California Department of Food and Agriculture
Center for Analytical Chemistry
Environmental Monitoring Section
3292 Meadowview Road
Sacramento, CA 95832

EMON-SM-52.6
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Title: Determination of Pyrethroids in Sediment

1. Scope:

   This section method (SM) documents selective pyrethroids analysis in sediment and is followed by all authorized EMON personnel.

2. Principle:

   The samples are homogenized and extracted with acetonitrile. The filtered extracts are salted out with sodium chloride. An aliquot of acetonitrile extract is taken and evaporated to dryness for solvent exchange to hexane. 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 Acetonitrile and hexanes are flammable and toxic solvents; they should be handled with care in a ventilated area.

4. Interferences:

   The electron capture detector (ECD) is not truly an element specific detector, it will also respond to compounds containing S, NO_2 or conjugated C=O functional groups, so it may be necessary to confirm samples on the mass selective detector.

5. Apparatus and Equipment:

   5.1 Shaker, (Lab-Line Force Orbital Air Shaker 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 (GC) equipped with ^{63}Ni ECD detector
   5.6 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 Acetonitrile, nanograde or equivalent pesticide grade
6.8 Mason jars, pint size with lids
6.9 Hexane, nanograde or equivalent pesticide grade
6.10 Sodium Chloride, ACS grade
6.11 Whatman filter paper, #1, 15 cm
6.12 Funnels, long stem, 60°, 10 mm diameter
6.13 Graduated cylinders with stopper, 100-mL
6.14 Pipettes, 10-mL and 1-mL
6.15 Graduated conical tubes with glass stopper, 15-mL
6.16 Disposable Pasteur pipettes, and other laboratory ware as needed
6.17 Recommended analytical columns:

For ECD - 100% Dimethylpolysiloxane (HP-1 or equivalent) fused silica column, 30 m x 0.53 mm id x 0.88 um film thickness.

For MSD - 5% (Phenyl)-methylpolysilioxane (HP-5MS or equivalent) 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 ng/uL with hexanes for identification purposes.

A combination standard of 1 mg/mL, obtained from CDFA/CAC Standard Repository was diluted to 10 ng/uL with acetone to be used for fortification. The combination standard of 1 mg/mL was also diluted to the following concentrations: 0.05, 0.1, 0.2, 0.5, 1.0 ng/uL in hexanes for instrument calibration.

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 freezer. If samples are to be extracted the next day, they may be stored in the refrigerator. Sample extracts shall be stored in the refrigerator (32-40 °F).

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the Sacramento River sediment for background to be used in method validation and QC. Moistures were performed on the background sediment and DPR determined that water should be added to the sediment sub-samples at the time of analysis to help better represent the samples. (9.1.3 Moistures) The background sediment was provided in a 50 gal bucket. The sediment was mixed well with a paddle attached to a drill and then 50 g sub-samples were taken and stored in pint mason jars in the freezer for use as QC at a later date.

9.1.1 Blank

Take a 50 g background sub-sample from the freezer and allow it to come to room temperature. Add 20 mL of American river water and cover with aluminum foil. Place on shaker and shake for 30 minutes at ~180 rpm. Proceed to step 9.2.2 of section 9.2.

9.1.2 Spike

Take a 50 g background sub-sample from the freezer and allow it to come to room temperature. Add 20 mL of American river water and cover with aluminum foil. Place on shaker and shake for 30 minutes at ~180 rpm. The background sample is now ready to fortify at a level requested by client. After fortification proceed to step 9.2.2 of section 9.2.

9.1.3 Moistures

9.1.3.1 Mix sample in its container to achieve a uniform mixture.

9.1.3.2 Weigh out a 20 – 30 g sub-sample into a pre-weighed aluminum weighing pan.

9.1.3.3 Dry pan with sediment for at least 6 hours in a ~ 110 °C oven.

9.1.3.4 Reweigh sediment after cooling in a dessicator.

9.1.3.5 Report the wet and dry weights on Chain of Custody sample sheets.

9.2 Test Sample Extraction

9.2.1 Mix the sediment sample in its mason jar to achieve a uniform mixture. Weigh out a 70 ± 0.5 g sub-sample into a pint mason jar.

9.2.2 Add 100 mL of acetonitrile to each sample and cover with aluminum foil and cap it. Place the sample on the shaker and shake for 30 minutes at ~180 rpm.

9.2.3 Filter sediment mixture through Whatman # 1 filter paper placed in a glass funnel into a 100 mL graduated cylinder containing ~ 10 g sodium chloride.

9.2.4 Collect approximately 80 mL of filtrate.

9.2.5 Cap the cylinder and hand shake vigorously for 1 minute.

9.2.6 Allow the acetonitrile and water phase to separate for approximately 20 minutes.

9.2.7 Pipette a 10 mL aliquot of the organic phase (upper layer) into a 15 mL test tube.

9.2.8 Evaporate to dryness in a water bath of ~ 40 °C under a gentle stream of nitrogen.

9.2.9 Pipette 1 mL of hexanes into the test tube, cap immediately and vortex well. Transfer into two autosampler vials.

10. Instrument Calibration:

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

recommended concentrations levels of standards are 0.05, 0.1, 0.2, 0.5, or 1ng/uL.

10.2 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 the pyrethroids extracts by a gas chromatograph equipped with a electron capture detector (ECD).

11.2.2 Recommended instrument parameters: Injector 220 °C; detector 300 °C Initial column temperature 150 °C, hold 2 min., ramp at 40 °C/min to final temperature 280 °C and hold for 20 min.; injection volume 1 uL.

11.3 Confirmation Instrumentation

11.3.1 Confirm pyrethroids by mass selective detector

11.3.2 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, 183 start time 11.00 min.
Lambda Cyhalothrin	181, 197, 199, 225 start time 11.80 min.
Permethrin	163, 165, 183, 184 start time 12.60 min.
Cyfluthrin	163, 199, 206, 226 start time 13.20 min.
Cypermethrin	163, 165, 181, 209 start time 13.70 min.
Fenvalerate	167, 181, 225, 419 start time 14.80 min.

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 at 0.03 ppm with a combination of pyrethroid 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 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 RL is choose in a range 1-5 times the MDL, as agreed upon per client agreement. The reporting limit for all the pyrethriods is 0.01ppm.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included three levels of fortification (0.025, 0.05, and 1.0 ppm) and a method blank. All spikes and method blanks were processed through the entire analytical method. Recoveries for the pyrethroids are shown 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 warning and control limits are set at $\pm$ 2 and 3 standard deviations of the % recovery, respectively, shown in Appendix 2.

12.5 Acceptance Criteria

12.5.1 Bracketing standard curves should have a percent change less than 15%. 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 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 chemist discretion, concentrations may be calculated using the response factor for the standard whose value is closest to the level in the sample.

$$\text{ppm} = \frac{\text{(sample peak area or ht) x (std conc) x (std vol. Injected) x (100 mL) x (final vol of sample)}}{\text{(std.peak area or ht) x (sample vol injected) x (sample wt (g)) x (aliquot vol.)}}$$

14. Reporting Procedure:

14.1 The ECD is used as the primary instrument with any positives being confirmed on the MSD.

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

15. Discussion:

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. So 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 0.05 ppm spike level and 4 replicates over a 30 day period. Twenty 50 g background sub-samples were taken from the freezer and allow it to come to room temperature. Each sub-sample had 20 mL of American river water added to it and was covered with aluminum foil. The 20 samples were placed on the shaker and shaked for 30 minutes at 180 rpm. The samples were then spiked at a 0.05 ppm level and stored in the freezer till analyzed on 0, 3, 9, 16, and 30 days. The study showed no degradation for any of the compounds. Results shown in Appendix 3.

15.3 The MDL and validation samples were also analyzed on an ECD equipped with a DB-17 and MSD equipped with an HP-5MS. The ranges for the MDL and validation data was much greater than those obtained on the ECD with an HP-1 column. At this time the MSD will be used for confirmation only.

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

16. References:

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

16.2 White, Jane, *Determination of Permethrin and Esfenvalerate/Fenvalerate in Sediment Water,* 2000, 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	Bifenthrin	Lambda cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Fenvalerate/ Esfenvalerate
0.03 ppb spk1	0.0321	0.0342	0.0312	0.0332	0.0332	0.0332
0.03 ppb spk2	0.0283	0.0300	0.0278	0.0398	0.0296	0.0304
0.03 ppb spk3	0.0331	0.0361	0.0321	0.0361	0.0360	0.0367
0.03 ppb spk4	0.0338	0.0375	0.0322	0.0356	0.0352	0.0354
0.03 ppb spk5	0.0348	0.0378	0.0328	0.0372	0.0366	0.0374
0.03 ppb spk6	0.0318	0.0334	0.0301	0.0321	0.0331	0.0327
0.03 ppb spk7	0.0339	0.0367	0.0325	0.0360	0.0353	0.0359
SD	0.002144	0.002778	0.001771	0.002662	0.002400	0.002508
MDL	0.006739	0.008731	0.005566	0.008367	0.007543	0.007883
RL	0.01	0.01	0.01	0.01	0.01	0.01

Appendix 2

Method Validation Data and Control Limits

	Bifenthrin	Lambda Cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Fenvalerate /Esfenvalerate
Spike Level (ppm)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)
0.025	101	105	94	101	99	102
	112	119	104	115	119	115
	116	127	120	122	164	120
	118	126	111	121	123	120
	105	110	98	105	106	105
0.05	111	118	93	111	104	111
	104	110	89	104	109	103
	128	143	117	137	134	134
	132	146	118	140	141	138
	103	114	93	108	106	107
1.0	96	98	89	97	89	94
	114	122	108	121	117	117
	97	103	92	101	99	99
	103	110	98	110	104	104
	108	116	101	114	109	110
Mean	109.9	117.8	101.7	113.8	114.9	111.9
SD	10.48	13.57	10.76	12.6	19.2	12.4
UCL	141.3	158.5	134	151.7	172.6	149.0
UWL	130.8	144.9	123.2	139.1	153.4	136.7
LWL	88.9	90.7	80.1	88.5	76.4	87.2
LCL	78.4	77.1	69.4	75.9	57.1	74.9

Appendix 3

Storage Stability Study

	Bifenthrin	**Lambda Cyhalothrin**	**Permethrin**	**Cyfluthrin**	**Cypermethrin**	**Fenvalerate /Esfenvalerate**
(ppm)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)
Day 0						
0.03	105	113	96	110	107	119
0.05 spk1	103	111	93	113	106	114
0.05 spk2	105	112	90	112	105	116
0.05 spk3	110	121	99	123	116	125
0.05 spk4	105	114	93	114	108	118
Day 3						
0.03	94	104	89	105	96	112
0.05 spk1	94	104	89	108	98	108
0.05 spk2	97	108	90	116	102	118
0.05 spk3	106	119	101	132	117	131
0.05 spk4	105	119	97	138	114	131
Day 9						
0.03	104	108	102	98	101	104
0.05 spk1	104	108	100	104	104	105
0.05 spk2	103	107	97	102	102	103
0.05 spk3	100	102	91	96	96	94
0.05 spk4	111	116	104	111	110	111
Day 16						
0.03	94	119	90	104	100	108
0.05 spk1	108	131	103	125	118	124
0.05 spk2	99	120	95	113	108	114
0.05 spk3	97	116	89	107	101	104
0.05 spk4	75	50	72	83	80	83
Day 30						
0.03	82	89	82	84	88	90
0.05 spk1	108	114	103	113	111	112
0.05 spk2	97	100	94	98	98	98
0.05 spk3	100	105	97	104	103	106
0.05 spk4	103	108	102	108	109	109

Author:

Jane White — 4/2/03
Jane White — Date
Agricultural Chemist II

Author:

Hsiao Feng — 4/2/03
Hsiao Feng — Date
Agricultural Chemist II

Approved By:

Catherine Cooper — 4/2/03
Catherine Cooper — Date
Section Supervisor

Reviewed By:

Terry Jackson — 4/8/03
Terry Jackson — Date
Quality Assurance Officer

Approved By:

Lilia Riveria — 4/8/03
Lilia Riveria — Date
Program Supervisor

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