Title: Determination of Pyrethroids in Sediment Water Using Triple Quadrupole GC/MS/MS

1. Scope:

This section method (SM) documents a selective pyrethroid analysis in sediment water and is followed by all authorized EMON personnel. This method uses the triple quadrupole to improve sensitivity and enables the lowering of the reporting limit over the previous method which used the ECD and MSD.

2. Principle:

The SM describes the method for determination of resmethrin, bifenthrin, fenpropathrin, lambda cyhalothrin epimer, lambda cyhalothrin, permethin cis, permethrin trans, cyfluthrin, cypermethrin, fenvalerate/ esfenvalerate and deltamethrin in sediment water. The pyrethroids are extracted from the sediment water using liquid-liquid extraction with hexane. The extracts are concentrated and then cleaned up with florisil before being analyzed with a gas chromatography equipped with triple quadrupole detector. The reporting limit is 10 ppt for resmethrin, 2 ppt for bifenthrin and 5 ppt for all the rest of the compounds.

3. Safety:

3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.

3.2 Hexane is a flammable and toxic solvent; it should be handled with care in a ventilated area.

4. Interferences:

There were no interferences at the time of validation for the background water provided.

5. Apparatus and Equipment:

5.1 Rotary Evaporator (Buchi/Brinkman or equivalent)
5.2 Nitrogen evaporator (Meyer N-EVAP Organomation Model #112 or equivalent)
5.3 Balance, (Mettler PC 4400 or equivalent)
5.4 Vortex-vibrating mixer
5.5 Gas Chromatograph equipped with a triple quadrupole

6. Reagents and Supplies:

6.1	Bifenthrin	CAS#42576-02-3
6.2	Fenpropathrin	CAS#39515-41-8
6.3	Lambda cyhalothrin epimer	CAS# unknown
6.4	Lambda cyhalothrin	CAS#91465-08-06
6.5	Permethrin cis	CAS#54774-45-7
6.6	Permethrin trans	CAS#51877-74-8
6.7	Cyfluthrin	CAS#68369-37-5
6.8	Cypermethrin	CAS#52315-07-8
6.9	Fenvalerate	CAS#51630-58-1
6.10	Deltamethrin	CAS#52918-63-5
6.11	Resmethrin	CAS#10453-86-8

6.12 Hexanes, nanograde or equivalent pesticide grade
6.13 Diethylether, nanograde or equivalent pesticide grade
6.14 Separatory funnel, 2 L
6.15 Boiling flask, 500 mL
6.16 Sodium Sulfate, ACS grade
6.17 Funnels, short stem, 60°, 10 mm diameter
6.18 Glass wool, Pyrex® fiberglass slivers 8 microns
6.19 Beaker, 1 L
6.20 Florisil SPE cartridge, 2 grams with 20 mL reservoir
6.21 Volumetric Pipette, 1 mL
6.22 Test tube, 50 mL
6.23 Test tube, 15 mL
6.24 Disposable Pasteur pipettes, and other laboratory ware as needed
6.25 Recommended analytical columns:
Varian –VF-5ms arylene stabilized phase equivalent to 5% phenyl, 95% dimethylpolysiloxane fused silica column, 30 m x 0.25 mm id x 0.25 um film thickness.

7. Standards Preparation:

7.1 The individual pyrethroid stock standards of 1.0 mg/mL were obtained from the CDFA/CAC Standards Repository. The standards were diluted to 10 µg/mL with hexanes for identification purposes.

A combination standard of 10 µg/mL was prepared from individual mg/mL standards with acetone to be used for fortification. Another 10 µg/mL combination standard was prepared in hexanes and was diluted to the following

concentrations: 0.005, 0.01, 0.025, 0.05, 0.1, 0.2, 0.5 µg/mL in hexanes for instrument calibration. The calibration standards are added to blank matrix extracts to correct for matrix background response enhancement.

7.2 Keep all standards in the designated refrigerator for storage.

7.3 The expiration date of each standard is six months from the preparation date.

8. Sample Preservation and Storage:

Store all samples waiting for extraction in a separate refrigerator (32-40 °F)

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the sediment water for background to be used in method validation and QC. The sediment water was prepared by adding 5 g of soil to approximately a liter of American river water.

9.2 Spike

Take a liter of background sediment water from refrigerator and allow it to come to room temperature. Fortify at a level requested by client. After fortification mix well and process same as samples.

9.3 Test Sample Extraction

9.3.1 Remove water samples from refrigerator and allow samples to come to room temperature before weighing them. Record weight.

9.3.2 Transfer the water sample to a 2 L separatory funnel leaving as much of the sediment as possible in the sample bottle.

9.3.3 Add 60 mL of hexanes to the sample bottle and manually shake for 30 seconds.

9.3.4 Transfer hexane and sediment into the separatory funnel and shake for 2 min., venting frequently.

9.3.5 Allow the layers to separate, drain the lower aqueous layer into a 1L beaker. Pour the hexane layer through a funnel containing a plug of glasswool and approximately 40 g sodium sulfate into a 500 mL boiling flask.

9.3.6 Transfer the water from the beaker into the separatory funnel and repeat steps 9.3.3 – 9.3.6 two more times shaking for 1 min. Combine the extracts in the same boiling flask. Record sample bottle weight.

9.3.7 Rotory evaporate to ~ 5 mL under vacuum at approximately 20-24 inch Hg in a water bath at 42-45° C.

9.3.8 Transfer the extract to a 15 mL test tube. Rinse flask 3 times with approximately 2 mL of hexane and transfer each rinsate to the same test tube.

9.3.9 Place the test tube on a nitrogen evaporator under a gentle stream of nitrogen with water bath set at 40-45° C and concentrate to ~ 2 mL final volume.

Cleanup

9.3.10 Condition a 2 g florisil SPE cartridge with 10 mL of 15% diethylether in hexane followed by 20 mL hexane. Do not allow cartridges to go to dryness.

9.3.11 Carefully load the sample extract onto the conditioned florisil SPE cartridge. Rinse the tube that previously contained the extract twice with 2 mL hexane. Add rinses to florisil cartridge.

9.3.12 Elute the pesticides from the cartridge with 30 mL of 15% diethylether in hexane and collect in a 50 mL tube.

9.3.13 Evaporate the sample eluants to dryness under a gentle stream of nitrogen in a 40-45° C water bath.

9.3.14 Pipet 1mL of hexane into the test tube and vortex well. Vial extract into 2 autosampler vials with inserts.

10. Instrument Calibration:

10.1 The calibration standards are added to blank matrix extracts to correct for matrix background response enhancement.

10.2 The calibration standard curve consists of a minimum of three levels. The recommended concentrations levels of standards are 0.001, 0.005, 0.01, 0.025, 0.05, 0.1, 0.2, or 0.5 µg/mL. Calibration is obtained using a linear or quadratic regression with the correlation coefficient (r) equal to or greater than 0.995.

11. Analysis:

11.1 Injection Scheme

The instrument may need to be conditioned with a matrix blank or old sample before running the following sequence of Standard Curve, Hexane, Matrix Blank, Matrix Spike, Test Samples (maximum of 10 – 12) and Standard Curve.

11.2 GC-Triple Quadrupole Instrumentation

11.2.1 Gas Chromatograph: Varian CP-3800
Column: Varian Factor Four VF-5ms 30M x 0.25mm x 0.25um.

Temperature Program: initial column temperature 80 °C, hold 1 min., ramp at 40 °C/min. to 180 °C hold for 0 min., ramp at 5 °C/min. to 305 °C hold for 0.5 min..

Injector Temperature: 250°C
Injection Volume: 1 µL
Carrier Gas:Helium 1mL/min.

Triple Quadrupole: Varian Triple Quad 320-MS
Ionization: Positive Electron Impact
Transfer Line: 300°C
Source Temp: 200°C
Collision Gas: Argon @ 1.8 mTorr

Compound	Retention Time (min.)	Precursor Ion	Product Ion	Collision Energy/-ev
Resmethrin 1	16.3	171	128,143	12
Resmethrin 2	16.5	171	128,143	12
Bifenthrin	17.3	181	166	15
Fenpropathrin	17.6	265	210	15
λ Cyhalothrin epimer	18.8	208	181	10
λ Cyhalothrin	19.2	208	181	10
Permethrin cis	20.7	183	168	23
Permethrin trans	20.9	183	168	23
Cyfluthrin 1,	21.7	226	206	15
Cyfluthrin 2	21.9	226	206	15
Cyfluthrin 3	22.0	226	206	15
Cyfluthrin 4	22.1	226	206	15
Cypermethrin 1,	22.3	181	152	20
Cypermethrin 2	22.5	181	152	20
Cypermethrin 3	22.6	181	152	20
Cypermethrin 4	22.7	181	152	20
Fenvalerate	24.2	167	125	15
Esfenvalerate	24.4	167	125	15
Deltamethrin	25.5	253	174	10

12. Quality Control:

12.1 Method Detection Limits (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 sediment water samples are spiked at 5 ppt except resmethrin, which was spiked at 10 ppt and processed through the entire method along with a blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL for each analyte using the following equation:

MDL = tS

Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL, t=3.143. The results for the standard deviations and MDL are in Appendix 1.

12.2 Reporting Limit (RL)

Reporting limit (RL) refers to a level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. The reporting limit for resmethrin is 10 ppt, bifenthrin is 2 ppt and for all other compounds is 5ppt.

12.3 Method Validation

The method validation consisted of three sample sets. Each set included three levels of fortification and a method blank. All spikes and method blanks were processed through the entire analytical method. Spike levels and recoveries for the pyrethroids are tabulated in Appendix 2.

12.4 Control Charts and Limits

Control charts were generated using the data from the method validation for each analyte. The upper and lower control limits are set at ± 3 standard deviations of the % recovery, shown in Appendix 2.

12.5 Acceptance Criteria

12.5.1 Each set of samples will have a matrix blank and a spiked matrix sample.

12.5.2 The retention time should be within ± 2 per cent of that of the standards.

12.5.3 The recoveries of the matrix spikes shall be within the control limits.

12.5.4 The sample shall be diluted if results exceed the calibration curve.

13. Calculations:

Lambda cyhalothrin/epimer, cyfluthrin, cypermethrin and fenvalerate are expressed as the sum of their isomers. Therefore, the total residues should be calculated using the sum of their peak responses.

Quantitation is based on external standard (ESTD) calculation using either the peak area or height. The MSD uses linear regression fit, with all levels weighted equally. Alternatively, at chemist discretion, concentrations may be calculated using the response factor for the standard whose value is closest to the level in the sample.

$$ppt = \frac{\text{(sample peak area or ht) x (std conc) x (std vol. Injected) x (final vol of sample)(1000)(1000)}}{\text{(std.peak area or ht) x (sample vol injected) x (sample wt (g))}}$$

14. Reporting Procedure:

Sample results are reported in accordance with the client's analytical laboratory specification sheets.

15. Discussion:

15.1 This method was developed to lower the reporting limit for the pyrethroids by using triple quadrupole mass spectrometry. The only change from the previous method EMON-SM-05-003 is the instrumentation. Since the extraction procedure is the same as the previous method a reduced number of spikes were analyzed for validation.

15.2 Negative chemical ionization (NCI) in selected ion monitor mode was also tried for the pyrethroids and showed some promise for all compounds except resmethrin which provided no signal. Method detection limits and validation resembled those found in EI mode. Future samples that have high background noise will be analyzed by both techniques since in chemical ionization mode the background noise has a different chemical origin and might offer some improvement. In the case of the background matrix provided for the QC there was little benefit observed by running samples under CI mode.

15.3 The sample matrix may require that the injector liner be changed more frequently and the column trimmed to maintain sensitivity. The ion volume and the source may also need to be cleaned more frequently.

15.4 This method was adapted from the methods listed in the references below.

16. References:

16.1 J. White, *Analysis of Pyrethroids in Sediment Water* Emon-SM-05-003, 2006, California Department of Food and Agriculture, Center for Analytical Chemistry, Environmental Analysis Section, 3292 Meadowview Road, Sacramento, California 95832

16.2 J. You, D.P. Weston, M. J. Lydy, *A Sonication Extraction Method for the Analysis of Prethroid, Organophosphate, and Organchlorine Pesticides from Sediment by Gas Chromatography with Electron-Capture Detection,* Archives Environmental Contamination and Toxicology 47, 141-147 (2004)

16.3 J. You, M. J. Lydy, *Evaluation of Desulfuration Methods for Pyrethroid, Organophosphate, and Organochloride Pesticides in Sediment with High Sulfur Content,* Archives Environmental Contamination and Toxicology 47, 148 -153 (2004)

16.4 J. White, H. Feng, Determination of Pyrethroids in Sediment Water, EMON-SM-52-7.1, 2004, California Department of Food and Agriculture, Center for Analytical Chemistry, Environmental Monitoring Laboratory, 3292 Meadowview Road, Sacramento, California 95832

Appendix 1

The determination of Method Detection Limit (MDL) and Reporting Limit (RL)

Spike level is 5 ppt for all compounds except Resmethrin, which is 10 ppt

	Bifenthrin	Fenopropathrin	λcyhalothrin Epimer/ λcyhalothrin	Permethrin cis	Permethrin trans	Cyfluthrin
	ppt	ppt	ppt	ppt	ppt	ppt
blk sed	n/d	n/d	n/d	n/d	n/d	n/d
spk1	5.74	5.51	5.87	5.72	4.10	5.68
spk2	4.98	4.57	5.23	6.04	4.06	5.23
spk 3	5.57	5.00	5.46	5.54	4.39	5.46
spk 4	5.36	4.71	5.71	5.40	3.85	5.53
spk 5	5.46	5.58	6.76	6.01	4.75	6.32
spk 6	5.02	4.42	5.93	5.29	4.24	5.90
spk 7	4.86	5.07	5.39	5.36	3.92	5.04
Std dev	0.29	0.42	0.55	0.34	0.33	0.47
MDL	0.91	1.32	1.74	1.05	1.05	1.46
RL	**2 ppt**	**5 ppt**	**5 ppt**	**5 ppt**	**5 ppt**	**5 ppt**

	Cypermethrin	Fenvalerate/ Esfenvalerate	Deltamethrin	Resmethrin
	ppt	ppt	ppt	ppt
blk sed	n/d	n/d	n/d	n/d
spk1	6.27	5.11	4.86	9.22
spk2	4.99	4.44	5.29	8.68
spk 3	6.31	5.92	4.50	10.70
spk 4	5.02	4.75	4.93	10.20
spk 5	5.71	5.41	6.29	10.19
spk 6	5.45	4.99	5.14	12.79
spk 7	5.65	4.82	5.36	9.56
Std dev	0.49	0.53	0.56	1.33
MDL	1.54	1.66	1.77	4.18
RL	**5 ppt**	**5 ppt**	**5 ppt**	**10 ppt**

Appendix 2

Method Validation Data and Control Limits

Analyte	Spike ppt	Recovery % set 1	set 2	set 3		
Bifenthrin	5	88.6	93.0	75.8	Mean:	80.2
	10	79.9	83.3	75.7	SD:	6.81
	25	72.8	75.6	76.8	UCL:	101
					LCL:	59.7
Fenpropathrin	5	91.0	91.6	108	Mean:	91.0
	10	83.8	86.9	76.1	SD:	9.39
	25	88.8	102	90.8	UCL:	119
					LCL:	62.8
λ cyhalothrin /epimer	5	97.2	101	76.2	Mean:	87.4
	10	84.4	92	76.2	SD:	8.59
	25	90.4	85.2	83.6	UCL:	113
					LCL:	61.6
Permethrin cis	5	98.2	132	71.2	Mean:	93.9
	10	95.7	85.0	93.5	SD:	16.8
	25	82.4	88.4	98.8	UCL:	144
					LCL:	43.6
Permethrin trans	5	91.8	129	78.4	Mean:	96.2
	10	108	87.4	92.6	SD:	14.5
	25	92.8	91.6	94.0	UCL:	140
					LCL:	52.7
Cyfluthrin	5	105	120	103	Mean:	102
	10	101	103	89.4	SD:	8.43
	25	95.2	103	96.8	UCL:	127
					LCL:	76.5
Cypermethrin	5	102	113	74.6	Mean:	95.8
	10	101	96.1	101	SD:	10.7
	25	90.0	96.4	88.4	UCL:	128
					LCL:	63.5
Fenvalerate / Esf	5	96.8	122	93.8	Mean:	95.2
	10	90.7	100	89.9	SD:	11.0
	25	86.8	90.0	86.4	UCL:	128
					LCL:	62.2

Appendix 2 continued

Method Validation Data and Control Limits

Analyte	Spike ppt	Recovery % set 1	set 2	set 3		
Deltamethrin	5	110	104	95.6	Mean:	96.4
	10	93.5	99.0	64.5	SD:	13.4
	25	95.2	109	96.4	UCL:	137
					LCL:	56.2
Resmethrin	15	85.3	74.0	69.3	Mean:	74.5
	25	80.7	63.7	67.0	SD:	8.4
	50	79.8	65.7	84.8	UCL:	99.7
					LCL:	49.3

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