# Determination of Pyrethroid Pesticides in Sediment

## 1.0 Scope and Application

1.1 This method describes the sample preparation using an automated extraction system for the determination of trace residue levels of a selected list of pyrethroid pesticides in sediments by dual column high resolution gas chromatography using electron capture detection. Table 1 lists the target pesticide compounds currently analyzed with their method detection limits and reporting limits for sediments.

1.2 These procedures are applicable when low parts per billion analyses are required to monitor differences between burdens in soils and sediment from relatively uncontaminated reference areas and contaminated areas.

## 2.0 Summary of Method

2.1 Sets of 12-16 homogenized sediment samples are scheduled for extraction by the project lead chemist. Extraction methods employed were developed and validated by the Water Pollution Control Laboratory (WPCL). Extract cleanup and partitioning methods are modifications of the multi-residue methods for fatty and non-fatty foods described in the U.S. Food and Drug Administration, Pesticide Analytical Manual, Vol. 1, 3rd Edition 1994, Chapter 3, Multi-residue Methods, Section 303-C1.

Homogenized sediment samples are removed from the freezer and allowed to thaw. A 1-5 g (sediment homogenate) sample is weighed into a pre-weighed aluminum planchet and placed in a 70°C oven for 48 hours to determine moisture content. A 10 g sample is mixed using a clean glass stirring rod with approximately 7 g of pre-extracted Hydromatrix (Varian Corp) in a 250 mL Trace Clean Wide Mouth Jar until the mixture is free flowing. The mixture is then poured into a 33 mL stainless steel Dionex Accelerated Solvent Extractor (ASE 200) extractor cell and packed by tamping the mixture. A solution containing DBOB surrogate is added to the cell and the cap is screwed onto the cell. The extractor cells (maximum of 24) are placed on the ASE 200 autosampler rack and the samples are extracted with a 50/50 mixture of acetone/dichloromethane (DCM) using heat and pressure. The extracts are automatically collected in 60 mL VOA vials.

2.3 The extracts are dried using sodium sulfate, evaporated to approximately 0.5 mL using Kuderna-Danish (K-D) glassware equipped with 3-ball Snyder columns and micro-Snyder apparatus and diluted to 10 mL using DCM. The extracts are then filtered through a 0.45 µm syringe filter into J2 Scientific AccuPrep 170 (GPC) autosampler tubes.

**Table 1.** Pyrethroid Compounds Analyzed and Their Estimated Minimum Detection Limits (MDL) and Reporting Limits (RL) in Sediment.

| Target Analytes | MDL* ng/g Dry wt. | RL ng/g Dry wt. |
|---|---|---|
| Bifenthrin | 1.00 | 2.00 |
| Cyfluthrin-1 | 3.00 | 5.00 |
| Cyfluthrin-2 | 3.00 | 5.00 |
| Cyfluthrin-3 | 1.00 | 2.00 |
| Cyfluthrin-4 | 1.00 | 2.00 |
| Cypermethrin-1 | 2.00 | 5.00 |
| Cypermethrin-2 | 2.00 | 5.00 |
| Cypermethrin3 | 2.00 | 5.00 |
| Cypermethrin-4 | 2.00 | 5.00 |
| Es-fenvalerate-1 | 2.00 | 5.00 |
| Es-fenvalerate-2 | 2.00 | 5.00 |
| Lambda-cyhalothrin-1 | 2.00 | 5.00 |
| Lambda-cyhalothrin-2 | 2.00 | 5.00 |
| Permethrin-1 | 1.00 | 2.00 |
| Permethrin-2 | 1.00 | 2.00 |
| Dibromooctafluorobiphenyl (DBOB, surrogate) | NA | NA |

*Estimated MDL based on 50 percent moisture

2.4 The GPC autosampler tubes are then placed on the GPC autosampler for initial sample cleanup.

2.5 The cleaned-up extracts are evaporated using K-D apparatus and solvent exchanged into petroluem ether. The extracts are then fractionated using 25 grams of Florisil in a 11 mm x 300 mm column with a 250 mL reservoir. The Florisil columns are eluted with petroleum ether (PE) (Fraction 1), 6% diethyl ether/PE (Fraction 2) and 15% diethyl ether/PE (Fraction 3). The fractions are concentrated to an appropriate volume using K-D/micro K-D apparatus prior to analysis by dual column high resolution gas chromatography.

**Table 2.** Distribution of Pyrethroid analytes among the Florisil Fractions.

| **(0%) Fraction 1** | **(6%) Fraction 2** | **(15%) Fraction 3** |
|---|---|---|
| DBOB (surrogate) | Bifenthrin | Cyfluthrin |
| (No pyrethroids are eluted in Fraction 1.) | Permethrin | Cypermethrin |
| | | Es-fenvalerate |
| | | lambda-cyalothrin |

## 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.

3.3 Interferences co-extracted from sediment samples limit the method detection and quantitation limits. For this reason, sample extract cleanup is necessary to yield reproducible and reliable analyses of low level contaminants.

## 4.0 Apparatus and Materials

4.1 Wide mouth, borosilicate glass, pre-cleaned and certified, 250 mL, Qorpak or equivalent.

4.2 Chromatographic Column - (300 mm x 11 mm) borosilicate glass chromatography column with 250 mL reservoir and Teflon stopcock.

4.3 Glass wool, Pyrex - solvent washed prior to use.

4.4 Kuderna-Danish (K-D) Apparatus

4.4.1 Concentrator tube - 10 mL, graduate (Kontes K0570050-1025, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.

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

4.4.3 Snyder column - three ball (Kontes K-503000-0121, or equivalent).

4.4.4 Micro-Snyder column - (Kontes VWR KT569261-0319 or equivalent).

4.4.5 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.

4.5 Water bath, Organomation Assoc. Inc.(OA-SYS/S-EVAP-KD), 115 V, thermostatically controlled with stainless steel cover to fit 5 K-D apparatus, installed in a fume hood. Water bath is equipped with solvent recovery system.

4.6 Extractor, automated, Dionex Accelerated Solvent Extractor (ASE 200), Dionex P/N 047046.

4.6.1 Extraction Cells, 33 mL, Dionex P/N 049562

4.6.2 Filters, cellulose for ASE extraction cells, Dionex P/N 049458.

4.6.3 VOA Vials, 60 mL, pre-cleaned and certified.

4.7 Sample vials - glass, 2.5 mL with PTFE-lined screw cap.

4.8 Analytical balance - capable of weighing 0.1 mg.

4.9 Drying oven.

4.10 Balance - capable of 100 g to the nearest 0.01 g.

4.11 Disposable Pasteur Pipets - (rinsed with solvents before use).

4.12 Aluminum dishes for moisture and lipid determination.

4.13 Desiccator with indicating desiccant.

4.14 Glass funnel, 75 mm.

4.15 Graduated cylinder, 250 mL and 100 mL.

4.17 Culture tubes, 16 x 100 mm, with PTFE lined cap.

4.18 Gas chromatographs (2), Hewlett-Packard HP 6890 plus, equipped with two micro ECD detectors with EPC, split-splitless injector with EPC, and autosampler.

4.19 Capillary columns, 60 meter DB5 and 60 meter DB17 (J&W Scientific) (0.25 mm I.D. and 25 µm film thickness) connected to a single injection port using a "Y" press fit connector.

4.20 Data System, Hewlett-Packard, to collect and record GC data, generate reports, and compute and record response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.

4.21 Homogenizer, Brinkman Polytron or equivalent equipped Teflon and titanium generator assembly (for homogenization of small sample amounts).

4.22 Gel Permeation (size exclusion) Chromatograph, automated, J2 Scientific AccuPrep 170, equipped with 70 g S-X3 BioBeads J2 Scientific P/N C0100 (100% DCM).

## 5.0 Reagents

5.1 Petroleum ether (PE), Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.

5.2 Acetone. (Same as above).

5.3 Iso-Octane. (Same as above).

5.4 Diethyl ether preserved with 2% ethanol.(Same as above).

5.5 Dichloromethane (DCM). (Same as above).

5.6 Chem Elut-Hydromatrix, Varian P/N 0019-8003. Pre-extracted on ASE-200 with acetone/DCM prior to use.

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

5.8 Florisil, 60/100 mesh, PR grade, Floridin Corp.

5.9 Nitrogen, pre-purified grade (99.9999%) or better (used for ASE and GPC.)

5.10 Nitrogen, ultra-pure (99.99999%) for ECD makeup.

5.11 Helium, ultra-pure (99.99999%) for GC carrier gas.

5.12 Air, compressed, breathing quality, for ASE pneumatics.

5.13 Pyrethroid Surrogate Solution containing 20 ppb of DBOB obtained from Restek Corp, Bellefonte, PA, P/N 31040.

5.14 Pyrethroid Spiking Solution Mix: Individual compounds obtained from Chem Service, Inc., West Chester, PA. Solution made in acetone.

| **Compound** | **Chem Service P/N** | **Concentration (ppb)** |
|---|---|---|
| Bifenthrin | PS2003 | 20 |
| Permethrin | F2216S | 60 |
| Lambda-cyhalothrin | PS2018 | 20 |
| Cyfluthrin | F2460 | 40 |
| Cypermethrin | PS1068 | 40 |
| Es-fenvalerate | PS2004 | 20 |

5.15 Pyrethroid Instrument Calibration Standards: Individual compounds obtained from Chem Service (see 5.14) are mixed in iso-octane with concentrations ranging from 1.0 ppb to 100 ppb.

5.16 Second Source Standards: Pyrethroid analytes were obtained from AccuStandard, New Haven, CT and Ultra Scientific, North Kingstown, RI for verification of calibration standards.

**CAUTION**

***The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. 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 data handling Material Safety Data Sheets should also be made available to all personnel involved in these analyses.***

## 6.0 Sample Collection, Preparation, and Storage

6.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and/or motor vehicle engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination.

6.2 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned (e.g., stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, rinsed with a high-purity acetone, and finally rinsed with Type II water.

## 7.0 Sample Extraction

7.1 Remove sediment samples from freezer and allow to thaw. Prior to extraction, sediment samples are thoroughly mixed by hand using a clean glass rod or may be homogenized using a Polytron homogenizer equipped with stainless steel generator equipped with Teflon bearings. Sample sets of 12-16 should be extracted when possible. The ASE-200 extractor will extract 24 cells. Be sure to reserve enough cells for method blanks, matrix spikes, and laboratory control spikes.

7.2 A separate extraction bench sheet is started for each project, sample matrix type, and analysis type. Several bench sheets may be used for an extraction set.

7.3 Prepare a glass rod or Teflon spatula for each sample to be weighed by rinsing 3 times with petroleum ether using a Teflon wash bottle.

7.4 Label 60 mL VOA vials for the collection of the sample extract. The labels must be placed between 1.5" and 3" from the top of the VOA cap; if they are placed outside of this area, they will interfere with the ASE optical sensor. Use two VOA vials for each sample. Label the first VOA vial with the ASE position number, bench sheet number and the sample name. Label the second VOA vial the same but add “RE” to distinguish between the two vials. Label and weigh aluminum planchets for moisture determinations (samples ID can be made on the bottom of planchets using a ball point pen.

7.5 Tare a 250 mL glass jar. Using a clean (solvent rinsed) glass rod, stir the sediment so that the mixture is homogeneous. Weigh 10 g of sample into the jar, record the weight on the bench sheet, and add the twice-extracted Hydromatrix from one ASE cell. Stir the mixture thoroughly and go on to the next sample. After approximately 15 minutes

stir the sample again. Repeat this at 15 minute intervals two more times or until the sample mixture is free flowing.

7.6 Weigh 1-5 g of additional sample into a pre-weighed and tared aluminum planchet for % moisture analysis. Place planchets in 70$^{o}$C oven for 48 hours and re-weigh dry weight.

7.7 Place a pre-rinsed powder funnel on top of a 33 mL ASE cell containing a pre-extracted cellulose filter *(the filter is the one that was used to pre-extract the Hydromatrix).*

7.8 Pour the sediment/Hydromatrix mixture through the powder funnel back into the extraction cell that the Hydromatrix was poured from. Tap the cell against the counter top to settle the contents. The mixture will fill the cell and it may be necessary to pack it slightly using the glass rod and the end of the powder funnel. The cells used for the **method blank** and **laboratory control spike** and its duplicate *(if used)* will contain only Hydromatrix.

7.9 **All** of the extraction cells are spiked with the pesticide surrogate standard. Spike each cell with exactly **1.0 mL** of the pesticide surrogate solution (20 ng/mL). Surrogate spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.10 The extraction cells used for the matrix spike (MS) and duplicate matrix spike (MSD) and laboratory control spike (LCD) and its duplicate (LCSD) (if used) are spiked with exactly **1.0 mL** of the pesticide matrix spike solution (20 ng/mL). Matrix spikes must be witnessed, recorded and dated on the extraction bench sheet.

7.11 The extraction cells are capped *(Firmly tightened but do not overtighten)* and placed on the ASE 200 carrousel. The first set of labeled VOA collection vials are placed on the ASE 200 collection carrousel with the position numbers corresponding to the position numbers of the extraction cells. Make sure that the solvent reservoir contains enough solvent for the extraction.

7.12 Samples are extracted with acetone/methylene chloride (DCM) 50:50 using the following conditions:

| | |
|---|---|
| Pre-heat | 0 min. |
| Heat | 5 min. |
| Static | 5 min. |
| Flush | 60% |
| Purge | 300 sec. |
| Cycles | 1 |

| | |
|---|---|
| Pressure | 1500 psi |
| Temp | 100 °C |
| Sol A Other | 100% |

7.13 After the initial extraction is complete, remove full VOA vials and place in a Wheaton rack. Place the second set of collection VOA vials labeled "RE" on the ASE carrousel. Check each of the extraction cells to make sure that the caps are *(firmly tightened)* as they tend to loosen with the first extraction. Make sure that the replacement vials are in the correct order. Make sure that the solvent reservoir contains enough solvent for the re-extraction. Re-start the ASE-200.

7.14 When extraction is completed, place VOA vials in a Wheaton rack with the "RE" vials next to the vials from the first extraction. The extracts should be re-capped with solid green caps (Qorpak) and placed in a refrigerator for storage until they are removed for the GPC cleanup procedure.

## 8.0 Gel Permeation Chromatography

**IMPORTANT:** ***All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether before they are used for this procedure.***

8.1 Remove VOA vials containing the sample extracts from the refrigerator. Make sure the vials are capped with the green Qorpak caps. Allow them to sit out until they are at room temperature.

8.2 Set up and label pre-cleaned K-D flasks (4-6) with concentrator tubes attached on ring stands in the fume hood. Place a 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 pre-rinsed sodium sulfate to the funnel. Make sure that the level of the sodium sulfate is uniform across the funnel to prevent any possible splashing out.

8.3 Pour sample extracts from the VOA vials through sodium sulfate into the K-D flask. Add about 10 mL of DCM to the VOA vial, cap and shake 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 (~50 mL) by pouring from a clean and rinsed 400 mL beaker. After the solvent has completely drained through the sodium sulfate add one more additional rinse of DCM (~50 mL) from the beaker of clean DCM. Allow the DCM to completely drain through the sodium sulfate (~3-5 minutes).

8.4 Add 0.5 mL Iso-Octane using a macro-pipetter and a solvent rinsed boiling chip to each K-D flask. Place a Snyder column on the K-D flask and place the flask on the hot water bath set at 80-82°C. Evaporate the solvent until the reflux line falls below

the top of the Snyder column. At this point there should be between 1-5 mL visible in the concentrator tube while the K-D apparatus is still on the hot water bath and 10 mL or less of the solvent remaining after the K-D flask is removed from the hot water bath and the solvent drains from the Snyder column. Dry off the water using a WyPall X60 towel to remove any water from around the ground glass union of the concentrator tube and the K-D flask to prevent any of it from entering the concentrator tube upon removal.

8.5 After the K-D apparatus has cooled and all of the solvent has drained from the Snyder column, remove the Snyder column, label the concentrator tube and then remove the concentrator tube from the flask and place the tube in a test tube rack and cover with pre-rinsed aluminum foil. Rinse the Snyder column with petroleum ether and place back in the column rack for storage. After all of the flasks have been removed from the hot water bath, repeat steps 2-5 for the remaining samples extracted with this set.

8.6 Add a new micro-boiling stone and place a clean micro-Snyder column on the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75$^{o}$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. Evaporate the solvent until only 0.5 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

8.7 When the solvent has been evaporated to 0.5 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add DCM to the concentrator tube to reach a final volume of 10.0 mL.

8.8 Draw the sample up into a clean 10 mL syringe with a 0.45 µm filter attached. Filter the sample into a 12 mL culture tube. Using a volumetric pipet remove 2.0 mL of the filtered sample and place it in a pre-weighed aluminum planchet for lipid determination. Cap the culture tube with the Teflon-insert style caps. Mark the bottom of the meniscus with a pen in case of evaporation before clean-up on GPC.

8.9 All samples are cleaned using a $J_2$ Scientific GPC (Autoinject 110, AccuPrep 170, DFW-20 Fixed Wavelength Detector, 1" ID glass column with 70g Bio-Beads SX-3 in 100% DCM)

8.9.1 From the desktop double click on the AccuPrep.exe shortcut to open the program. Click on the Use Injector button and allow the instrument time to initialize. Activate the pump by using the top left hand button. A solvent Control Pump window will open up. Click on the Apply Defaults button and then OK on the Selected Pressure Limit 30 psi. The pump should audibly be heard coming on and the green light should show that the system is on line and status flowing. Make sure that the

bottle of clean DCM is full and the waste bottle is empty. Allow the system to pump for about 5 minutes before switching the column in-line (gray button next to Column that has 'Put in line' on it). The pressure will be observed to normally go up to the 12-16 psi range. Turn the power on to the detector to allow it at least 30 minutes of time to warm up before use. Because the scale is auto-adjusted in the software now it is no longer necessary to manually adjust the range on the unit itself.

8.9.2 While the system is equilibrating, the sequence can be entered. Click on the Seq button next to the Pump button. An 'Editing new sequence' window will pop up. This gives a view of the instrument which clearly shows the sample tray locations and the corresponding sample collection locations. By clicking on the sample tray position, a new window 'Adding sample at tray position #' will pop up. This allows information to be included about each specific sample. Sample position 1 will always be a calibration standard (CLP-340) which is run prior to any sequence of runs to verify instrument integrity. In the Sample ID field just type in 'CLP-340'. In the Descrip (optional), information pertaining to the project, laboratory control number, bench sheet number and date are typically added. The Method File needs to be changed to 'GPC-Sed' for only this sample and in the Sample Type field the 'Calibration' type can be chosen. After this information is completed click on the OK to continue. This returns you back to the main sequence window but now the first position will be highlighted in green. Continue by adding the next sample information to tray position 2, again following the same steps as before. The Method File name to be used is 'Sed-Pest'. Also by default, the Sample Type field will already be set at 'Sample'. This will not need to be changed until a duplicate sample (Duplicate), matrix spike (Matrix Spike), matrix spike duplicate (Spike Duplicate) and laboratory control spike (Spiked Blank) are encountered. After all the samples have been added to the sequence, save it as the WPCL log-in number (L-###-##). From the Editing sequence window print out the sample list. Compare the information to your original bench sheet to insure there are no mistakes. Make sure the GPC-Sed method is being used for the calibration standard and 'Sed-Pest' method is being used for the samples. Next verify that the samples are still at the marked line on the culture tubes (add DCM to the marked line if they are not). Place a tube with the GPC Calibration Standard Solution (CLP-340) in sample tray position 1 and then follow as the sequence was made in the remaining positions.

8.9.3 Get two boxes of the 125 mL Trace Clean amber bottles for sample collection. A bottle does not need to be placed in collection position #1 because that is the GPC Calibration Std (all goes to waste). Remove the white caps from the bottles and place them on top of the detector (so that Teflon side is not exposed to possible contamination). Label the boxes with laboratory control numbers and keep them for the post-GPC samples to be stored in. Now that the pump has had plenty of time to equilibrate the system and the detector has had plenty of time to warm up, in the Signal field click to adjust the setting to 'Absorbance Units' and click on the 'Zero

Signal' button to set the baseline.

8.9.4 If the pressure seems to be pretty stable between the 12-16 psi range and all the samples positions and collection positions have been loaded, then click on the large button with the stop watch to begin the program. A window will pop up asking if the correct column method is loaded (100%DCM). Click on 'yes' to engage the syringe pump to begin priming. The sample probe will move over to sample position #1 and aspirate the sample. After the samples have all been processed (~1 hour per sample), remove the label from the sample position and place it on the bottle in corresponding collection position. Cap the bottle and place it back in the box that was retained for their storage. At the end of the sequence there will be a window that pops up saying that the 'Sequence has been successfully completed'. The column will switch offline and the pump will automatically shut down. The only thing that has to manually be turned off is the power to the detector.

8.10 Pour the GPC eluate into a rinsed K-D flask. Rinse the bottle with some DCM and add that to the K-D flask. Add 0.5 mL Iso-Octane and a micro boiling chip to each K-D flask. Attach a Snyder column to the flask and place in the hot water bath. When the volume of the solvent in the concentrator tube is level with the base of the K-D flask, lift the K-D apparatus up enough to be able to angle it slightly and add 40-50 mL Petrolem Ether through the top of the Snyder column. By holding the K-D apparatus at an angle, it allows the solvent to more easily drain back into the flask. Return to the K-D apparatus back into the hot water bath. Repeat this step 2 more times to successfully solvent exchange the sample from DCM to Petroleum Ether. When the apparent volume in the concentrator tube is 5-10 mL remove it from the hot water bath. Wipe down the K-D apparatus with a WyPall X60 towel especially around the ground glass junction. Remove the Snyder column from the K-D apparatus and allow to completely drain into the concentrator tube. After it has finished cooling, remove the concentrator tube and place a micro-Snyder column on it. Add a new micro boiling chip and place it in a 400 mL beaker containing water heated to approximately 75$^{o}$C on a hot plate (4-5 tubes can be evaporated at one time). Evaporate the solvent down to 1-2 mL. Remove it from the water bath and allow it to cool.

8.11 Transfer the solution to a 13 x 100 culture tube with a Pasteur pipette, rinse the concentrator tube with 0.5 ml of Petroleum Ether, vortex, and transfer the rinse to the culture tube. Repeat the rinse step two more times, and add each rinse to the culture tube. Cap the culture tube with a Teflon faced cap. Place extracts in a refrigerator for storage until the final Florisil column cleanup is done.

## 9.0 Florisil Column Fractionation

**IMPORTANT:** ***All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether (PE) before they are used for this procedure. Florisil[7] must be activated in an oven at 130°C for at least 24 hours prior to use.***

9.1 This procedure is performed after the GPC cleanup procedure for all sediment samples analyzed for pyrethroids.

9.2 Prepare the reagents to be used for Florisil cleanup: 6% ethyl ether in petroleum ether and 15% ethyl ether in PE. Make an amount slightly in excess of what is actually needed to allow for any loss which may occur during solvent transfer. The required volume is 200 mL per sample for the 6%(F2) and 220 ml per sample for the 15%(F3).

9.3 Prepare the chromatography columns. Place a small piece of PE rinsed glass wool in the bottom of the column and tap into place with a PE rinsed glass rod. Cover with a small portion (0.5 inch) of sodium sulfate. Fill the column with 5 grams of Florisil that has been measured using a dedicated pre-calibrated culture tube. Tap column with rubber "mallet" to firmly settle the Florisil. Top the column with 3/4-1 inch of sodium sulfate. This will prevent the column from being disrupted when solvent is added and will remove any residual water.

9.4 Place a 600 mL beaker under the column and pre-wet the column with about 25 mL of petroleum ether.

**IMPORTANT:** ***From this point and through the elution process, the solvent level should <u>never</u> be allowed to go below the top of the sodium sulfate layer.***

9.5 When approximately 1 inch of PE remains above the surface of the column, place a K-D flask under column making sure that the stopcock is in the full open position. This will allow for a flow rate of about 2 to 3 mL/min. When the meniscus of the PE rinse reaches the column bed surface, introduce the sample on to the column using a long stem Pasteur pipette. Immediately add approximately 0.5 mL of PE to the tube, vortex, and add the rinse to the sample extract on the column. Add another 0.5 ml of PE to the tube, vortex, and add this final rinse to the sample extract on the column. Start the columns in a sequential fashion, and the lag time will be adequate to perform the necessary tasks for up to six columns.

9.6 When the combined sample and rinses reach the sodium sulfate layer, add 200 mls of Petroleum Ether that has been carefully measured out using a graduated cylinder to the column reservoir. Make sure that the stopcock is fully open in order to achieve the desired flow rate of 5 ml per minute. Collect directly into a clean PE rinsed 500 mL

KD flask. This fraction contains surrogate DBOB only. No pyrethroid compounds elute in this fraction. See Table 2.

9.7 Just as the last of the F1 solvent reaches the top of the sodium sulfate layer, change the K-D flask, add 200 ml of the 6% diethyl ether/PE mixture to the column reservoir, replace with a second clean PE rinsed 500 mL KD flask, and elute as before. Add a micro boiling stone and attach a Snyder column with a green clamp to the K-D flask containing the 0% (F1) fraction and place vessel in the hot water bath with the temperature set at 80-82 $^{o}$C and reduce volume to an apparent volume of 1 mL. Tap the Snyder column to make sure solvent is not trapped between the balls then remove the vessel from the bath and place in the vessel stand to cool.

9.8 Repeat the above using 220 mL of 15% diethyl ether/PE mixture.

9.9 When the vessels are cool, remove the concentrator tube from the K-D flask add a new micro boiling stone and attach a clean micro-Snyder column to the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75$^{o}$C on a hot plate. Evaporate the solvent until only 0.5-1 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.

9.10 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. 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. Transfer the extract to a clean labeled culture tube and cap.

9.11 Repeat for 6% (F2) and 15% (F3) extracts. The extracts are ready for analysis by GC-ECD.

## 10.0 Analytical Procedure

10.1 Before the sample extracts can be analyzed by the GC-ECD, a sequence listing the order of calibration standards, second source check standards, initial and continuing calibration blanks, initial and continuing calibration verification standards and sample extracts is written using Agilent Chemstation Software.

10.2 Each GC sequence includes a minimum of seven calibration standards. The calibration curve concentration for pyrethroids differs for different analytes, but in general the range is 1 ppb to 100 ppb.

10.3 To verify the calibration standards, second source pesticide check standards (see 5.16) are

analyzed.

10.4 An initial calibration blank and initial calibration verification standard is analyzed after the calibration standards and prior to the first sample extract. Continuing calibration blanks (CCBs) and calibration verification standards (CCVs) are analyzed after ten sample extracts have been analyzed. If a CCV fails, the five samples prior to the failed CCV and the five samples after the failed CCV are re-analyzed after a new calibration curve is analyzed.

10.5 The CCV analyte concentrations are mid-range of the calibration curve (20-60 ppb).

10.6 As the run proceeds, sample extracts are monitored for analyte concentrations that are greater than the calibration curve and need dilution.

10.7 Instrumentation

10.7.1 Agilent 6890*plus* gas chromatograph equipped with two $^{63}$Ni micro-electron capture detectors with EPC and autosampler. Two 60 meter, 0.25 mm ID, 0.25 um (film thickness) fused silica columns (J&W) are used. A 5 meter length of DB-5 column is connected to a press fit "Y" union which splits the column effluent into two 60 m columns, a DB-5 and a DB-17. The injector is a split-splitless injector with EPC.

10.7.2 Chromatograph conditions:
The injector is operated isothermal at 240$^{o}$C. The oven has an initial temperature of 130$^{o}$C which is held for 1 minute and then temperature programmed to 240$^{o}$C at a rate of 20$^{o}$C/min and held for 7 min. It is then programmed to 290$^{o}$C at a rate of 2$^{o}$C/min and is held for 16.50 min. Helium is used as the carrier gas at a linear velocity of 33 cm/sec. Nitrogen is used for the detector makeup at 30 mL/min. See Table 3 for elution order of pyrethroid analytes.

10.7.3 Sample volume:
Three microliters of samples and standards are injected and split approximately 50/50 onto the 60 m DB-5 and the 60 m DB-17.

10.7.4 Data processing:
An Agilent 3365 Series II Chemstation is used for detector signal acquisition and analysis.

**Table 3 Elution Times of Pyrethroid Analytes from J&W 60 Meter Columns**

| | Retention Time (minutes) | |
|---|---|---|
| **Pyrethroids** | **DB-5** | **DB-17** |
| Bifenthrin | 21.47 | 23.57 |
| Cyfluthrin-1 | 30.26 | 36.15 |
| Cyfluthrin-2 | 30.63 | 36.51 |
| Cyfluthrin-3 | 30.98 | 36.90 |
| Cyfluthrin-4 | 31.13 | NA |
| Cypermethrin-1 | 31.58 | 38.28 |
| Cypermethrin-2 | 31.98 | 38.70 |
| Cypermethrin3 | 32.32 | 39.08 |
| Cypermethrin-4 | 32.47 | NA |
| Es-fenvalerate-1 | 35.54 | 43.79 |
| Es-fenvalerate-2 | 36.42 | 45.12 |
| Lambda-cyhalothrin-1 | 24.25 | 27.29 |
| Lambda-cyhalothrin-2 | 24.98 | 28.11 |
| Permethrin-1 | 27.88 | 34.12 |
| Permethrin-2 | 28.38 | 34.73 |
| DBOB (surrogate) | 8.60 | 8.13 |

## 12.0 Method Performance

Average Percent Recoveries and Standard Deviations for LCS/LCSD and MS/MSD Pyrethroid Analytes

| Pyrethroids - sediment | Ave % Rec LCS/LCSD | Std Dev LCS/LCSD | Ave % Rec MS/MSD | Std Dev MS/MSD |
|---|---|---|---|---|
| Bifenthrin | 99.3 | 5.55 | 115.2 | 28.1 |
| Cyfluthrin-1 | 94.9 | 13.2 | 106.0 | 2.58 |
| Cyfluthrin-2 | 98.9 | 16.6 | 96.8 | 18.8 |
| Cyfluthrin-3 | 86.1 | 2.67 | 77.8 | 7.55 |
| Cyfluthrin-4 | 77.1 | 4.67 | 83.9 | 17.0 |
| Cypermethrin-1 | 88.4 | 3.26 | 105.5 | 17.6 |
| Cypermethrin-2 | 77.8 | 2.31 | 90.4 | 14.1 |
| Cypermethrin3 | 78.2 | 3.21 | 79.7 | 8.49 |
| Cypermethrin-4 | 76.2 | 1.91 | 106.0 | 21.2 |
| Es-fenvalerate-1 | 77.5 | 17.8 | 91.7 | 12.8 |
| Es-fenvalerate-2 | 89.7 | 16.9 | 112.3 | 4.35 |
| Lambda-cyhalothrin-1 | 96.3 | 14.3 | 99.1 | 11.5 |
| Lambda-cyhalothrin-2 | 91.9 | 17.8 | 101.7 | 15.7 |
| Permethrin-1 | 88.3 | 14.5 | 92.6 | 6.70 |
| Permethrin-2 | 83.0 | 6.61 | 75.1 | 16.9 |
| | | | | |
| DBOB (surrogate) | 104 | 12.6 | 87.3 | 10.2 |

LCS/LCSD – laboratory control spike/duplicate
MS/MSD – matrix spike/duplicate

## 12.0 References

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