Determination of Synthetic Pyrethroids in Water Samples

1.0 Scope and Application

1.1 This method 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. The following target analytes can be determined by this method:

Analyte	CAS No.
Bifenthrin	82657-04-3
Cyfluthrin	68359-37-5
Cypermethrin	52315-07-8
Deltamethrin	52918-63-5
Esfenvalerate/ Fenvalerate	66230-04-4/ 51630-58-1
Fenpropathrin	64257-84-7
Lambda-cyhalothrin	91465-08-6
Permethrin	52645-53-1

1.2 The method detection limit (MDL) 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 average recoveries greater than 75 percent.

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) and Reporting Limits (RL) in ppb.

Target Analytes	MDL (µg/l)	RL (µg/l)
Bifenthrin	0.001	0.002
Cyfluthrin	0.002	0.004
Cypermethrin	0.002	0.004
Deltamethrin	0.002	0.004
Es/Fenvalerate	0.001	0.002
Fenpropathrin*	0.002	0.004
Lambda-cyhalothrin	0.001	0.002
Permethrin	0.003	0.005

* Estimated MDL

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. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified for the information of the analyst.

5.0 Apparatus and Laboratory Supplies

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

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

5.3 Beakers. Borosilicate glass, 400 mL

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

5.5 Kuderna-Danish (K-D) Apparatus.

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

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

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

5.5.4 Micro-Snyder column. Alltech 9058 or equivalent.

5.5.5 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.

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

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

5.8 Analytical balance. Capable of weighing 0.1 mg.

5.9 Drying oven.

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

5.11 Glass filter funnel. Fluted, 75 mm or larger.

5.12 Graduated cylinder. 1000 ml, 250 mL and 100 mL.

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

5.14 Analytical systems

5.14.1 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.

5.14.2 Gas chromatograph. Varian 3800, equipped with a Varian Saturn model 2000 or 4000 Ion Trap Mass Spectrometer, split-splitless injector, LEAP Model CTC A200SE autosampler and a 30 meter capillary column (J&W Scientific). Table 3 lists the operating parameters.

5.14.3 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.

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 Isothermal: 240 ºC

Oven Program 1- Multi-residue
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
Runtime: 73.67 min

Oven Program 2-Pyrethroids only
Initial temperature: 130 ºC, hold for 1.00 min
Ramp 1: 20 deg/min, final temp 240ºC, hold time 7.00 min
Ramp 2: 2 deg/min, final temp 290ºC, hold time 16.50 min
Runtime: 55.00 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-Iron Trap Detector

Carrier gas: Helium

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

Varian 1078 Inlet: Isothermal @ 260 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

6.0 Reagents, materials, gases and standards

6.1 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.

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

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

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

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

6.6 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).

7. CALIBRATION

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 2. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane or other suitable solvent. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with hexane or other suitable

solvent. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 µL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = (A_s)(C_{is}) / (A_s)(C_{is})$$

where
A_s = *Response for the parameter to be measured*
A_{is} = *Response for the internal standard*
C_{is} = *Concentration of the internal standard, in µg/L*
C_s = *Concentration of the parameter to be measured, in µg/L*

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s / A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of the lauric acid value is suggested. This procedure determines the adsorption from hexane solution of lauric acid, in milligrams, per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the data from Table 3, estimate the recovery and single-operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If

the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3s
Lower Control Limit (1CL) = R - 3s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the

samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9.0 Sample Collection, Preservation, and Storage

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

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

10.0 Sample Extraction

10.1 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 Dibromooctafluorobiphenyl (DBOB, CAS# 10386-84-2) 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.

10.2 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.

10.3 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.

10.4 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.

10.5 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).

10.6 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.

10.7 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.

10.8 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.

10.9 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.

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

11.0 Cleanup Procedure

11.1 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.

12.0 Analytical Procedure

12.1 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.

12.2 GC acquisition

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

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

12.2.3 Pour standard curves into GC vials using 0.5, 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).

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

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

12.3 Analysis

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

13.0 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. 771. Method 1660, *The Determination of Pyrethrins and Pyrethroids in Municipal and Industrial Wastewater*.

U.S. Environmental Protection Agency, Office of Solid Waste, SW-846 On-Line, Method 3510C, *Separatory Funnel Liquid-Liquid Extraction*, Revision 3, December 1996, http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3510c.pdf, [06-27-06]

U.S. Environmental Protection Agency, Office of Solid Waste, SW-846 On-Line, Draft Update IVB, Method 8081B, *Organochlorine Pesticides by Gas Chromatography,* Revision 2, November 2000 http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8081b_ivb.pdf [06-27-06]

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