California Department of Food and Agriculture
Center for Analytical Chemistry
Environmental Monitoring Section
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Title: Determination of Pyrethroids in Sediment Water

1. **Scope**:

This section method (SM) provides stepwise procedure for selective pyrethroids analysis in sediment water and is followed by all authorized EMON personnel.

2. **Principle**:

The pyrethroids are extracted from sediment water samples with methylene chloride. The extract is passed through sodium sulfate to remove residual water. The anhydrous extract is evaporated on a rotary evaporator and then a solvent exchange is performed with hexane. The extract is concentrated to a final volume of 1 mL. The extract is ready for analysis.

3. **Safety**:

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

3.2 Methylene chloride is a regulated and controlled carcinogenic hazardous substance. It must be stored and handled in accordance with California Code of Regulations, Title 8, Subchapter 7, Group 16, Article 110, Section 5202.

4. **Interferences**:

There were no matrix interferences that caused quantitative problems during method development and validation, but upon running real samples there was an occasional interference that affected bifenthrin. See discussion.

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 mass selective detector (MSD)

6. Reagents and Supplies:

6.1	Bifenthrin	CAS#42576-02-3
6.2	Lambda cyhalothrin	CAS#91465-08-06
6.3	Permethrin	CAS#526454-53-1
6.4	Cyfluthrin1,2,3,4	CAS#68369-37-5
6.5	Cypermethrin 1,2,3,4	CAS#52315-07-8
6.6	Fenvalerate	CAS#51630-58-1

6.7 Methylene Chloride, nanograde or equivalent pesticide grade
6.8 Acetone, nanograde or equivalent pesticide grade
6.9 Hexane, nanograde or equivalent pesticide grade
6.10 Separatory funnel, 2 L
6.11 Boiling flask, 500 mL
6.12 Sodium Sulfate, ACS grade
6.13 Funnels, long stem, 60°, 10 mm diameter
6.14 Volumetric Pipette, 1 mL
6.15 Graduated conical tubes with glass stopper, 15 mL
6.16 Glass wool, Pyrex® fiber glass slivers 8 microns
6.17 Disposable Pasteur pipettes, and other laboratory ware as needed
6.18 Recommended analytical columns:

5% (Phenyl)-methylpolysiloxane (HP-5MS or equivalent) fused silica column, 30 m x 0.25 mm id x 0.25 μm 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 hexane for identification purposes.

A multi level combination standard was prepared from the individual stock standards in acetone to be used for fortification. The individual stock standards were also used to prepare a multi level combination standard in hexane, which was diluted to prepare the working standards used for instrument calibration. Appendix 1 shows the levels of the working standards.

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 the 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 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 100 mL of methylene chloride to the sample bottle and manually shake for 30 seconds.

9.3.4 Pour methylene chloride and sediment into the separatory funnel and then shake for 2 min, venting frequently.

9.3.5 After phase separation, drain lower methylene chloride layer through a funnel containing approximately 60 g sodium sulfate into a 500 mL boiling flask.

9.3.6 Repeat steps 9.3.3 – 9.3.5 two more times using 80 mL of methylene chloride each time and shake for 1 min. Combine the extracts in the same boiling flask. Record sample bottle weight.

9.3.7 After draining the final extraction, rinse the sodium sulfate with 25 mL of methylene chloride.

9.3.8 Evaporate the sample extract to approximately 1 mL on a rotary evaporator using a water bath at 35 °C ± 3 and approximately 15 – 20 inch Hg vacuum. Add approximately 2mL of hexane and rotoevaporate to approximately 2 mL. Transfer the extract to a calibrated 15 mL graduated test tube.

9.3.9 Rinse flask 3 more times with approximately 2 mL of hexane and transfer each rinse to the same test tube.

9.3.10 Evaporate the extract to a volume slightly less than 1 mL under a gentle stream of nitrogen in a 40 °C water bath. Bring to a final volume of 1 mL with hexane, mix well and transfer into two autosampler vials.

10. **Instrument Calibration**:

10.1 The calibration standard curve consists of a minimum of three levels.

10.2 The calibration curve is obtained using linear regression with a 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 Instrumentation

11.2.1 Analyze pyrethroids by a gas chromatograph equipped with a mass selective detector.

11.2.2 Recommended instrument parameters: Injector 250 °C; msd transfer line heater 280 °C; initial column temperature 70 °C, hold 1 min., ramp at 22 °C/min. to final temperature of 280 °C and hold for 9 min.; injection volume 2 uL.

Ions Selected for SIM Acquisition:

Bifenthrin	165, 166, **181**, 182 start time 11.00 min.
Lambda Cyhalothrin	**181**, 197, 199, 225 start time 11.80 min.
Permethrin	163, 165, **183**, 184 start time 12.50 min.
Cyfluthrin	163, 165, 206, **226** start time 13.10 min.
Cypermethrin	**163**, 165, 181, 209 start time 13.50 min.
Fenvalerate	167, 181, 225, **419** start time 14.50 min.

Quantition ions are 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 samples are spiked with a multi-level pyrethroid combination standard 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 2.

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 RL is chosen in a range 1-5 times the MDL, as per client agreement. The reporting limit for all the pyrethriods is listed in Appendix 2 along with the MDL.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included four 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 shown in Appendix 3.

12.4 Control Charts and Limits

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

12.5 Acceptance Criteria

12.5.1 Bracketing standard curves should have a percent change ≤ 20%. The % change in response was calculated as follows:

$$\% \text{ change in response} = \frac{\text{Absolute value of response (std before – std after)}}{\text{Std before}} \times 100$$

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

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

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

12.5.5 The sample shall be diluted if results fall outside of the calibration curve.

13. **Calculations:**

Permethrin, 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 an external standard (ESTD) calculation using either the peak area or height. The software uses a linear curve fit, with all levels weighted equally. Alternatively, at the chemist's discretion, concentrations may be calculated using the response factor for the standard whose value is < 30% to the level in the sample.

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

14. Reporting Procedure:

Sample results are reported out according to the client's analytical laboratory specification sheets.

15. Discussion and References:

15.1 The fenvalerate standard is a ratio of approximately 60% fenvalerate and 40% esfenvalerate. The compound of interest is the esfenvalerate, but it was found from other studies that esfenvalerate in sample matrix degraded to fenvalerate over time. Therefore the total of fenvalerate/esfenvalerate was calculated and reported.

15.2 A storage stability study was done with this project. The storage stability study consisted of a multi level combination spike level and 3 replicates over a 37 day period. Twenty-one bottles containing background water with sediment added were spiked and stored in the refrigerator until analyzed on 0, 3, 6, 9, 13, 30, and 37 days. Along with the storage spikes a blank and method control spike were also extracted. The study showed degradation of lambda cyhalothrin and permethrin on day 3. The rest of the compounds didn't start to show degradation until day 30. The results are shown in Appendix 4.

15.3 Bifenthrin occasionally had an interference that affected quantitation, so the program rate in the method was decrease from 22 °C/min to 10 °C/min so as to separate out the interference. This adjustment worked well for bifenthrin, but gave less sensitivity for the other pyrthroids, so it was only used for bifenthrin when an interference occurred.

15.4 The sample matrix may require that the injector liner be changed more frequently and the column trimmed to maintain sensitivity.

15.5 References:

15.51 *Determination of Asana Insecticide Residues In Crops, Animal Tissues, Soil and Water: Electron-Capture Gas Chromatographic Method,* (MMS-R-581-1), Modesto, California

15.52 White, Jane, *Determination of Permethrin and Esfenvalerate/Fenvalerate in Sediment Water,* EMON-SM-52.5, 2000, California Department of Food

and Agriculture, Center for Analytical Chemistry, Environmental Monitoring Laboratory, 3292 Meadowview Road, Sacramento, California 95832

Appendix 1

Working Standard levels (ng/μL)

Level	Bifenthrin	Lambda cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Fenvalerate/ Esfenvalerate
5	0.1	0.4	1	2	1.6	1
4	0.05	0.2	0.5	1	0.8	0.5
3	0.025	0.1	0.25	0.5	0.4	0.25
2	0.01	0.04	0.1	0.2	0.16	0.1
1	0.004	0.016	0.04	0.08	0.064	0.04

Appendix 2

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

	Bifenthrin	**Lambda cyhalothrin**	**Permethrin**	**Cyfluthrin**	**Cypermethrin**	**Fenvalerate/ Esfenvalerate**
Spike Level	10 ppt	40 ppt	100 ppt	200 ppt	160 ppt	100 ppt
blk	N/D	N/D	N/D	N/D	N/D	N/D
spk1	8.46	38.8	98.5	187	162	87.0
spk2	7.48	37.8	98.9	169	157	80.9
spk3	8.43	37.6	94.2	188	161	88.7
spk4	9.70	43.6	107	215	192	96.4
spk5	8.13	39.6	97.4	200	187	90.6
spk6	8.46	43.2	109	221	202	103
spk7	7.93	41.3	101	191	167	89.8
SD	0.687	2.468	5.318	17.799	17.859	7.047
MDL (ppt)	2.159	7.756	16.71	55.94	56.13	22.15
RL (ppt)	5	20	50	80	80	50

Appendix 3

Method Validation Data and Control Limits

Analyte	Spike Level ppt	Recovery % Set 1	Set2	Set 3	Set 4	Set 5	
Bifenthrin	10	95.9	107	105	96.0	120	SD = 12.29
	20	88.5	95.9	90.2	108	97.5	Mean = 92.07
	50	86.2	78.7	75.7	82.4	92.4	UCL 128.9
	100	101	76.2	73.0	83.9	87.9	LCL 55.21
Lambda cyhalothrin	40	131	116	127	115	121	SD =13.55
	80	104	118	116	130	108	Mean = 108.51
	200	102	98.1	83.0	105	104	UCL 149.2
	400	113	88.8	89.6	104	96.7	LCL 67.84
Permethrin	100	123	118	120	111	116	SD = 11.46
	200	108	120	111	123	111	Mean = 107.3
	500	104	101	84.2	99.6	106	UCL 141.7
	1000	114	92.7	87.9	97.1	99.4	LCL 72.9
Cyfluthrin	200	122	104	132	117	117	SD = 13.02
	400	99.8	117	113	125	121	Mean = 108.14
	1000	102	95.7	85.7	103	111	UCL 147.2
	2000	114	89.8	87.9	110	95.9	LCL 69.07
Cypermethrin	160	130	130	144	120	121	SD = 16.54
	320	120	125	111	136	124	Mean = 113.395
	800	110	104	81.8	104	108	UCL 163.0
	1600	121	93.7	89.9	103	91.5	LCL 69.07
Fenvalerate/ Esfenvalerate	100	121	111	111	106	107	SD = 13.02
	200	102	111	103	117	107	Mean = 108.14
	500	97.7	87.4	70.8	95.2	99.2	UCL 147.2
	1000	102	84.7	85.8	93.7	86.1	LCL 69.07

Appendix 4

Storage Stability Study

Analyte	Spike Level	Recovery % Day 0	Day 3	Day 6	Day 9	Day 13	Day 30	Day 37
Bifenthrin	Blk	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	Spk 25 ppt	87.1	108	101	88.0	83	85	70.7
	Spk1 25 ppt	90.6	77.3	84.6	78.4	85.2	72.8	66.0
	Spk2 25 ppt	100	77.0	69.8	65.7	77.2	84.2	59.7
	Spk3 25 ppt	88.0	89.2	74.5	100	74..8	75.9	69.8
Lambda Cyhalothrin	Blk	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	Spk 100 ppt	104	110	102	98.1	94.6	92.4	82.7
	Spk1 100 ppt	114	50.2	50.3	49.4	51.5	45.0	39.8
	Spk2 100 ppt	124	54.8	45.0	40.7	55.3	48.9	37.0
	Spk3 100 ppt	111	58.6	50.8	74.4	50.0	38.3	37.1
Permethrin	Blk	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	Spk 250 ppt	104	117	104	107	109	92.0	85.6
	Spk1 250 ppt	112	53.7	47.0	30.2	27.8	15.6	14.1
	Spk2 250 ppt	120	56.8	32.1	27.7	28.3	22.8	15.0
	Spk3 250 ppt	112	60.6	38.4	41.8	25.1	17.3	20.5
cyfluthrin	Blk	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	Spk 500 ppt	109	126	101	110	118	115	93.5
	Spk1 500 ppt	126	89.9	85.4	78.7	79.4	58.0	56.1
	Spk2 500 ppt	139	95.0	77.0	63.3	79.6	68.2	53.9
	Spk3 500 ppt	119	104	82.0	96.0	82.8	55.4	62.5
cypermethrin	Blk	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	Spk 400 ppt	112	125	110	117	130	108	110
	Spk1 400 ppt	128	91.0	80.0	82.0	81.0	60.8	65.0
	Spk2 400 ppt	135	91.0	72.0	66.0	75.3	71.1	68.1
	Spk3 400 ppt	128	98.0	78.0	91.0	80.5	53.4	55.4
Fenvalerate/ Esfenvalerate	Blk	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	Spk 250 ppt	85.4	112	104	106	110	95.8	85.6
	Spk1 250 ppt	95.3	78.8	90.5	90.2	95.6	77.5	71.3
	Spk2 250 ppt	111	83.6	77.1	75.9	76.8	95.5	65.9
	Spk3 250 ppt	110	92.8	81.1	106	80.0	82.3	76.1

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