117	19
fenamiphos sulfone	32.0	102	3.5
fenamiphos sulfoxide	32.0	148	0.57
phorate sulfone	32.0	83	15
phorate sulfoxide	32.0	80	12
disulfoton sulfoxide	32.0	3.8	7.3
thiodicarb	32.0	87	12
clethodim total	32.0	0.0	**
bensulide	32.0	0.0	**
tebufenozide	12.8	20	12
chlorpyrifos oxon	32.0	172	26
fenamiphos	12.8	0.0	**
phorate	32.0	0.0	**
tebuconazole	12.8	113	4.7
disulfoton	32.0	0.0	**
chlorpyrifos	32.0	15	27

The data in Table 4 were from chlorinated tap water samples spiked with target analytes and immediately extracted by SPE. Results demonstrate that removal of free chlorine from the sample is necessary to prevent degradation of nine of the method analytes (highlighted in yellow). Recoveries for fenamiphos sulfoxide and chlorpyrifos oxon (highlighted in blue) are believed to be high due to degradation of the parent analyte. Therefore, the preservatives listed in Figure 1 were used in all the remaining studies presented here.

Results (continued)

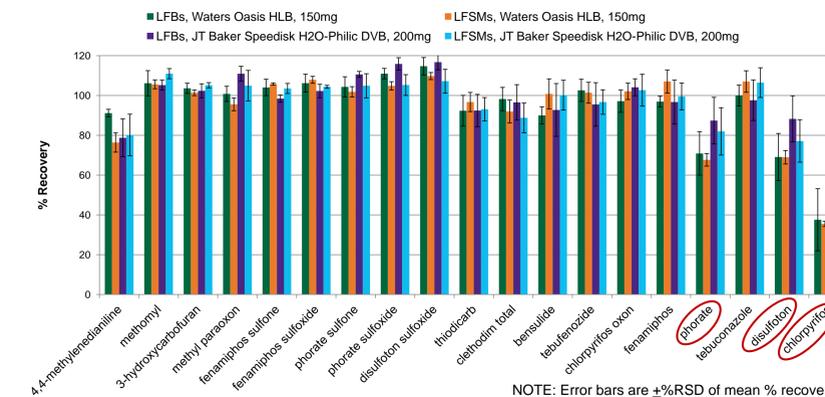
The chromatogram in Figure 2 illustrates the separation achieved with the conditions used to gather performance data. Although chromatographic resolution of all the analytes is not necessary, analytes must be adequately resolved in order to permit the mass spectrometer to dwell on a minimum number of compounds eluting within a retention time window. Instrumental sensitivity (or signal-to-noise) will decrease if too many compounds are permitted to elute within a retention time window. Approved drinking water LC/MS/MS methods specify a minimum of 10 scans across the peak to meet precision DQOs.^{3,4}

Figure 2. Chromatogram of a Mid-level Calibration Standard



The demonstration of capability data in Figure 3 meets DQOs of 70-130% recovery with <30% RSD for all CCL 3 analytes fortified at the mid-level calibration level (concentrations in Table 4) in both laboratory fortified blanks (LFBs) and laboratory fortified sample matrices (LFSMs) using Waters Oasis HLB and Baker Speedisk H₂O-Philiic divinylbenzene (DVB) cartridges. Several NHSRC analytes (circled in red) did not elute above minimal DQO limits from the Oasis HLB cartridge using methanol, however recoveries above 70% were obtained using the Speedisk H₂O-Philiic DVB cartridge. In the case of the LFSMs, chlorinated tap water from a surface water source was used.

Figure 3. Demonstration of Capability (n=4) preserved and fortified at 12.8 - 32 ng/L



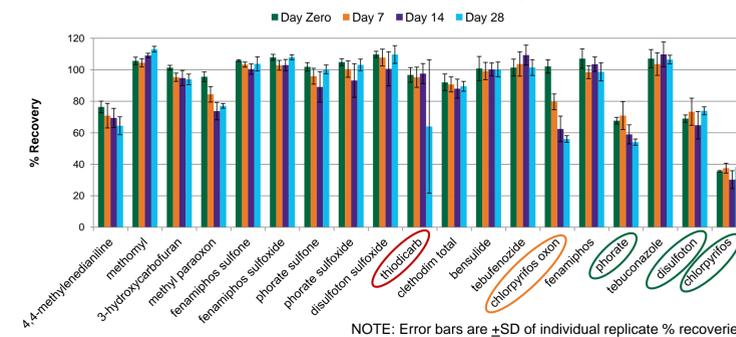
Conclusions

- An SPE-LC/MS/MS (draft EPA Method 540) has been nearly completed for the analysis of 18 analytes in drinking water which meets DQOs on at least one SPE cartridge.
- Preservation of samples was accomplished with Trizma, 2-chloroacetamide, and ascorbic acid.
- Matrix effects were reduced across a broad elution range by using three surrogate standards and two internal standards.
- Holding times were established at 28 days for all analytes except thiodicarb (14 days) and chlorpyrifos oxon (7 days).
- Single laboratory LCMRLs were obtained that are below HRLs for CCL 3 analytes.

Disclaimer: Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Chlorinated tap water samples were buffered with Trizma at pH 7 to minimize aqueous hydrolysis of some analytes. Additional preservation with 2-chloroacetamide (antimicrobial) and ascorbic acid (dechlorination) was performed before fortification. Samples were stored at 10 °C for 48 hrs to simulate shipping conditions, then at 4 °C for the remaining days. As observed in Figure 4, all CCL 3 and NHSRC analytes will have a 28 day aqueous holding time with two exceptions. Thiodicarb (circled in red) will be limited to a 14 day holding time, while chlorpyrifos oxon (circled in orange) will be limited to seven days. Some NHSRC recoveries (circled in green), indicate low recovery on Oasis HLB but no significant loss over time.

Figure 4. Aqueous Holding Time Study (n=4) chlorinated tap water, preserved, then fortified at 12.8-32ng/L, Oasis HLB SPE



The single laboratory LCMRLs, using the described method, are shown in Table 5. An LCMRL value for chlorpyrifos could not be generated due to low recovery at all levels (Oasis HLB cartridges). For the CCL 3 chemicals, the LCMRLs are below the known health reference levels (HRLs). Additional LCMRL data will be generated using the Speedisk H₂O-Philiic DVB cartridges.

Table 5. Single Laboratory DLs^a and LCMRLs^b Oasis HLB cartridges

Analyte	Fortified Conc. ng/L ^c	DL (ng/L)	LCMRL (ng/L)	HRL (µg/L) ^d
3-hydroxycarbofuran	1.60	0.45	1.3	0.42 (noncancer)
4,4'-methyleneedianiline	1.60	0.62	0.86	0.022 (cancer, 10 ⁻⁶)
bensulide	1.60	0.51	1.2	35 (noncancer)
chlorpyrifos oxon	1.60	1.0	2.0	
clethodim total	1.60	0.47	1.2	70 (noncancer)
disulfoton	1.60	0.34	2.7	
disulfoton sulfoxide	1.60	0.68	2.0	
fenamiphos	1.60	0.30	0.64	0.7 (noncancer)
fenamiphos sulfone	1.60	0.88	1.0	
fenamiphos sulfoxide	1.60	0.39	0.86	
methomyl	1.60	0.39	1.2	
methyl paraoxon	1.60	0.53	0.87	
phorate	1.60	0.29	1.1	
phorate sulfone	1.60	0.57	0.99	
phorate sulfoxide	1.60	0.70	2.0	
tebuconazole	0.64	0.25	2.0	210 (noncancer)
tebufenozide	0.64	0.26	0.81	126 (noncancer)
thiodicarb	1.60	0.67	2.4	1.86 (cancer, 10 ⁻⁶)

^a Detection limits (DLs) were determined by analyzing seven replicates over three days according to the procedure in reference 5.
^b LCMRLs were calculated according to the procedure in reference 6.
^c Spiking concentration used to determine DL.

References

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