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 a GC/MS/MS instrument to improve sensitivity and enables the lowering of the reporting limit over the previous method.

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 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, 5 ppt for fenpropathrin, cypermethrin, fenvalerate,4 ppt for deltamethrin, 2 ppt for lambda cyhalothrin, cyfluthrin and 1 ppt for bifenthrin, permethrin cis and permethrin trans.

3. Safety:

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

3.2 Hexane and ethyl ether are flammable and toxic solvent; it should be handled with care in a fume hood.

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 Agilent 7010 GC/MS/MS system or equivalent.

6. Reagents and Supplies:

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

6.11 Hexanes, nanograde or equivalent pesticide grade
6.12 Diethylether, nanograde or equivalent pesticide grade
6.13 Separatory funnel, 2 L
6.14 Boiling flask, 500 mL
6.15 Sodium Sulfate, ACS grade
6.16 Funnels, short stem, 60°, 10 mm diameter
6.17 Glass wool, Pyrex® fiberglass slivers 8 microns
6.18 Beaker, 1 L
6.19 Phenomenex Strata Florisil SPE cartridge, 2 grams with 12 mL reservoir
6.20 Volumetric Pipette
6.21 Test tube, 50 mL
6.22 Test tube, 15 mL
6.23 Disposable Pasteur pipettes, and other laboratory ware as needed
6.24 Recommended analytical columns:

Two Agilent -5ms arylene stabilized phase equivalent to 5% phenyl, 95% dimethylpolysiloxane fused silica column, 15 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: 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1, 0.2 µg/mL in

hexanes for instrument calibration. The calibration standards are added to blank matrix extracts to correct for matrix enhancement.

7.2 Keep all standards in the designated refrigerator for storage.

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

8. Sample Preservation and Storage:

Store all samples waiting for extraction in a sample refrigerator (4 ± 3°C).

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 0.5 g of background 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 37- 42°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 ± 2°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 enhancement.

10.2 Quantitation is generally performed using calibration curves. A minimum of three data points shall be used to construct a linear curve. The quadratic curve requires a minimum of five points to define the curve.

The recommended concentrations levels of standards are 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1 or 0.2 µg/mL. Calibration is obtained using a linear or quadratic regression with the correlation coefficient (r^2) ≥0.990.

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 and Bracketing Standard.

11.2 GC-Triple Quadrupole Instrumentation

11.2.1 Gas Chromatograph: Agilent 7890B or equivalent

Column1: Agilent -5ms 15M x 0.25mm x 0.25um
Inlet Mode: Splitless
Inlet Temp: 240°C
Flow: 1.3 mL/min.
Column2: Agilent-5ms 15M x0.25mm x 0.25um.
Flow 1.5 mL/min
Control Mode: Constant Flow

Temperature Program: 60°C(1 min.)
40°C/min. to 170°C (0 min.)
10°C/min. to 310°C (0.5 min.)

Injection Volume: 1 µL
Carrier Gas: Helium 1.3 mL/min

Post Column Backflush
Backflush Start Time: 18.25 min

Post Run Duration: 1.0 min.
Oven Temp.: 325°C
Restrictor Temp: 325°C
Void Volume Backflushed: 4.0

Triple Quadrupole: Agilent 7010B
Acquisition Mode: Multi Reaction Monitoring (MRM)
MS Source: EI
Aux Temp: 310°C
Source Temp: 280°C
MS Quad 1 Temp: 150°C
MS Quad 2 Temp: 150°C
Collision Gas: Nitrogen

MRM Transitions

Compound	RT	Precursor Ion	Product Ion	Dwell (ms)	Collision Energy (v)
Resmethrin	12.96	171	143	124.3	10
		171	**128**	124.3	15
Bifenthrin	13.57	181.2	**166.2**	46	10
		181.2	165.2	46	25
		166.2	165.2	46	20
Fenpropathrin	16.69	264.9	**210**	45.9	10
		207.9	181	45.9	5
lambda Cyhalothrin	14.53	208.1	**181.1**	61.7	10
		181.1	152.1	61.7	30
		181.1	127.1	61.7	30
Permethrin Cis	15.25	183.1	**153.1**	58.7	15
		182.9	155.1	58.7	10
		162.9	91.1	58.7	15
Permethrin Trans	15.37	183.1	168.1	48.3	10
		163	**127**	48.3	5
		163	91	48.3	15
Cyfluthrin 1-4	15.93	226	206	61.6	25
		162.9	**127**	61.6	5
		162.9	90.9	61.6	15

Cypermethrin 1-4	16.21	181	152.1	61.6	25
		163	**127**	61.6	5
		163	91	61.6	10
Fenvalerate	17.1	419	225	58.5	2
		224.9	119	58.5	15
		167	**125.1**	58.5	5
Deltamethrin	17.74	252.9	**174**	48.2	5
		252.9	93	48.2	15
		250.7	172	48.2	5

Quant Ions in bold

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 2 and 5 ppt for different analytes 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 single tailed 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 is 10 ppt for resmethrin, 5 ppt for fenpropathrin, cypermethrin, fenvalerate,4 ppt for deltamethrin, 2 ppt for lambda cyhalothrin, cyfluthrin and 1 ppt for bifenthrin, permethrin cis, permethrin trans.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included four or five 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 percent recovery, shown in Appendix 2.

12.5 Acceptance Criteria

12.5.1 Each set of samples will have a matrix blank and a matrix spike. Solvent is injected after the highest level standard and before the first sample to demonstrate that no carryover of the standard has occurred.

12.5.2 The retention time of the analyte must match within ± 0.15 min. of the analyte in the standards within the same sequence.

12.5.3 Presence of the Quan and Qual ion overlay should be aligned. Ions used for monitoring must have intensities greater than 3x signal/noise.

12.5.4 The relative abundances of structurally significant ions used for confirmation must be within ± 30% relative when compared to a standard injected during the same run.

12.5.5 The sample shall be diluted if results exceed the 10% of the highest calibration standard in the curve.

12.5.6 The recoveries of the matrix spikes shall be within the control limits

13. Calculations:

Cyfluthrin, cypermethrin, fenvalerate and resmethrin 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 triple quadrupole uses linear or quadratic regression fit 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.

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

14. Reporting Procedure:

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

15. Discussion:

15.1 An instrument method was developed using Agilent Triple quadrupole GCMSMS. This method was updated and validated to lower the reporting limit for permethrin cis and trans to 1ppt and deltamethrin to 4ppt.

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

15.3 The new source of background water provided by DPR must be checked, to assure there is no low-level pyrethroid contamination before analysis and make matrix matching working standards.

15.4 The method detection limit (MDL) is used as a guide to determine the reporting limit (RL). The RL is chosen in a range 1 - 5 times of the MDL to meet the DPR's requirements.

15.5 It may be necessary to dilute samples due to matrix interference. Then the reporting would have to raise according the dilution factor.

15.6 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)

	Bifenthrin	Fenopropathrin	Λ-cyhalothrin	Permethrin cis	Permethrin trans	Cyfluthrin
	2 ppt	5 ppt	5 ppt	2 ppt	2 ppt	5 ppt
blk sed	ND	ND	ND	ND	ND	ND
spk1	1.64	4.93	3.89	1.68	1.77	4.33
spk2	2.04	5.20	4.23	1.95	2.06	5.30
spk 3	1.79	4.02	3.07	1.76	1.97	3.72
spk 4	1.86	4.43	3.52	1.69	1.71	4.17
spk 5	1.62	4.53	3.58	1.55	1.56	4.60
spk 6	1.19	4.04	3.20	1.30	1.33	3.31
spk 7	1.24	4.82	4.09	1.32	1.39	4.39
Std dev	0.31	0.45	0.44	0.24	0.28	0.64
MDL	0.99	1.40	1.37	0.74	0.87	2.00
RL	**1 ppt**	**5 ppt**	**2 ppt**	**1 ppt**	**1 ppt**	**2 ppt**

	Cypermethrin	Fenvalerate	Deltamethrin	Resmethrin
	5 ppt	5 ppt	5 ppt	5 ppt
blk sed	ND	ND	ND	ND
spk1	4.55	4.67	5.04	4.66
spk2	5.57	5.43	4.75	4.96
spk 3	4.46	3.73	4.37	3.09
spk 4	4.33	4.46	4.79	3.50
spk 5	4.78	4.74	5.04	4.36
spk 6	3.61	3.24	2.59	3.56
spk 7	4.57	5.05	3.80	3.98
Std dev	0.58	0.76	0.89	0.68
MDL	1.83	2.38	2.78	2.13
RL	**5 ppt**	**5 ppt**	**4 ppt**	**10 ppt**

Appendix 2

Method Validation Data and Control Limits

	Spike	Recovery %						
Analyte	**ppt**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**		**%**
Bifenthrin	2	82.0	102	89.5	93.0	81.0	Mean:	85.1
	5	94.3	97.2	69.8	85.8	84.2	SD:	9.10
	10	85.0	92.1	80.7	78.1	67.3	UCL:	112
	25	89.6	96.4	80.8	75.6	69.6	UWL:	103
	100	95.6	90.2	85.4	84.9	77.5	LWL:	66.9
							LCL:	57.8
Fenpropathrin	2						Mean:	92.5
	5	98.6	104	80.4	88.6	90.6	SD:	8.44
	10	88.7	96.6	87.1	81.9	76.0	UCL:	118
	25	100	102	90.0	88.0	85.6	UWL:	109
	100	108	99.0	99.5	95.8	89.1	LWL:	75.6
							LCL:	67.1
λ cyhalothrin	2	79.0	77.5	73.0	67.5	60.5	Mean:	73.2
	5	77.8	84.6	61.4	70.4	71.6	SD:	7.96
	10	75.7	78.0	69.6	64.9	59.2	UCL:	97.1
	25	82.8	83.6	69.6	65.2	65.6	UWL:	89.2
	100	88.8	78.3	79.0	77.4	69.6	LWL:	57.3
							LCL:	49.3
Permethrin cis	2	84.0	97.5	88.0	84.5	77.5	Mean:	90.9
	5	85.0	106	80.0	89.0	97.4	SD:	9.88
	10	92.2	93.0	90.1	79.3	71.6	UCL:	121
	25	100	102	88.4	89.6	78.4	UWL:	111
	100	113	103	99.4	93.2	91.0	LWL:	71.2
							LCL:	61.3
Permethrin trans	2	88.5	103	98.5	85.5	78.0	Mean:	93.2
	5	87.0	114	83.0	89.0	97.4	SD:	11.00
	10	78.4	97.2	94.7	78.9	74.6	UCL:	126
	25	92.0	110	94.4	95.2	82.0	UWL:	115
	100	115	103	102	95.4	92.3	LWL:	71.2
							LCL:	60.2

	Spike	Recovery %						
Analyte	**ppt**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**		**%**
Cyfluthrin	2	86.5	92.5	84.0	76.5	77.0	Mean:	90.3
	5	86.6	106	74.4	83.4	92.0	SD:	11.29
	10	80.4	94.3	84.5	76.4	77.9	UCL:	124
	25	95.6	108	87.6	93.2	87.2	UWL:	113
	100	119	106	101	94.4	93.2	LWL:	67.7
							LCL:	56.4
Cypermethrin	2						Mean:	92.9
	5	91.0	111	89.2	86.6	95.6	SD:	10.64
	10	82.5	96.7	94.5	78.5	72.6	UCL:	125
	25	96.4	107	88.4	95.6	81.6	UWL:	114
	100	112	107	83.7	94.3	93.00	LWL:	71.6
							LCL:	60.9
Fenvalerate	2						Mean:	92.4
	5	93.4	109	74.6	89.2	94.8	SD:	11.99
	10	84.8	94.3	86.0	84.3	76.4	UCL:	128
	25	97.2	110	83.2	82.0	80.8	UWL:	116
	100	117	112	95.5	96.5	87.8	LWL:	68.5
							LCL:	56.5
Deltamethrin	2						Mean:	90.0
	5	101	95.0	87.4	95.8	101	SD:	9.62
	10	105	89.9	77.8	82.8	80.5	UCL:	119
	25	100	99.8	77.6	78.4	80.8	UWL:	109
	100	104	84.2	93.9	84.8	80.3	LWL:	70.8
							LCL:	61.1
Resmethrin	2						Mean:	79.6
	5	93.2	99.2	61.8	70.0	87.2	SD:	11.89
	10	74.7	78.0	68.9	62.6	60.2	UCL:	115
	25	78.0	92.4	75.2	74	78.00	UWL:	103
	100	94.7	95.0	91.8	80.1	76.8	LWL:	55.8
							LCL:	43.9

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Revision Log:

Date	What was Revised? Why?
7/1/2019	A request has been made to lower the reporting limit for Permethrin-cis and trans to 1ppt and Deltamethrin to 4ppt.
	Section 11.2.1 GCMSMS instrument changed from Varian or Bruker to Agilent.
	Section 12.1 MDL determination study: changed the spike level from 5ppt and 10ppt to 2ppt and 5ppt.
	Section 12.2.1 MRM transition changed or added one more transition for analytes
	Section 12.2 update the reporting limit.
	Section 12.3 Method validation -Validated 5 sets and each set included 4 to 5 levels of fortification.
	Section 12.5 updated the method acceptance criteria.
	Section 15.2 Remove Negative chemical ionization techniques, it doesn't apply to the instrument used in this method.
11/5/2019	Section 3.2 Added ethyl ether and use in fume hood
	Section 6.19 added brand name
	Section 12.1 Added single tailed t test