

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

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Key Words

Environmental analysis, online SPE, pesticide analysis, drinking water testing, EPA 543

Goal

To assist in the validation of 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).

Introduction

Traditionally, United States Environmental Protection Agency (EPA) methods for drinking water analysis have required offline SPE to concentrate analytes to a level that can be detected using LC-MS instrumentation. This process requires the collection of large volumes of sample, typically between 250 and 1000 mL. These large samples are extracted using offline SPE; the analytes are eluted from the SPE column, dried down before being reconstituted in an appropriate solvent and analyzed using an LC-MS. 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 and cost 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) avoids these disadvantages by utilizing a smaller sample volume collected in the field and eliminating the manual offline SPE step. Using the LC to conduct the SPE step 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.

This method updates EPA Method 540, which uses offline SPE and increases the compound list to include 3-hydroxycarbofuran, bensulide, fenamiphos, fenamiphos sulfone, fenamiphos sulfoxide, tebuconazole, and tebufenozide, as well as the internal standards methomyl- $^{13}\text{C}_2$, ^{15}N , carbofuran- $^{13}\text{C}_6$, and bensulide- d_{14} .

Experimental

Sample Preparation

Drinking water samples were collected from a municipal tap (San Jose Municipal Water System, San Jose, CA). Calibration and validation samples were prepared from reagent water, as specified in EPA Method 543. 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 1 mL. The EPA's method allows for injection volumes as high as 2 mL, although lower injection volumes are permitted. No further treatment to the samples was conducted prior to injection.

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 |

Liquid Chromatography

Online SPE was conducted using the Thermo Scientific™ EQUAN MAX Plus™ system. The EQUAN MAX Plus system uses two UHPLC pumps (Thermo Scientific™ Dionex™ UltiMate™ 3000 Series 3400RS and 3400SD pumps), a large-volume-capable autosampler fitted with a 5 mL sample loop, and a thermostatted column compartment. Two different columns were used, an online SPE cartridge for trapping the analytes and internal standards as detailed in EPA Method 543, followed by a Thermo Scientific™ Hypersil GOLD™ aQ analytical HPLC column (3 µm particle size, 2.1 x 50 mm, P/N 25303-052130). The column was held at 25 °C. The mobile phases for both columns were water with 20 mM ammonium acetate and acetonitrile. The LC gradients for each column are shown in Table 2 and Table 3.

Table 2. LC gradient and flow rates for the loading pump. Flow was reduced to 0.1 mL/min between 5.2 and 10 minutes while the online SPE column was in line with the analytical pump to conserve mobile phase. Flow was increased to 4 mL/min from 10 to 17.1 minutes to expedite the wash and re-equilibration of the online SPE column, and reduced back to 2.0 mL/min at 21.2 minutes.

| Time (min) | Flow Rate (µL/min) | % Water with 20 mM 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 analytical column.

| Time (min) | Flow Rate (mL/min) | % Water with 20 mM 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 |

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

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, and the masses of the analytes and internal standards 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™ software. Method detection limits were calculated for each analyte based on 5 replicate injections of calibration levels. A student's t-test was used to calculate the IDLs for each analyte. The calculation for the MDL was $MDL = \text{Standard Deviation for the 5 replicate injections} \times 3.747$. ($3.747 \sqrt{n-1}$ where $n=5$).

Table 4. Mass spectrometer source conditions.

| Parameter | Value |
|-------------------|---------------------|
| Ionization | Heated electrospray |
| Polarity | Positive |
| Spray Voltage | 3500 V |
| Sheath Gas | 50 arb |
| Aux Gas | 8 arb |
| Sweep Gas | 2 arb |
| Ion Transfer Tube | 300 °C |
| Vaporizer Temp | 300 °C |
| Cycle Time | 0.25 s |

Table 5. Optimized MS transitions for each compound analyzed in this experiment. 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 and Discussion

Calibrations, Replicates, and Spiked Water Sample Analysis

A 12-point calibration curve was created for the analytes. This large number of calibration points was chosen to cover a wide range of concentrations to investigate detection limits for all of the analytes. Thus, some of the calibration points were below the method detection limit and were excluded while analyzing the data. The internal standards were spiked into every sample at a level between 64 and 160 ng/L for each of the three internal standard compounds listed in Table 5. The calibration curve ranged from 0.01 ng/L (ppt) to 80 ng/L for fenamiphos, tebufenozide, and tebuconazole, and from 0.025 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 was 0.9974 for tebufenozide. Chromatograms for the analytes and internal standard are shown in Figure 2.

Each calibration level was injected five separate times to calculate the method detection limit (MDL). The calculated detection limits and the calibration level used to determine the MDL is listed in Table 7. Although the calibration standards went as low as 0.025 ng/L, not every compound was detected at this concentration. The calibration standard range was chosen to determine the MDL of each individual compound in the method. Calibration levels where there was no response detected or with a signal to noise ratio less than 3:1 were excluded from the calibration curve.

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

Table 6. Linearity results for each analyte.

| Compound | ISTD | R ² Value |
|----------------------|--|----------------------|
| 3-Hydroxycarbofuran | Methomyl- ¹³ C ₂ ¹⁵ N | 0.9986 |
| Bensulide | Bensulide- <i>d</i> ₁₄ | 0.9977 |
| Fenamiphos | Bensulide- <i>d</i> ₁₄ | 0.9989 |
| Fenamiphos sulfone | Carbofuran- ¹³ C ₆ | 0.9993 |
| Fenamiphos sulfoxide | Carbofuran- ¹³ C ₆ | 0.9983 |
| Tebuconazole | Bensulide- <i>d</i> ₁₄ | 0.9970 |
| Tebufenozide | Bensulide- <i>d</i> ₁₄ | 0.9981 |

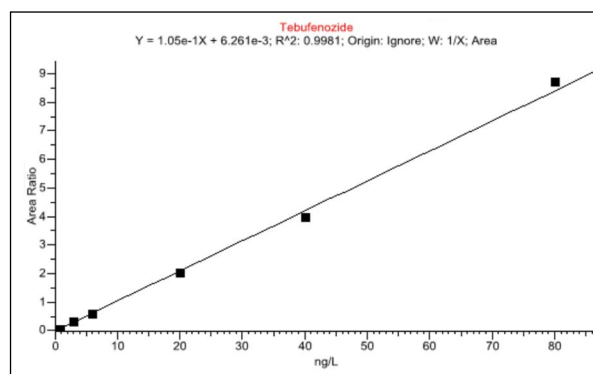


Figure 1. Calibration line for the compound tebufenozide. Calibration concentrations covered 12 points from 0.02 ng/L to 80 ng/L.

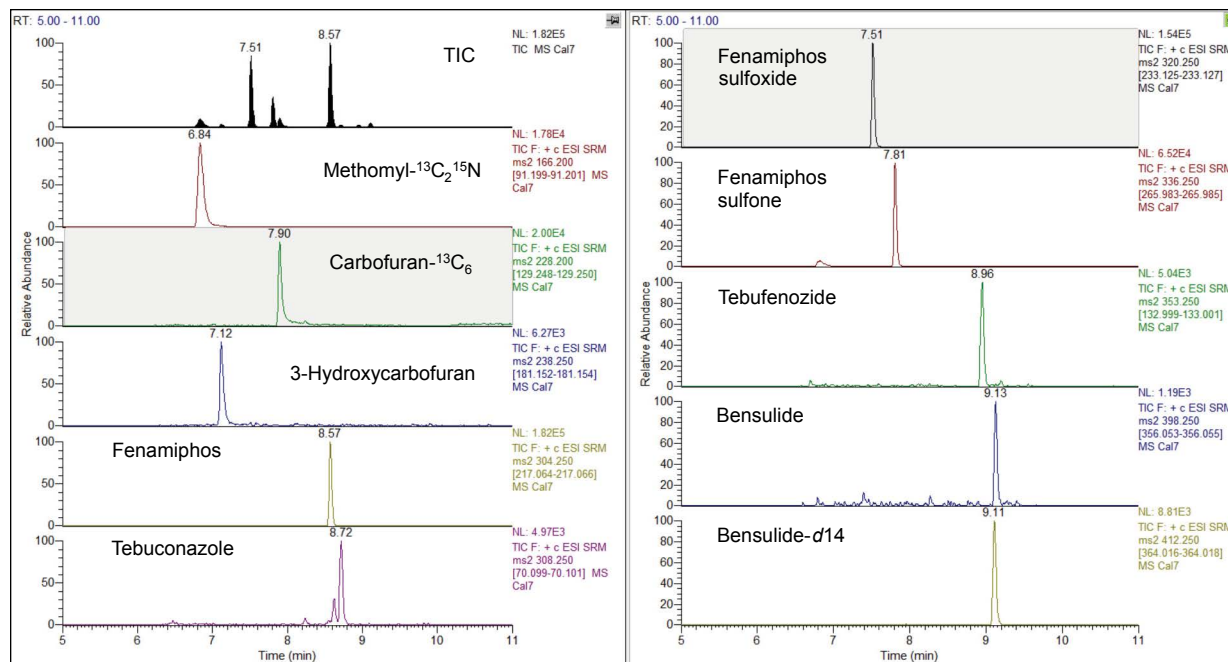


Figure 2. Total ion chromatogram and individually extracted chromatograms for each analyte and internal standard. This is a mid-level calibration at 15 ng/L for 3-hydroxycarbofuran, bensulide, fenamiphos sulfone, and fenamiphos sulfoxide and 6 ng/L for the others.

Table 7. Method detection limits and lowest calibration level values for each compound.

| Compound | MDL (ng/L) | Level used to calculate MDL (ng/L) | Lowest Calibration Level (ng/L) |
|----------------------|------------|------------------------------------|---------------------------------|
| 3-Hydroxycarbofuran | 0.946 | 1 | 0.5 |
| Bensulide | 2.356 | 2 | 1 |
| Fenamiphos | 0.007 | 0.04 | 0.025 |
| Fenamiphos sulfone | 0.074 | 0.1 | 0.025 |
| Fenamiphos sulfoxide | 0.037 | 0.1 | 0.025 |
| Tebuconazole | 0.246 | 0.4 | 0.01 |
| Tebufenozide | 0.186 | 0.2 | 0.02 |

Conclusion

The analysis of organic chemicals in drinking water pursuant to the US EPA's Method 543 has been presented, showing the following:

- Excellent linearity over the calibration range
- Sensitivity in the low ppt and ppq range (ng/L–pg/L) meeting or exceeding the EPA's requirements
- Online SPE analysis of these compounds in a 21 minute run time

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