

CALIFORNIA DEPT. OF FOOD & AGRICULTURE  
Center for Analytical Chemistry  
Environmental Monitoring Section  
3292 Meadowview Road  
Sacramento, CA 95832  
(916) 262-2080 FAX (916) 262-1572

Method #: 16.0  
Original Date: November 9, 2001  
Revised Date:  
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### GC Determination of Cyfluthrin in Surface Water

**Scope:** This method is for the determination of cyfluthrin in surface water. The method reporting limit is 0.05 ppb.

**Principle:** Cyfluthrin in water is extracted with hexane. After concentrating, the sample is redissolved in hexane and analyzed by gas chromatography using an electron capture detector (ECD).

**Safety:**

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

**Interference:**

A cleanup procedure was used because interference for cyfluthrin was found in the water used to validate this method.

**Reagents, Equipment and Instrument:**

*Reagents:*

1. Cyfluthrin Standard, 1.0 mg/mL in acetone, obtained from CDFA Standard Repository (Center for Analytical Chemistry, California Department of Food and Agriculture)
2. Hexane, pesticide residue grade
3. Acetone, pesticide residue grade
4. Sodium sulfate, anhydrous, granular, ACS 10-60 mesh

*Equipment:*

1. Separatory funnels, 2000 mL
2. Boiling flasks, flat-bottomed, 24/40 joints, 500 mL
3. Rotary evaporator, Büchi-Brinkmann, Model R 110
4. Nitrogen evaporator, Organomation, Model 112
5. Vortex mixer, Thermolyne, Model 37600
6. Beaker, 1500 mL
7. Funnel, long stem, 60°, 100 mm diameter
8. Conical test tubes, graduated, 15 mL
9. Mega Bond Elut® 6cc/1grm Flourisil Cartridge
10. Sepelco Visiprep 24 Manifold
11. In house vacuum
10. Analytical balance; Mettler, SM; 3000 g

**Reagents, Equipment and Instrument:** continued*Instrument:*

1. GC: Hewlett-Packard 5890 Gas Chromatograph with ChemStation and ECD detector.

**Standard Preparation:**

1. Dilute the 1 mg/mL cyfluthrin standard with hexane to make up a series of standard solutions. These standard solutions will be used for instrument calibration and sample calculation.
2. Dilute the 1 mg/mL cyfluthrin standard with acetone to make up needed standard solutions for spiking.
3. Keep all prepared standards in the designated refrigerator for storage when not in use.
4. The expiration date of each standard is six months from the preparation date.

**Sample Preservation and Storage:**

1. Check the temperature of ten percent of the samples upon arrival and record it.
2. Sign the sample chain of custody and obtain the EMON number.
3. Store all samples waiting for extraction in the walk-in refrigerator.
4. Store all samples waiting for analysis in a refrigerator.

**Analysis:***Sample Extraction:*

1. Remove samples from refrigerated storage and allow them to come to room temperature ( $\pm 5$  °C).
2. Shake each sample well. Record weight of water by weighing sample bottle before and after water has been transferred into a 2 L separatory funnel.
3. Extract samples by adding 100 mL of hexane and shaking vigorously for two minute. **Vent frequently to relieve pressure.**
4. After the layers have separated, drain the aqueous layer into a 1500 mL beaker.
11. Pour the organic layer from the top of separatory funnel into a 500 mL boiling flash through a funnel filled with 20 g of anhydrous sodium sulfate.
12. Repeat steps 3, 4 and 5 two more times using 80 mL portions of hexane and shaking the samples for one minute.
7. After draining the final extract, rinse the sodium sulfate with ~ 25 mL of hexane.
8. Concentrate the extract to 2 ~ 3 mL on a rotary evaporator using 40 °C water bath and a vacuum of 20 inches Hg. Transfer the extract to a calibrated 15 mL graduate test tube.
9. Rinse the flask three times, each time with approximately 3 mL of hexane and transfer each rinse into the same test tube.
13. Under a gentle stream of nitrogen with a water bath at 40 °C, evaporate the extract to a volume of 2 to 3 mL. Vortex for 15 sec.

**Sample clean up procedure**

14. Condition a Flourisil cartridge with 3 mL hexane (*do not allow cartridge to dry*), 5 mL of 12% acetone in hexane (*do not allow cartridge to dry*), and 5 mL hexane using a vacuum manifold with no vacuum applied. (*do not allow cartridge to dry*).
12. Load the sample in the preconditioned flourisil cartridge. Discard the eluant.

**Sample clean up procedure:** continued

13. Rinse the test tube well with 3 mL of hexane and vortex for 15 sec. Load the rinsing on the same cartridge. Discard the rinse.
14. Repeat step twelve one more time. *Allow cartridge to dry using vacuum for 30 sec.*  
Everything on step 10 to 13 go to waste, **DO NOT COLLECT IN TEST TUBE**
15. Collect sample into a calibrated 15 mL graduated test tube by eluting cartridge with 5 mL of 8% acetone in hexane. *Dry cartridge using vacuum (5 to 10 psi).*
16. Under a gentle stream of nitrogen with and water bath at 40°C, evaporate the extract to a volume ~0.1 mL. Vortex for 15 sec.
17. Bring to a final volume of 1.0 mL with hexane.
18. Put sample into two autosampler vials, one with insert and one without insert.
19. Storage vials in freezer for GC analysis.

**Preparation of blanks and spikes and cleanup solutions**

Blank: Weigh out 1000 ±0.1 g of American River background water into a 2 L separatory funnel. Follow the sample extraction procedure outlined above.

Spike: Weigh out 1000 ±0.1 g of American River background water into a 2 L separatory funnel. Spike a known amount of cyfluthrin into the sample. Mix well and let stand for at least 1 minute, and then follow the sample extraction procedure outlined above.

**Cleanup Solutions**

8% Acetone/Hexane      8 mL of acetone into a 100 mL volumetric flask. Fill to volume with hexane

12% Acetone/Hexane      12 mL of acetone into a 100 mL volumetric flask. Fill to volume with hexane

**Instrument Conditions**

Instrument:	GC: Hewlett-Packard 1050, controlled by ChemStation
Column:	HP-1 (100% dimethylpolysiloxane) 30m x 0.25mm x 0.25um.
Injection volume:	2 µL
Retention Time:	Cyfluthrin I ~ 31.15 minutes
	Cyfluthrin II ~ 31.72 minutes
	Cyfluthrin III ~ 32.31 minutes
	Cyfluthrin IV ~ 32.60 minutes

**Instrument calibration:**

Load a method and run a set of calibration standards (0.025 ng/µL, 0.05 ng/µL, 0.1 ng/µL, 0.5 ng/µL, and 1.0 ng/µL) to check system linearity.

**Method Performance:***Quality Control:*

1. A 5-point calibration curve of 0.025, 0.05, 0.1, 0.5 and 1.0  $\eta\text{g}/\mu\text{L}$  is obtained at the beginning and the end of each sample set. The Chemstation software is used to calculate sample result in  $\mu\text{g}$ .
2. Each sample shall be injected two times to insure reliability of the analysis. If the signal of a sample is greater than that of the highest standard in the calibration curve, dilute the sample. Re-inject the diluted sample two more times together with standards.
3. Sample storage: All field samples shall be kept refrigerated at 5 °C.
4. Sample extracts: All extracts shall be kept refrigerated at 5 °C until analyzed.
5. Refrigerator temperature shall be monitored and recorded daily.
6. For each set of samples, one matrix blank and one matrix spike shall be included. Each set of samples shall not contain more than twelve samples.
7. To avoid cross-contamination, glassware shall be washed following Environmental Monitoring standard operation procedure (SOP 502.6).
8. At least ten percent of the sample results shall be manually calculated to check instrument results. Hand calculated results are based on a single calibration point using the following equation:

$$\text{ppb} = \frac{(\text{sample peak ht.})(\text{std. conc., } \mu\text{g/mL})(\text{std. vol. inj., } \mu\text{L})(\text{sample final vol., mL})(1000 \eta\text{g}/\mu\text{g})}{(\text{Std. peak ht.})(\text{sample vol. injected, } \mu\text{L})(\text{sample wt., g})}$$

*Recovery Data:*

The method was validated by preparing five sets of spiked samples. Each set contained a blank and five levels of spikes. The background waters (American River water) were obtained from the Department of Pesticide Regulation. Sets were processed through the entire analytical method on separate days. Calculated recoveries for cyfluthrin are tabulated in Appendix I.

*Method Detection Limit (MDL):*

The MDL refers to the minimum concentration of cyfluthrin that can be detected in surface and well waters with 99% confidence. The MDL was computed based on the following procedure:

- a) Prepare 7 replicates of cyfluthrin at 0.1 ppb for each matrix.
- b) Process each sample through the entire method along with a blank.
- c) Calculate the percent recovery for each sample.
- d) Calculate the standard deviation (S) for the percent recovery.
- c) Compute the MDL as follows:

$$\text{MDL} = t \times S$$

where;

$t$  is the Student 't' value for the 99% confidence level with n-1 degrees of freedom (n-1,  $1 - \alpha = 0.99$ ) where n represents the number of replicates. For n=7,  $t=3.143$ .  
 $S$  denotes the standard deviation obtained from replicate analyses.

The results for the standard deviations and MDL calculations are tabulated in Appendix II.

**Method Performance:** continued*Reporting Limit (RL):*

The RL refers to level above which quantitative results are reported. The MDL is used as a guide to determine the RL. In this method, the RL is set at 5 times or less the MDL. The cyfluthrin RL is 0.05 ppb.

**Calculations:**

Samples are calculated using a multilevel calibration curve of response versus concentration. The multilevel calibration curve is used to confirm linearity over the calibration range. The method of linear fit ignoring the origin is selected to create the curve. Each calibration level corresponds to a calibration standard of known concentration. Calibration standards should be prepared so that the cyfluthrin concentration varies across the range of concentrations expected in the samples.

$$\text{ppb} = \frac{(\text{instrument calculated results, } \mu\text{g}) (1000 \text{ ng}/\mu\text{g})}{(\text{sample wt., g})}$$

where instrument calculated results (ICR):

$$\text{ICR } (\mu\text{g}) = \frac{(\text{sample peak. ht.}) (\text{RF, } \mu\text{g/mL}) (\text{std. vol. injected, } \mu\text{L}) (\text{FV., mL})}{(\text{sample vol. injected, } \mu\text{L})}$$

and

$$\text{RF } (\mu\text{g/mL}) = \frac{\Sigma [ (\text{std. conc., } \mu\text{g/mL}) / (\text{std. peak ht.,}) ]}{n}$$

n = number of standards

**Acceptance Criteria:**

1. The standard curves at the beginning and the end of each sample set should not have an average percent change greater than 15%. The % change in response is calculated as follows:

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

2. Results are calculated by the Chemstation software using the calibration curve run before the samples. If the results for replicate injections for a sample differ by less than 10 %, either result can be reported. If the difference is greater than 10 % with no known cause, more work shall be done to obtain reproducible results.

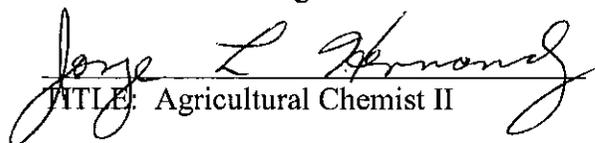
**Discussion:**

Cyfluthrin is a mixture of four diastereo-isomeric enantiomer pairs. For the analysis, the four isomers are separate and calculate. Then, the peaks are added to get the reported result. Since the final result contain the variability associate with each peak, the control limits for cyfluthrin are greater than when a single peak is calculated. It is our experience that cyfluthrin is heat sensitive. To achieve acceptable recoveries, prolonged heating must be avoided and the recommended temperature must be followed during evaporation to prevent low recoveries. The imidacloprid calibration curve is linear in the selected range and any of the calibration points may be used for manual calculation. However, it is recommended for this calculation to use a standard with a peak height close to the peak height of the calculated sample.

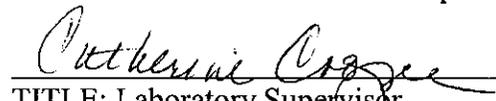
**References:**

1. *Current SOP for Method Validation, Environmental Monitoring Section 1997*
2. *Farm Chemical Handbook, 2001*
3. Keith, Lawrence H; *Principals of Environmental Analysis, Anal. Chem.*, 1983,55, 2210-2218

WRITTEN BY: Jorge L. Hernandez

  
TITLE: Agricultural Chemist II

REVIEWED BY: Catherine Cooper

  
TITLE: Laboratory Supervisor  
Environmental Monitoring

**Appendix I: Method Validation Results and Recoveries**

Spike Level (ppb)	Cyfluthrin	
	Results (ppb)	%
0.2	0.209	105
	0.204	102
	0.198	98.8
	0.199	100
	0.192	96.0
0.5	0.490	98.0
	0.540	108
	0.525	105
	0.392	78.4
	0.535	107
1.0	0.893	89.3
	1.17	117
	0.919	91.9
	0.966	96.6
	1.01	101
5.0	4.34	86.8
	4.83	96.6
	4.25	85.0
	4.60	92.0
	4.78	95.6

**Appendix II: MDL Determination**

Spike	Cyfluthrin	
	ppb	% Recovery
Blank	ND	
Spike 1	0.194	97.0
Spike 2	0.195	97.5
Spike 3	0.221	111
Spike 4	0.201	101
Spike 5	0.212	106
Spike 6	0.210	105
Spike 7	0.197	98.5
<b>STDEV</b>	0.0081	
<b>MDL</b>	0.025	
<b>RL</b>	0.05	