Determination of Synthetic Pyrethroids in Water Samples

1.0 Scope and Application

1.1 This is a modified EPA Method 8081A and describes the sample preparation and quantitative analysis of trace level synthetic pyrethroids in surface, municipal and wastewater using liquid-liquid extraction and high resolution gas chromatography with electron capture detector (GC/ECD) and gas chromatography with mass spectrometer and ion trap detector (GC/MS-ITD) for confirmation.

1.2 The estimated detection limit for each analyte is listed in Table 1. The actual MDL may differ from those listed, depending upon the nature of interferences in the sample matrix. Validation of the target analytes produced recoveries greater than 65 percent. The mean percent recoveries for each analyte are also included in Table 1.

1.3 If possible, unknowns in the sample will be qualitatively confirmed for compound identification by gas chromatography equipped with an ion trap mass spectrometer detector (GC/MS-ITD).

2.0 Summary of Method

2.1 A measured volume of sample (1000 ml) is extracted with methylene chloride (DCM) using a separatory funnel. The DCM extract is dried with sodium sulfate, evaporated using Kuderna-Danish (K-D) and solvent exchanged into petroleum ether. The extract is concentrated with micro-snyder (micro K-D) apparatus to approximately 1 ml and adjusted to 2.0 ml with iso-octane. The extracts are analyzed by gas chromatography using conditions which permit the separation and measurement of the target analytes in the extracts by GC/ECD.

2.2 Interferences in analyses may be encountered in very dirty samples and cleanup may be needed to aid in the elimination or reduction of these interferences. Florisil column cleanup or Gel Permeation Chromatography (GPC) procedures will be followed.

Table 1. Synthetic Pyrethroids Analyzed by GC/ECD, their Minimum Detection Limits (MDL), Reporting Limits (RL) and Mean Percent Recovery in water.

Target Analytes	MDL (µg/l)	RL (µg/l)	Mean % Recovery
Bifenthrin	0.002	0.005	101
Cyfluthrin	0.002	0.005	79.0
Cypermethrin	0.005	0.010	83.3
Esfenvalerate/fenvalerate	0.002	0.005	91.5
Lambda cyhalothrin	0.002	0.005	96.7
Permethrin	0.005	0.010	80.9

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4.

Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination. Na_2SO_4 can be solvent rinsed to eliminate contaminants.

3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. A Florisil or GPC cleanup procedure can be used to overcome many of these interferences, but unique samples may require

additional cleanup approaches to achieve the MDL listed in Table 1.

4.0 Apparatus and Laboratory Supplies

4.1 Separatory funnel. 2000-ml, with TFE-fluorocarbon stopcock, ground glass or TEF stopper.

4.2 Automatic shaker designed to fit 2 liter separatory funnels with rpm and timer controls.

4.3 Beakers. Borosilicate glass, 400 mL

4.4 Glass wool. Pyrex - solvent washed prior to use.

4.5 Kuderna-Danish (K-D) Apparatus.

4.5.1 Concentrator tube. 15 mL, graduate (Kontes K0570012-0500, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

4.5.2 Evaporation flask. 500 mL (Kontes K-570050-0500, or equivalent), attached to concentrator tube with blue clamp (Kontes K-662750-0012).

4.5.3 Snyder column. Three ball (Kontes K-503000-0121, or equivalent).

4.5.4 Micro-Snyder column. Alltech 9058 or equivalent.

4.5.5 Boiling chips. Hengar granules, high purity amphoteric alundum - extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.

4.6 Water bath. Blue M, 115 V, thermostatically controlled with stainless steel cover to fit K-D apparatus, installed in a fume hood.

4.7 GC vials. GC autosampler vials, borosilicate glass, 2 mL with PTFE-lined screw cap.

4.8 Analytical balance. Capable of weighing 0.1 mg.

4.9 Drying oven.

4.10 Disposable Pasteur Pipettes. 2 mL, rinsed with solvents before use.

4.11 Glass filter funnel. Fluted, 75 mm or larger.
4.12 Graduated cylinder. 1000 ml, 250 mL and 100 mL.

4.13 Culture tubes. 13 x 100 mm with PTFE lined screw cap.

4.14 Analytical systems

4.14.1 Gas chromatograph. Agilent 6890 equipped with dual ^{63}Ni micro electron capture detectors (ECD) with EPC, split-splitless injector, a 7683 autosampler and dual capillary columns (J&W Scientific) connected to a single injection port using a 5 meter pre-column with a "Y" press fit connector. Section 9 describes the acquisition and analysis procedures while Table 2 lists the operating parameters.

4.14.2 Gas chromatograph. Varian 3400, equipped with a Varian Saturn model 2000 Ion Trap Mass Spectrometer, split-splitless injector, LEAP Model CTC A200SE autosampler and a 30 meter capillary column (J&W Scientific).

Table 2 Operating Parameters for Agilent 6890 GC/ECD

Gases: Carrier: Helium, 1 mL/min
Makeup: Nitrogen, 1 mL/min

Columns
DB-5, 60 m x 0.25 mm I.D. x 0.25 µm film thickness
DB-17-MS, 60 m x 0.25 mm I.D. x 0.25 µm film thickness

Mode: Constant flow
Initial flow: 1.9 mL/min

Inlet Isocratic temp: 240 ºC

Oven
Initial temperature: 80 ºC, initial time: 1.00 min
Ramp 1: 15.0 deg/min, final temp 210 ºC, hold time 10.00 min
Ramp 2: 2.0 deg/min, final temp 290 ºC, hold time 14.00 min

Detectors (^{63}Ni µECD)
Temperature: 310 ºC
Combined Flow: 31.0 mL/min (column + make-up flow)

Injection Volume: 3 µL

Table 3 Operating Parameters for Varian Saturn GC/MS-Ion Trap Detector

Carrier gas: Helium

Columns: DB5MS, 30 m x 0.25 mm I.D. x 0.25 µm film thickness

Varian 1078 Inlet: Isocratic temp @ 200 ºC

Injection Volume: 2 µL

Oven:

Initial temperature: 80 ºC

Initial time: 1.00 min

Ramp 1: 15.0 deg/min, final temp 210 ºC, hold time 10.00 min
Ramp 2: 2.0 deg/min, final temp 280 ºC, hold time 0.50 min

MS-MS Operating Conditions

Trap Temperature: 240 ºC
Manifold Temperature: 80 ºC
Transferline Temperature: 280 ºC

Ionization mode: EI Auto
Ion preparation: MS/MS
Scan time: 3µ scan/sec
Emission current; 50 µAmps
Waveform type: Non-resonant
Filament delay: 5 minute

4.14.3 Data System. Hewlett-Packard, to collect and record GC/ECD data, generates reports, computes and records response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards and calculating in external standard mode.

5.0 Reagents, materials, gases and standards

5.1 Reagent water is defined as water in which an interferent is not observed at method detection limit of each parameter of interest. Deionized (DI)

water was used for method validation and as method blank.

5.2 Petroleum ether (PE), acetone, methylene chloride (DCM), diethyl ether, isooctane. Pesticide residue quality or equivalent.

5.3 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.

5.4 Nitrogen. Ultra-pure (99.99999%) for GC/ECD

5.5 Helium. Ultra-pure (99.99999%) for GC/ECD

5.6 Stock standards. Individual stock standards (100 µg/ml) are purchased as certified solutions from AccuStandard (New Haven, CT), ChemService (West Chester, PA) and Ultra Scientific (North Kingstown, RI).

6.0 Sample Collection, Preservation, and Storage

6.1 Samples are collected in one liter amber glass bottles and iced or refrigerated at 4 °C from time of collection until extraction.

6.2 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

7.0 Sample Extraction

7.1 Remove water samples from refrigerator and allow samples to reach room temperature prior to extraction. Transfer contents to a pre-cleaned 2-liter separatory funnel. Immediately add 1.0 ml of the 20 ppb DBOB surrogate solution to every sample. For laboratory control spike (LCS) and matrix spikes (MS/MSD) also add 1.0 ml of 20 ppb pyrethroid spiking solution.

7.2 Add 60 ml of methylene chloride (DCM) to the empty bottle, replace the cap and rinse the bottle. Pour the DCM into the separatory funnel and repeat with another 60 mL aliquot of DCM. Extract the sample by shaking the funnel for 5 minutes on the auto-shaker with periodic venting to release excess pressure. Allow organic layer to separate from the water phase for a minimum of 10 minutes. Collect the methylene chloride extract in a 400 ml beaker.

7.3 Add a second 120 ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the beaker.

7.4 Set up and label pre-cleaned K-D flasks with concentrator tubes and

attached with a blue clamp on ring stands in the fume hood. Add 0.5 ml iso-octane as “keeper” and a solvent rinsed micro-boiling chip to each K-D concentrator tube. Place a filter funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of solvent rinsed sodium sulfate to the funnel.

7.5 Pour the combined extracts from the beaker through sodium sulfate into the K-D flask. Rinse the beaker with about 10 mL of DCM and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~10-20 mL).

7.6 Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 78-82 ^{o}C. Evaporate solvent on the hot water bath. When the apparent volume of solvent in the concentrator tube is 5-10 mL, add 20-30 mL of petroleum ether through the top of the Snyder column. Repeat this procedure when the apparent volume is again at 5-10 mL. When the reflux line falls below the top of the Snyder column, the K-D apparatus should be removed from the hot water bath. Dry the outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Upon cooling, remove the concentrator tube from the K-D apparatus.

7.7 Place a clean micro-Snyder column on the concentrator tube with a blue clamp, add a new micro boiling chip and place in a 400 mL beaker containing water heated to approximately 78 ^{o}C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath.

7.8 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Dry the outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Remove the micro-Snyder column and add iso-octane to the concentrator tube to reach a final volume of 2.0 mL. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex Genie mixer may be used for this step.

7.9 Transfer the solution from the concentrator tube to a culture tube and cap with a Teflon™ faced cap. Place extracts in a refrigerator for storage until analysis or cleanup, if necessary.

7.10 When ready for analysis, transfer extract to labeled GC vials and cap.

8.0 Cleanup Procedure

8.1 Cleanup of dirty samples may be necessary due to interferences in the analysis of baseline or co-elution with target analytes of the sample extract. Follow the in-house SOP for Florisil® column or GPC method, as needed.

9.0 Analytical Procedure

9.1 The final extract will be analyzed on an Agilent 6890 GC/ECD. Chromatographic conditions for operating the Agilent 6890 GC/ECD are found in Table 2.

9.2 GC acquisition

9.2.1 Analyze a Pesticide Degradation Check Solution (Ultra Scientific) at the beginning and end of each run to ensure GC performance.

9.2.2 Pour several isooctanes into GC vials using the same lot as used for samples with each GC run.

9.2.3 Pour standard curves into GC vials using 1.0, 2.0, 5.0, 10, 20 and 50 ppb Pyrethroid Std in isooctane. Pour extra vials of a mid-level concentration for use as CCV (to be analyzed every 20 samples or less).

9.2.3 Create sequence file and sequence table on computer. Use the WPCL login number for “Data Subdirectory” and “Save As” sequence name.

9.2.4 Acquire data and recap each vial daily to preserve sample integrity.

9.3 Analysis

9.3.1 Recalibrate pyrethroid curves and analyze samples in external standard mode. Add a printed chromatogram and report for each standard and sample to folder.

9.3.2 Certain analytes will co-elute on a given column. However, using two columns with different polarities will allow for confirmation of target analytes.

10.0 References

U.S. Environmental Protection Agency, Office of Water, EPA 821-R-92-002, April 1992, Methods For The Determination of Nonconventional Pesticides In Municipal And Industrial Wastewater, p. 227.
Method 622, *The Determination of Organochlorine Pesticides in Municipal and Industrial Wastewater*.