EPA Method 538: Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection with the Agilent 6460 Triple Quadrupole LC/MS System

Application Note

Environmental

Authors

Imma Ferrer and Michael Thurman
Center for Environmental
Mass Spectrometry
University of Colorado in Boulder,
Colorado, USA

Abstract

A new method from the U.S. EPA (538) on 11 selected organic contaminants in drinking water by direct aqueous injection liquid chromatography/mass spectrometry/mass spectrometry has been developed using UHPLC and the Agilent Model 6460 Triple Quadrupole LC/MS System. One advantage of the EPA method is that solid phase extraction is no longer needed for sample preparation. This means that the analysis time is reduced by at least half. Furthermore, run time was cut by almost two-thirds by the use of UHPLC, and the method provided excellent linearity ($R^2 \geq 0.9999$) for all analytes, with limits of detection from 1 to 500 ng/L.

Introduction

Pesticides and other organic contaminants in drinking water pose potential human health risks. Agricultural and industrial uses of these chemicals are major sources of such contamination. To ensure the quality of drinking water in the US, the Environmental Protection Agency (EPA) has a number of monitoring requirements. EPA Method 538 has been developed and implemented for the determination of selected organic contaminants in drinking water, most of which are organophosphate pesticides.

EPA Method 538 involves analysis of water by direct aqueous injection and liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). The method measures the presence of 11 target analytes, using five deuterated internal standards. The analytes are separated and identified by comparing the acquired transition ions and retention times to calibration standards obtained under identical LC/MS/MS conditions. The concentration of each analyte is determined by internal standard calibration following standard procedures. Because the method requires no sample extraction, it is rapid and inexpensive relative to other LC/MS/MS methods.

This application note describes an Agilent implementation of EPA Method 538, which is demonstrated with the Agilent 1290 Infinity LC System and an Agilent 6460 Triple Quadrupole LC/MS System using Jet Stream technology. The 10-minute UHPLC chromatographic analysis is more than twice as fast as the original EPA Method 538, saving time and solvent costs. The method was modified by adding a second transition for all analyte ions for confirmation, which satisfies the European Union (EU) specifications for unequivocal identification by mass spectrometry. This gives an even greater assurance of correct identification than prescribed by the EPA. The utility of the method was demonstrated using local water samples.

Experimental

Reagents and Standards

All standard solutions (100 µg/mL) were purchased from Accustandards (New Haven, CT). The deuterated standards were obtained from Cambridge Isotopes (Cambridge, MA). HPLC grade acetonitrile and methanol were obtained from Burdick and Jackson (Muskegon, MI, USA). Formic acid was obtained from Sigma-Aldrich (St. Louis, MO, USA). Individual stock solutions (1 µg/mL) were prepared in pure methanol and stored at -18 °C. From these solutions, working standard solutions were prepared by dilution with acetonitrile and water.

Instruments

The method was run on the 1290 Infinity LC System with a 100 µL sample loop, coupled to the 6460 Triple Quadrupole LC/MS System with Jet Stream Technology. The instrument conditions are listed in Table 1.

Table 1. LC and MS Instrument Conditions

LC conditions	
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm (p/n 959757-902)
Column temperature	25 °C
Injection volume	100 µL
Mobile phase	A = Acetonitrile B = 0.1% acetic acid in water
Run time	10 min
Flow rate	0.4 mL/min
Gradient	90% B at time 0, and hold for 1.7 min. Gradient to 100% B at 10 min.
MS conditions	
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Gas temperature	250 °C
Desolvation gas flow rate	10 L/min
Nebulizer pressure	45 psi
Capillary voltage	4000 V
Nozzle voltage	0 V
Delta EMV	200 V

Sample Preparation

Method 538 calls for a 40 mL water sample, preserved with sodium omadine and ammonium acetate. Remove a 950 µL aliquot and place it in a vial, along with a 50 µL aliquot of five deuterium labeled internal standards. The organic solvent content of the sample should not exceed 5%. Collect the samples in baked amber glass bottles and store at 4 °C until analyzed. Pass the water sample through a PFTE filter (0.2 µm) before addition of internal standards, in order to prevent plugging of the analytical column. The sample is then ready for direct injection into the LC/MS/MS system. Blanks should also be passed through the filter to check for interferences.

Results and Discussion

Method 538

Table 2 shows the 10 organophosphate analytes included in EPA Method 538 along with the polynuclear aromatic heterocycle, quinoline. These 11 analytes represent important possible drinking water contaminants [1]. Five deuterated standards are also part of the method and are shown in Table 3.

Table 2. Ten Organophosphate Pesticides and Quinoline are the 11 Compounds Measured in EPA Method 538 as Drinking Water Contaminants

Analyte	**Chemical Abstract Services Registry Number (CASRN)**
Acephate	30560-19-1
Aldicarb	116-06-3
Aldicarb sulfoxide	1646-87-3
Dicrotophos	141-66-2
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Fenamiphos sulfone	31972-44-8
Fenamiphos sulfoxide	31972-43-7
Methamidophos	10265-92-6
Oxydemeton-methyl	301-12-2
Quinoline	91-22-5
Thiofanox	39196-18-4

One advantage of the EPA method is that solid phase extraction (SPE) is no longer needed for sample preparation, which means that total analysis time is cut at least in half. In addition, suppression from the sample matrix is reduced because the matrix is not concentrated as may occur with SPE. Although concentration of the sample may enable lower detection limits, this advantage will be mitigated by suppression effects. In addition, the sensitivity of the instrument negates the need for concentration of the sample. The method is quite simple, requiring only the addition of the internal standard mixture to the water sample.

Table 3. The Five Deuterated Internal Standards Used in EPA Method 538 [1]

Internal standards
Acephate-d_6
Diisopropyl methylphosphonate-d_{14} (DIMP-d_{14})
Metamidophos-d_6
Oxydemeton-methyl-d_6
Quinoline-d_7

Limits of Detection and Linearity

The EPA Method 538 calls for one MRM transition per compound [1]. The adaptation of the method described includes a second transition in order to provide a confirmation ion for each detected compound. This change also conforms to standard analytical procedures that call for a second confirming transition for analysis by LC/MS/MS using triple quadrupole methods, as well as ion-ratio percentages. Table 4 shows the transitions for each of the 11 compounds, along with the fragmentation and collision energies. Table 5 shows the transition used for each of the deuterated labeled standards used for quantitation, as well as their fragmentation and collision energies.

Table 5. Transitions, Fragmentation Energies, and Collision Energies for Each of the 5 Labeled Standards for EPA Method 538

Compound	Transition	Fragmentation energy	Collision energy
Acephate-d_6	190→149	50	0
DIMP-d_{14}	195→99	70	5
Methamidophos-d_6	148→97	70	10
Oxydemeton-methyl-d_6	253→175	70	10
Quinoline-d_7	137→81	110	35

Table 4. Transitions, Fragmentation Energies, and Collision Energies Used for Each of the 11 Standards for EPA Method 538

Compound name	Precursor ion	Product ion	Dwell	Fragmentor (V)	Collision energy (V)	Polarity
Acephate	206	165	10	90	5	Positive
Acephate	184	143	10	50	0	Positive
Aldicarb	213	116	10	90	5	Positive
Aldicarb	213	89	10	90	15	Positive
Aldicarb-sulfoxide	229	166	10	70	5	Positive
Aldicarb-sulfoxide	229	109	10	70	10	Positive
Dicrotophos	238	193	10	70	0	Positive
Dicrotophos	238	112	10	70	5	Positive
DIMP	181	139	10	70	0	Positive
DIMP	181	97	10	70	5	Positive
Fenamiphos-sulfone	336	308	10	110	10	Positive
Fenamiphos-sulfone	336	266	10	110	15	Positive
Fenamiphos-sulfoxide	320	292	10	110	10	Positive
Fenamiphos-sulfoxide	320	233	10	110	20	Positive
Methamidophos	142	125	10	70	10	Positive
Methamidophos	142	94	10	70	10	Positive
Oxydemeton-methyl	269	191	10	110	5	Positive
Oxydemeton-methyl	247	169	10	70	10	Positive
Quinoline	130	103	10	110	25	Positive
Quinoline	130	77	10	110	35	Positive
Thiofanox	241	184	10	90	5	Positive
Thiofanox	241	57	10	90	15	Positive

The extracted ion chromatogram (EIC) for the 11 compounds of EPA Method 538 is shown in Figure 1, using a 10-minute rapid gradient with UHPLC (Table 1). The 11 compounds elute in approximately 6 minutes. The more polar compounds, such as methamidophos, acephate, and aldicarb sulfoxide, elute in the first minute of the chromatogram. The more hydrophobic compounds, such as diisopropyl methylphosphonate (DIMP), aldicarb, fenamiphos sulfoxide, and sulfone, along with thiofanox, elute at the end of the chromatographic run. Good peak shape, which improves sensitivity and increases the limit of detection, was accomplished with this gradient.

The limits of detection (LODs) for the 11 analytes varied from 1 ng/L for aldicarb sulfoxide, which was the most sensitive compound, to 500 ng/L for acephate, which was the least sensitive compound (Table 6). The wide variation in LODs reflects the ability of each analyte to form ions in electrospray. The most polar analytes such as acephate and methamidophos were the least sensitive, while fenamiphos sulfone, and thiofanox were some of the most sensitive compounds and also the most hydrophobic. The LODs for 9 of the 11 compounds were lower than those posted in Table 5 of Method 538, and the MRLs for the same nine compounds were also equal to or lower than those listed in Table 5. Quinoline in particular is much more sensitive using the Agilent 6460 Triple Quadrupole LC/MS System with Jet Stream Technology because it is a stable compound (PNA) with a nitrogen heteroatom. Thus, this adaptation of Method 538 meets the criteria for a sensitive method for organophosphate pesticides in drinking water.

The extra MRM transition used in this adaptation of Method 538 is an important component of a valid method for water quality analysis of pesticides in water samples. The European Union (EU) specifications for unequivocal identification by mass spectrometry require two transitions, and this procedure has become an unofficial standard worldwide.

Table 6. Limits of Detection for EPA Method 538

Compound	**Fortified conc. (ng/L)[a]**	**LOD (ng/L)[b]**	**MRL (ng/L)[c]**
Acephate	500	500	1000
Aldicarb	5	2	5
Aldicarb sulfoxide	5	1	2
DIMP	10	10	20
Dicrotophos	10	10	20
Fenamiphos sulfone	5	5	10
Fenamiphos sulfoxide	5	5	10
Methamidophos	50	50	100
Oxydemeton-methyl	5	5	10
Quinoline	10	10	20
Thiofanox	5	2	5

a. Spiking concentration used to determine LOD

b. Limit of Detection (determined as three times signal-to-noise)

c. Method Reporting Limit (determined as six times signal-to-noise with two transitions per compound taken into account)

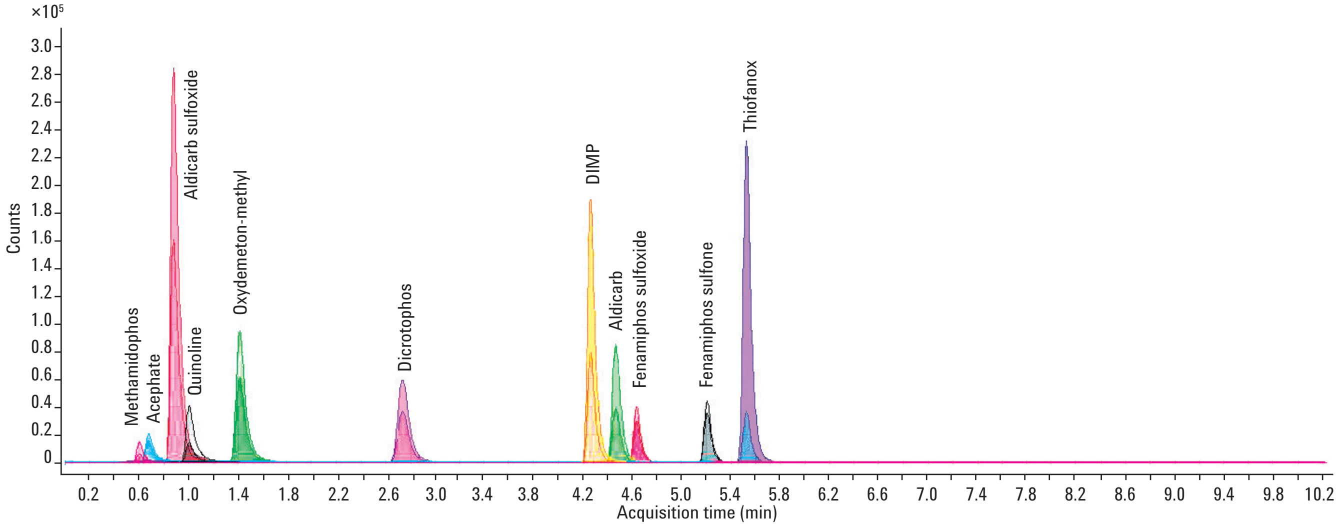


Figure 1. UHPLC extracted ion chromatogram (EIC) with the Agilent 1290 Infinity LC System, for the 11 analytes of EPA Method 538.

Figure 2 shows the excellent linearity that was achieved with the direct injection method for two of the analytes, quinoline and fenamiphos sulfone. In fact, the R^2 values are ≥0.9999 for all compounds in this method.

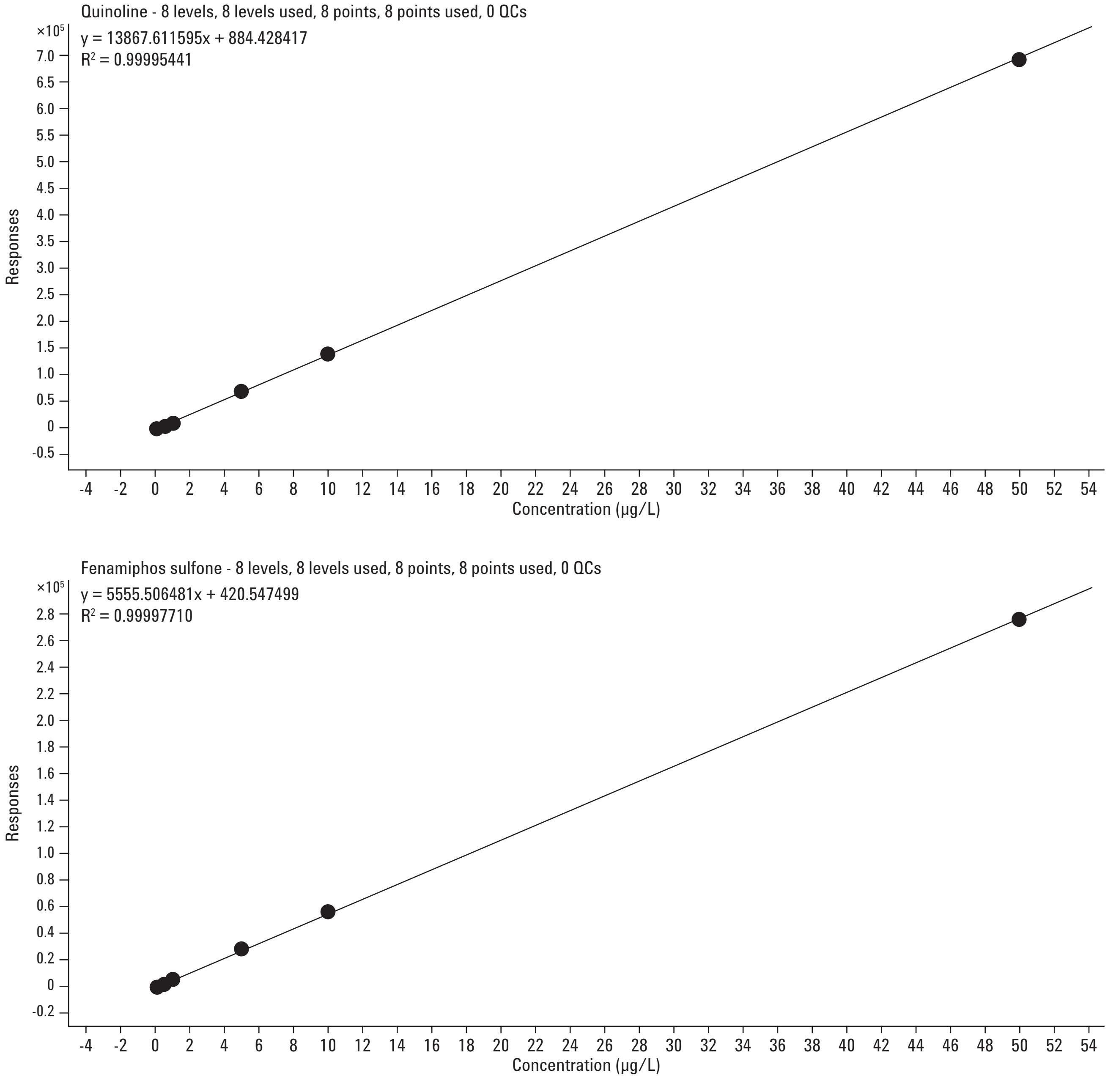
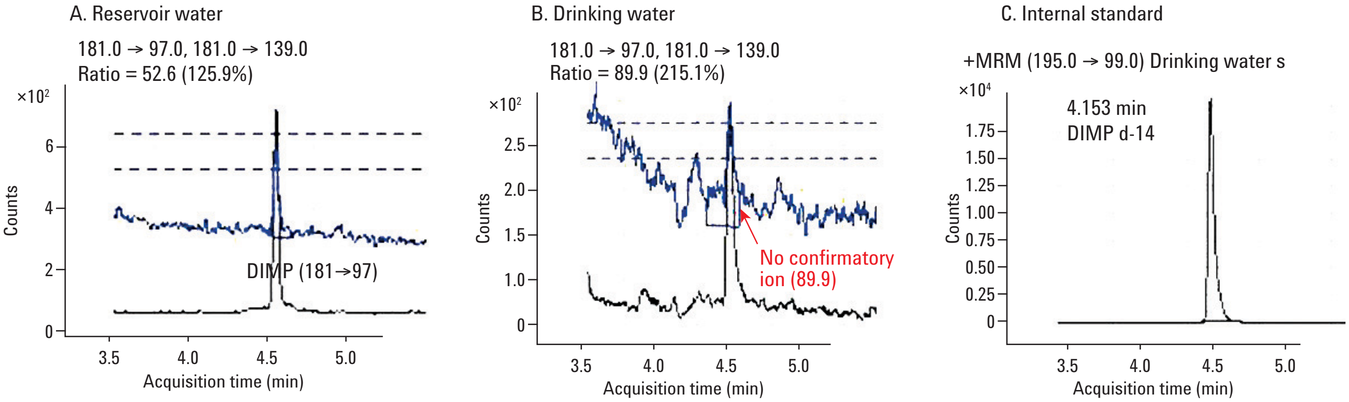


Figure 2. Calibration curves for quinoline and fenamiphos sulfone.

Testing of Drinking Water Samples

EPA Method 538 was tested on several samples from river, reservoir and drinking water sources. The method detected only DIMP in one of the samples taken from a reservoir prior to drinking water treatment (Figure 3). The treated drinking water contained no detectable organophosphate pesticides. Thus, the method was found to be reliable and useful for the analysis of drinking water contaminants, as well as rapid and sensitive.



A. Reservoir water

B. Drinking water

C. Internal standard

Figure 3. Analysis of the reservoir water prior to treatment (A.) and treated water (B.) for the pesticide DIMP using the modified Method 538. DIMP is detected using the 181→97 transition as the quantifier ion, and the 181→139 transition as the qualifier ion. In the case of the drinking water, the qualifier (confirmatory) ion is not present, resulting in a quantifier to qualifier ion ratio that is much too high, indicating the absence of DIMP in the drinking water. The deuterated DIMP internal standard is shown in C.

Conclusions

Running EPA Method 538 on the Agilent 1290 Infinity LC System and the Agilent 6460 Triple Quadrupole LC/MS System with Jet Stream Technology shortens time-to-results by almost a factor of three, and increases reliability of the method by adding a second transition. In addition, the detection limits and adaptations conform to the requirements of this method [1].

Reference

1. J.A. Shoemaker, 2009, EPA Method 538: Determination of selected organic constituents in drinking water by direct aqueous injection-liquid chromatography/tandem mass spectrometry, EPA/600/R-09/149, 40p.

For More Information

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