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DETERMINATION OF PERMETHRIN AND ESFENVALERATE / FENVALERATE IN SEDIMENT WATER

Scope: This method is for the determination of permethrin (cis and trans), esfenvalerate and its isomer fenvalerate in sediment water. The reporting limits of this method is 0.05 ppb for these compounds using the electron capture detector and 0.1 ppb using the mass selective detector.

Principle: The sediment water was extracted using hexanes. After concentrating the hexanes, the extracted residues were analyzed by gas chromatograph equipped with an electron capture detector (ECD) or by a mass selective detector (MSD). Permethrin was reported as the total of the cis and trans isomers and esfenvalerate was reported as the total of esfenvalerate and its isomer fenvalerate.

Reagents:

1. Permethrin, CAS#52645-53-1, (combination of isomers cis and trans), 1.0 mg/mL in acetone, obtained from CDFA Standard Repository (Center for Analytical Chemistry, California Department of Food and Agriculture)
2. Fenvalerate, CAS#51630-58-1, (combination of isomers fenvalerate and esfenvalerate), 1.0 mg/mL in acetone, obtained from CDFA Standard Repository (Center for Analytical Chemistry, California Department of Food and Agriculture)
3. Hexanes, pesticide residue grade
4. Acetone, pesticide residue grade
5. Sodium sulfate, anhydrous granular (ACS)

Safety:

Most of the reagents used and analyzed for in this method have not been completely characterized. All general laboratory safety rules must be followed.

Equipment:

1. Separatory funnels, 2 L
2. Boiling flasks, flat bottom, 24/40 joints, 500 mL
3. Beakers, 1 L
4. Funnels, glass short stemmed 100 mm diameter
5. Rotary evaporator, Buchi/Brinkmann, R110

Equipment: continued

6. Conical test tubes, graduated, calibrated 15 mL
7. Nitrogen evaporator, Organomation, Model 12

Instruments:

1. GC-ECD: Hewlett-Packard 5890 Gas Chromatograph equipped with an electron capture detector
2. GC/MSD: Hewlett-Packard 6890 Gas Chromatograph equipped with a series 5973 Mass Selective Detector

Interference:

The background had small peaks on the GC-ECD that fell close to the retention times of the compounds of interest but didn't interfere with the quanitation at this time. The MSD has no interferences at this time.

Standard Preparation:

1. The 1mg/mL standards are diluted to 10ug/mL with acetone for spiking purpose.
2. Dilute the mg/mL standards into a series of desired standard sets that will be used for instrument calibration and sample calculation.
3. Keep all prepared standards in the designated refrigerator for storage while not in use.
4. The shelf life of each prepared standard is six months.

Sample Preservation and Storage:

1. Check the temperature of samples upon arrival and record it.
2. Sign the chain of custody and obtain the EMON number.

Procedure:

1. Remove samples from the refrigerator and allow them to come to room temperature before weighing them. Record weight.
2. Transfer water sample to a 2 L separatory funnel leaving as much of the sediment as possible in the bottle.
3. Add 20 mL acetone to the bottle and shake for 10 seconds.
4. Add 100 mL hexanes to the bottle and shake for 30 seconds.
5. Pour acetone, hexanes and sediment to the separatory funnel and shake for 1 min.
5. After phase separation, drain water layer into a 1 L beaker then drain the hexanes layer through glass wool and ~ 45 g sodium sulfate into a 500 mL flask.
6. Pour the sample layer back into the separatory funnel.
7. Repeat the steps 3-6 two more times using 20 mL acetone and 80 mL hexanes.
8. Rinse the sodium sulfate with ~ 20 mL hexanes.
9. Weigh empty bottles and record the weight.
10. Rotoevaporate the extract to ~ 1 mL at 50 ° C under approximately 20 inches of Hg vacuum.
11. Transfer the extract to a 15 mL graduated test tube and rinse the flask twice with approximately 2 mL of hexane and add to the test tube.

Procedure: continued

12. Evaporate the extract to a final volume of 1 mL under a gentle stream of nitrogen in a 50 ° C waterbath. Vortex to mix well.

Preparation of blanks and Spikes

Blank: American River water with 5 grams of sediment added to the bottle. (Prepared by the Department of Pesticide Regulations)

Spike: Spike standard directly into the bottle containing American River water with 5 grams of sediment added. Mix well and let sediment settle before extracting.

Instrument Conditions:

Instrument: HP 5890 Gas Chromatograph equipped with an electron capture detector
Column: HP-1 (Crosslinked methyl silicone gum) 30 m x 0.53 mm x 0.88 μm
Carrier gas: Helium, 5 psi
Injector temperature : 220 °C
Detector temperature: 300 °C
Column oven temperature:
Initial temperature: 150 °C hold for 2 min.
Rate: 40 °C/min.
Final temperature: 280 °C for 20 min.
Injection volume: 1 μL
Retention times: Permethrin (cis & trans): ~ 10.3 & 10.4 minutes
Esfenvalerate (fenvalerate & esfenvalerate):~13.5 & 13.9 minutes

Instrument: HP 6890 Gas Chromatograph equipped with a 5973 Mass Selective Detector
Column: HP-5MS (5% Phenyl Methyl Siloxane), 30m x 0.25 mm x 0.25 μm
Carrier: Helium, 6.4 psi
Column oven temperature:
Initial temperature: 70 °C hold for 1.0 min.
Program Rate 25 °C/ min.
Final temperature: 280 °C hold for 8.00 min.
Injecture temperature: 250 °C
Transfer Line Temperature: 280 °C
Ions Selected for SIM Acquistion: Permethrin cis 163, 165, 183, 184
Permethrin trans 163, 165, 183, 184
Fenvalerate 181, 225, 419
Esfenvalerate 181, 225, 419

Retention times: Permethrin cis ~12.5 minutes
Permethrin trans ~12.6 minutes
Fenvalerate ~15.1 minutes
Esfenvalerate~15.4 minutes

Volume Injected: 2 μL

Instrument Calibration:

1. Load a method, set the desired condition for analysis.
2. Run 0.05, 0.1, 0.25, and 0.5 ηg/uL to check the system linearity

Analysis:

Quality Control:

1. A 4-point calibration curve of 0.05, 0.1, 0.25 and 0.5 ηg/uL for permethrin and esfenvalerate/fenvalerate were obtained at the beginning and the end of each set of samples.
2. Each sample shall be injected two times to insure reliability of the analysis. Results obtained using a calibration curve shall lie within the range of the calibration curve. If results fall outside the calibration curve, the sample must be concentrated/diluted or the calibration curve extended. A sample set is usually comprised of 10 samples, a blank and a spike.

Method Detection Limit (MDL):

Method Detection Limit (MDL) refers to the lowest concentration of analyte that a method can detect reliably in either a sample or a blank. To determine the MDL, spike 7 samples, with 0.1 ppb of permethrin and esfenvalerate/fenvalerate and process through the entire method along with a blank. The standard deviation derived from the 7 spike results was used to calculate the MDL using the following equation:

$$MDL = tS$$

Where: t = the student " t" value for the 99% confidence level with n-1 degrees of freedom (t=3.143 for 6 degrees of freedom). n= the number of replicates.
S = the standard deviation obtained from the 7 replicates analysis

The results for the standard deviations and MDL are in Appendix 1.

Reporting Limit (RL):

RL refers to level above which quantitative results may be obtained. The MDL was used as a guide to determine the RL. The reporting limit is 0.05 ppb for permethrin and esfenvalerate/fenvalerate using the ECD and 0.1ppb for permethrin and esfenvalerate/fenvalerate using the MSD.

Recovery Data:

The analytical method was validated using five sets of spike samples. Each set contained a blank and five levels of spikes. Each set was processed through the entire analytical method. Recoveries of permthrin and esfenvalerate/fenvalerate are shown in Appendix 2.

Calculations:

$$ppb = \frac{(\text{peak ht sample})(\text{response factor, } \eta g)\ (\text{sample final volume, mL})(1000\ \mu L/mL)}{\text{---}}$$

(sample vol. Injected, μL)

Calculations: continued

$$\text{where: response factor}(\eta g) = \frac{[\text{(std. Conc.}_n, \eta g/\mu L)(\text{std. Vol. Injected, } \mu L)/(\text{std. Peak ht..}_n)]}{n}$$

n=number of standards

Acceptance Criteria:

1. The standard curves at the beginning and end of each sample set should not have a percent change greater than 10 % for the ECD and 20% for the MSD. The % change in response was calculated as follows:

   % Change in response = absolute value of [response of (std before - std after)/ std before] x 100

2. The samples were calculated using the response factor average of the curves. If the results between the two injections differ less than 10 % for ECD and 15 % for MSD either result can be reported. A change greater than 10 % for ECD and 15 % for MSD with no known reason requires a third injection.

Discussion: In this project a storage stability study was done. The storage stability study consisted of 0.5 ppb spike level and 3 replicates over a 13 day period. The spiked samples were stored in the refrigerator and then analyzed on days 0, 3, 5, 7, 10, and finally with day 13. It was noticed that by day 3 the esfenvalerate spike had started to transform to its isomer fenvalerate. At the beginning of this project we were just going to analyze for esfenvalerate since that was the analyte being applied in the environment. However, after the transformation of the esfenvalerate to it's isomer fenvalerate it was decided to add the two together and report the total. All the mdl and validation data was recalculated to report the total of fenvalerate and esfenvalerate. A new standard was prepare using fenvalerate which is a ratio of approximately 60% fenvalerate to 40% esfenvalerate, compared to the esfenvalerate standard which was approximately 10% fenvalerate to 90% esfenvalerate. It was also noticed that permethrin over the 13 day storage study showed a little degradation. The results for the storage stability study are shown in appendix 3.

The results for the GC-ECD were calculated using height to minimize any interferences that might be caused by the background. The background had small peaks that fell close to the retention times of the compounds permethrin cis and trans , but didn't interfere with quanitation at this time. The MSD has no interferences.

References:

Determination of Asana Insecticide Residues In Crops, Animal Tissues, Soil And Water: Electron-Capture Gas Chromatographic Method,(MMS-R-581-1) February, 1986, Shell Development Company Biological Sciences Research Center Modesto, California

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Appendix: 1

Permethrin and Esfenvalerate/Fenvalerate MDL Results (ppb) for sediment water on GC-ECD

Spike #	Permethrin	Esfenvalerate/Fenvalerate
1	0.0972	0.0938
2	0.0944	0.112
3	0.0945	0.0864
4	0.0952	0.0925
5	0.0969	0.0871
6	0.0937	0.0891
7	0.0930	0.0881
S=	0.00157	0.009
MDL = 3.143 x S	0.00493	0.028

Permethrin and Esfenvalerate/Fenvalerate MDL Results (ppb) for sediment water on MSD

Spike #	Permethrin	Esfenvalerate/Fenvalerate
1	0.117	0.097
2	0.124	0.129
3	0.113	0.128
4	0.099	0.110
5	0.121	0.108
6	0.091	0.113
7	0.105	0.129
S=	0.012	0.013
MDL = 3.143 x S	0.037	0.04

Appendix: 2

Permethrin and Esfenvalerate/Fenvalerate Method Validation Results and Recoveries for sediment water on GC-ECD

	Permethrin (cis & trans)		Esfenvalerate/Fenvalerate	
Spike Level (ppb)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)
0.1	0.0988	98.8	0.089	89
	0.113	113	0.112	112
	0.110	110	0.109	109
	0.106	106	0.109	109
	0.086	86	0.080	80
0.5	0.474	94.8	0.483	96.6
	0.520	104	0.530	106
	0.513	103	0.513	103
	0.483	96.6	0.542	108
	0.495	99	0.520	104
1.0	0.962	96.2	1.00	100
	1.08	108	1.11	111
	1.06	106	1.07	107
	1.11	111	1.18	118
	1.01	101	1.05	105
5.0	4.80	96.0	4.47	89.4
	4.68	93.6	4.73	94.6
	4.30	86.0	3.67	73.4
	3.94	78.8	3.76	75.2
	4.11	82.2	4.77	95.4
10.0	8.43	84.3	10.9	109
	8.87	88.7	9.10	91.0
	8.23	82.3	8.90	89.0
	9.98	99.8	11.0	110
	8.25	82.5	8.69	86.9

Appendix 2: continued

Permethrin and Esfenvalerate/Fenvalerate Method Validation Results and Recoveries for sediment water on MSD

Permethrin			Esfenvalerate/Fenvalerate	
Spike Level (ppb)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)
0.1	0.0827	82.7	0.0860	86.0
	0.103	103	0.110	110
	0.0975	97.5	0.0944	94.4
	0.100	100	0.0973	97.3
	0.0820	82.8	0.0736	73.6
0.5	0.469	93.8	0.586	117
	0.536	107	0.612	122
	0.441	88.2	0.408	81.7
	0.477	95.4	0.504	100.8
	0.455	91.0	0.403	80.6
1.0	0.963	96.3	0.993	99.3
	1.04	104	1.05	105
	0.907	90.7	0.993	99.3
	1.05	105	1.06	106
	0.821	82.1	0.757	75.7
5.0	4.47	89.4	4.08	81.6
	4.73	94.6	5.05	101
	3.67	73.4	3.58	71.6
	3.76	75.2	3.95	79.0
	4.77	95.4	4.74	94.7
10.0	8.57	85.7	7.61	76.1
	9.10	91.0	8.95	98.5
	9.00	90.0	8.18	81.8
	10.1	101	10.1	101
	7.05	70.5	6.58	65.8

Appendix 3

Permethrin and Esfenvalerate/Fenvalerate Storage Study Results and Recoveries for sediment water on GC-ECD

	Permethrin		Esfenvalerate/Fenvalerate	
Spike Level (ppb)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)
Day 0				
0.1 ppb	0.120	120	0.112	112
spk 1 0.5 ppb	0.483	96.6	0.480	96.0
spk 2 0.5 ppb	0.549	110	0.560	112
spk 3 0.5 ppb	0.571	114	0.582	116
Day 3				
0.1 ppb	0.101	101	0.100	100
spk 1 0.5 ppb	0.382	76.4	0.481	96.2
spk 2 0.5 ppb	0.461	92.2	0.514	103
spk 3 0.5 ppb	0.471	94.2	0.528	106
Day 5				
0.1 ppb	0.099	99	0.088	88
spk 1 0.5 ppb	0.321	64.2	0.456	91.2
spk 2 0.5 ppb	0.387	77.4	0.468	93.6
spk 3 0.5 ppb	0.355	71.0	0.424	84.8
Day 7				
0.1 ppb	0.104	104	0.102	102
spk 1 0.5 ppb	0.289	57.8	0.419	83.8
spk 2 0.5 ppb	0.353	70.6	0.447	89.4
spk 3 0.5 ppb	0.399	79.8	0.459	91.8
Day 10				
0.1 ppb	0.114	114	0.103	103
spk 1 0.5 ppb	0.372	74.4	0.466	93.2
spk 2 0.5 ppb	0.370	74.0	0.468	93.6
spk 3 0.5 ppb	0.372	74.4	0.459	91.8
Day 13				
0.1 ppb	0.103	103	0.0938	93.8
spk 1 0.5 ppb	0.192	38.4	0.466	93.2
spk 2 0.5 ppb	0.345	69.0	0.517	103
spk 3 0.5 ppb	0.331	66.0	0.493	98.6

Spike # 1 for days 0-13 used sediment water that had been stored for sometime, this might have something to do with the lower recoveries.