

EPA Draft Method 543 Quantitation of Organic Pesticides in Drinking Water Using Online Pre-concentration/Solid Phase Extraction and Tandem Mass Spectrometry

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Overview

Purpose: Validate a new EPA method for the analysis of organic pesticides in drinking water. This method utilizes large volume injections for online preconcentration and eliminates offline solid phase extraction (SPE).

Methods: Water samples containing the 7 analytes of interest are injected onto an online SPE cartridge, washed, then backflushed onto an analytical column prior to analysis by LCMS.

Results: Detection limits are determined for the 7 compounds and presented in this poster.

Introduction

Traditionally, EPA methods for drinking water analysis have required offline SPE to concentrate analytes to a level that can be detected using LCMS instrumentation. This process requires collection of large volumes of sample, typically between 250 and 1000mL. These large samples are then extracted using offline SPE, and the analytes are eluted from the SPE column and dried down before being reconstituted in an appropriate solvent and analyzed using LCMS. Several disadvantages to this technique include shipping costs and difficulty handling a large amount of samples collected in the field, as well as a substantial amount of sample preparation time using SPE cartridges. Furthermore, SPE cartridges are a one-time use consumable, and must be disposed of after each sample is treated.

Online Pre-concentration and Solid Phase Extraction (Online SPE) can eliminate these challenges by utilizing a smaller sample volume collected in the field and eliminating the manual offline SPE step. Using the LC to conduct the SPE eliminates intra-assay variability that can arise from manual processing of samples or inconsistencies between individual SPE cartridges. Also, the cost of consumables is lowered for the analysis since the online SPE cartridge is reused. Finally, the samples can be loaded onto the autosampler prior to analysis, and the experimental batch can be run with little to no user intervention.

The analytes analyzed in this method include 3-Hydroxycarbofuran, Bensulide, Fenamiphos, Fenamiphos Sulfone, Fenamiphos Sulfoxide, Tebuconazole, and Tebufenozide.

Methods

Sample Preparation

Drinking water samples were collected from a municipal tap (San Jose water, San Jose CA). Calibration and validation samples were prepared from reagent water, as specified in the draft EPA 543 method. All samples were treated with preservatives as shown in Table 1. The analytes of interest and internal standards were provided by the US EPA National Exposure Research Laboratory. In this experiment, all sample injections were 2 mL. No further treatment to the samples was conducted prior to injection.

Liquid Chromatography

Online SPE was conducted using the Thermo Scientific™ EQuan MAX Plus™ system. The EQuan MAX system uses two UHPLC pumps, a large volume capable autosampler fitted with a 5mL sample loop, and a thermostatted column compartment. Two different columns were used, a SPE cartridge (Waters Oasis HLB 20 μm, 2.1x30 mm) followed by an analytical column, (Thermo Scientific™ Hypersil GOLD™ aQ analytical HPLC column, 3μm, 2.1x50 mm). The column was held at 25C. The mobile phases for both columns were Water with 20mM ammonium acetate and acetonitrile. The LC gradients for each column are shown in Table 2 and Table 3.

Online SPE was conducted using a series of valves on the autosampler. There were distinct steps. Step 1, 2mL of sample is loaded into the 5mL sample loop. Step 2, the sample is transferred from the loop to the online SPE cartridge and washed. Step 3 (after 5 minutes), the SPE cartridge is switched inline with the analytical pump and the sample is transferred from the online SPE cartridge to the analytical column. Step 4 (after 5 more minutes), the SPE is switched back to the loading pump to be washed and re-equilibrated while the analytical column undergoes the same process. After re-equilibration, the next sample is injected.

TABLE 1. Sample preservation and treatment. All standards, QC samples, and drinking water samples were treated as shown.

| Compound | Amount | Purpose |
|-------------------|----------|----------------------|
| 2-Chloroacetamide | 2.0 g/L | Antimicrobial |
| L-Ascorbic Acid | 200 mg/L | Dechlorinating agent |
| Trizma | 7.75 g/L | Buffering reagent |

TABLE 2. LC Gradient and flow rates for the loading pump. Flow was reduced from 5.2 to 10 minutes while the column was in line with the analytical pump to conserve mobile phase. Flow was increased at 10 minutes to expedite the wash and re-equilibration of the column.

| Time (min) | Flow Rate (mL/min) | % Water with 20mM Ammonium Acetate | % Acetonitrile |
|------------|--------------------|------------------------------------|----------------|
| 0.0 | 2.0 | 100 | 0 |
| 5.1 | 2.0 | 100 | 0 |
| 5.2 | 0.1 | 100 | 0 |
| 10.0 | 0.1 | 100 | 0 |
| 10.1 | 4.0 | 100 | 0 |
| 14.0 | 4.0 | 100 | 0 |
| 14.1 | 4.0 | 0 | 100 |
| 17.1 | 4.0 | 0 | 100 |
| 17.2 | 4.0 | 100 | 0 |
| 21.2 | 2.0 | 100 | 0 |

TABLE 3. LC Gradient and flow rates for the analytical pump. Flow was increased from 10.1 to 17.1 minutes to expedite the wash and re-equilibration of the column.

| Time (min) | Flow Rate (mL/min) | % Water with 20mM Ammonium Acetate | % Acetonitrile |
|------------|--------------------|------------------------------------|----------------|
| 0.0 | 0.4 | 98 | 2 |
| 5.1 | 0.4 | 98 | 2 |
| 10.0 | 0.4 | 5 | 95 |
| 10.1 | 0.6 | 0 | 100 |
| 14.0 | 0.6 | 0 | 100 |
| 14.1 | 0.6 | 50 | 50 |
| 17.1 | 0.6 | 50 | 50 |
| 17.2 | 0.4 | 98 | 2 |
| 21.2 | 0.4 | 98 | 2 |

Mass Spectrometry

Mass spectrometry was performed with the Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer. The MS source parameters are listed in Table 4, while the analytes and internal standard's masses monitored are shown in Table 5. The LC flow was diverted to waste from 0 to 6.2 minutes and from 10 minutes to the end of the run.

Data Analysis

Data were analyzed using Thermo Scientific™ TraceFinder™ 3.2 Software. EPA LCMRL were calculated using the EPA's LCMRL Version 2.0 calculator.

TABLE 4. Mass spectrometer source conditions. Heated electrospray ionization was used.

| Parameter | Value |
|-------------------|--------------|
| Polarity | Positive |
| Spray Voltage | 3500 V |
| Sheath Gas | 50 |
| Aux Gas | 8 |
| Sweep Gas | 2 |
| Ion Transfer Tube | 300 C |
| Vaporizer Temp | 300 C |
| Cycle Time | 0.25 seconds |

TABLE 5. Optimized MS transitions for each compound analyzed in this experiment. As per the EPA method, only one product ion was monitored for each precursor ion.

| Compound | RT (min) | Precursor Ion | Product Ion | Collision Energy (V) | RF Lens (V) |
|--|----------|---------------|-------------|----------------------|-------------|
| 3-Hydroxycarbofuran | 7.2 | 238.25 | 181.15 | 11 | 57 |
| Bensulide | 9.1 | 398.25 | 356.05 | 7 | 60 |
| Fenamiphos | 8.6 | 304.25 | 217.06 | 22 | 81 |
| Fenamiphos sulfone | 7.9 | 336.25 | 265.98 | 19 | 89 |
| Fenamiphos sulfoxide | 7.6 | 320.25 | 233.13 | 24 | 84 |
| Tebuconazole | 8.8 | 308.25 | 70.10 | 21 | 85 |
| Tebufenozide | 9.0 | 353.25 | 133.00 | 7 | 60 |
| Methomyl- ¹³ C ₂ ¹⁵ N | 6.9 | 166.20 | 91.20 | 9 | 41 |
| Bensulide- <i>d</i> 14 | 9.1 | 412.25 | 364.02 | 7 | 69 |
| Carbofuran- ¹³ C ₆ | 8.0 | 228.20 | 129.25 | 21 | 58 |

Results

Calibrations, Replicates, and Spiked Water Sample Analysis

A 15 point calibration curve was created for the analytes. The internal standards were spiked into every sample at a level between 64 and 160 ng/L for each of the 3 internal standard compounds listed in Table 5. The calibration curve ranged from 0.2 ng/L (ppt) to 80 ng/L for Fenamiphos, Tebufenozide and Tebuconazole and from 0.5 ng/L to 200 ng/L for 3-Hydroxycarbofuran, Bensulide, Fenamiphos sulfone, and Fenamiphos sulfoxide. The curves were a linear fit with a 1/X weighting factor. Table 6 lists each compound, its associated internal standard, and the R² value for the calibration line. Figure 1 shows the calibration line for Tebufenozide. Excellent linearity was demonstrated over the entire range for all compounds, the lowest R² value being 0.9974 for Tebufenozide. Chromatograms for the analytes and internal standard are shown in Figure 2.

Each calibration level was injected five separate times in order to calculate the single laboratory lowest concentration minimum reporting level (LCMRL). The LCMRL calculator tool can be found online at http://water.epa.gov/scitech/drinkingwater/labcert/analyticalmethods_ogwdw.cfm

The calculated detection limits and LCMRL levels are listed in Table 7. Where the LCMRL is not shown, the calibration curve and replicate injections did not go low enough to calculate a LCMRL. Thus, the LCMRL is below the lowest calibration level, and will be calculated at a later date after injecting lower concentrations.

Additionally, tap water was analyzed for the presence of any of the analytes contained in the method. None of the contaminants were detected San Jose, California municipal tap water.

TABLE 6. Linearity results. Compounds, associated internal standard and calibration line R² values for each analyte.

| Compound | ISTD | R ² Value |
|----------------------|--|----------------------|
| 3-Hydroxycarbofuran | Methomyl- ¹³ C ₂ ¹⁵ N | 0.9994 |
| Bensulide | Bensulide- <i>d</i> 14 | 0.9983 |
| Fenamiphos | Bensulide- <i>d</i> 14 | 0.9986 |
| Fenamiphos sulfone | Carbofuran- ¹³ C ₆ | 0.9966 |
| Fenamiphos sulfoxide | Carbofuran- ¹³ C ₆ | 0.9983 |
| Tebuconazole | Bensulide- <i>d</i> 14 | 0.9975 |
| Tebufenozide | Bensulide- <i>d</i> 14 | 0.9974 |

FIGURE 1. Calibration line for the compound Tebufenozide. Calibration concentrations covered 15 points from 0.2 ng/L to 80 ng/L.

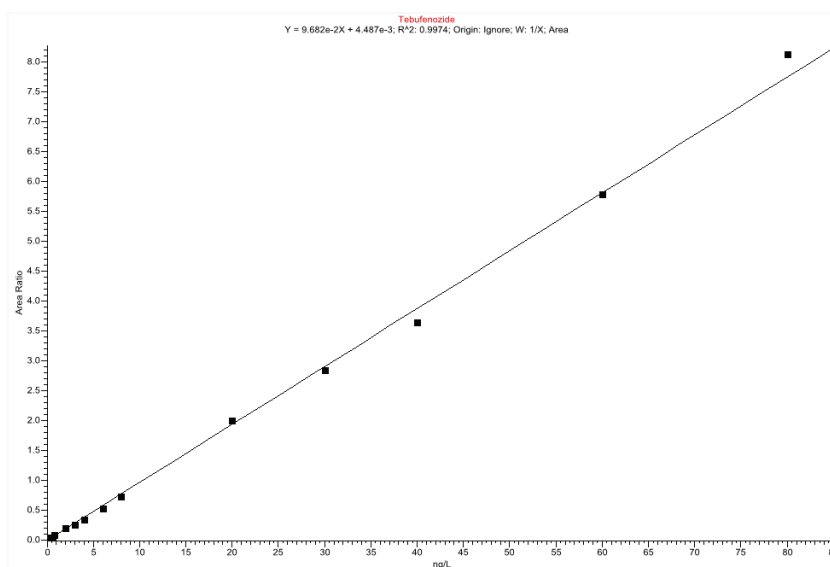
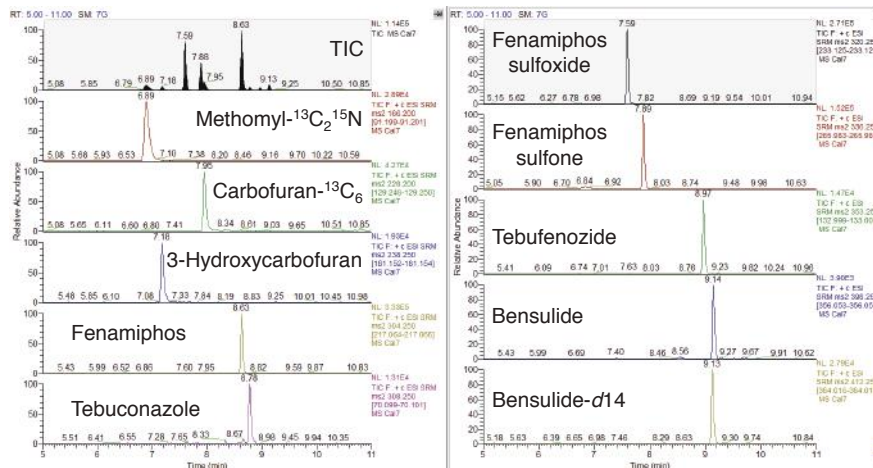


TABLE 7. Detection limits and LCMRL values for each compound. If the LCMRL value is listed with a less than symbol (<), the LCMRL lies below the lowest calibration level analyzed in this experiment.

| Compound | LCMRL (ng/L) | Detection Limit (ng/L) |
|----------------------|--------------|------------------------|
| 3-Hydroxycarbofuran | < 0.5 | 0.31 |
| Bensulide | 2.1 | 0.71 |
| Fenamiphos | <0.2 | 0.061 |
| Fenamiphos sulfone | <0.5 | 0.11 |
| Fenamiphos sulfoxide | <0.5 | 0.10 |
| Tebuconazole | 0.46 | 0.41 |
| Tebufenozide | 14 | 1.2 |

FIGURE 2. Total ion chromatogram and individually extracted chromatograms for each analyte and internal standard. This is a mid level calibration at 15 ng/L for 3-Hydroxycarbofuran, Bensulide, Fenamiphos sulfone, Fenamiphos sulfoxide and 6 ng/L for the others.



Conclusion

This poster has demonstrated the analysis of organic chemicals in drinking water pursuant to the US EPA's draft method 543.

- Excellent linearity over the calibration range.
- Sensitivity in the low ppt and ppq range (ng/L-pg/L)
- Further work will determine LCMRL levels for those compounds not determined in this experimental work.
- Demonstrated the online SPE analysis of these compounds in a 21 minute run time.

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