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Center for Analytical Chemistry
Environmental Analysis Section
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Title: Determination of Fipronil and Metabolites in Surface Water using Gas Chromatography/Mass spectrometry

1. Scope:

   This section method (SM) documents the procedure for Fipronil pesticide analysis in surface water by all authorized section personnel.

2. Principle:

   The surface water sample is extracted with methylene chloride. The extract is passed through sodium sulfate to remove residual water. The anhydrous extract is evaporated to almost dryness on a rotary evaporator and diluted to a final volume of 0.5mL with methylene chloride. The extract is then analyzed by a gas chromatograph/ mass selective detector (MSD) in selected ion monitoring (SIM) mode.

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.

   3.3 All solvents should be handled with care in a ventilated area.

4. Interferences:

   There are matrix interferences that cause quantitative problems. Therefore the calibration standards will be made up in appropriate matrix.

5. Apparatus and Equipment:

   5.1 Rotary evaporator (Büchi/Brinkman or equivalent)
   5.2 Nitrogen evaporator (Meyer N-EVAP Organomation Model # 112 or equivalent)
   5.3 Vortex-vibrating mixer
   5.4 Balance (Mettler PC 4400) or equivalent
   5.5 Gas Chromatograph equipped with mass selective detector (MSD)

6. Reagents and Supplies

6.1 Methylene Chloride, nanograde or equivalent pesticide grade
6.2 Acetone, nanograde or equivalent pesticide grade
6.3 Anhydrous Sodium Sulfate, granular
6.4 Fipronil CAS# 120068-37-3
6.5 Fipronil Amide CAS#
6.6 Fipronil DeSulfinyl CAS#
6.7 Fipronil DeSulfinylamide CAS#
6.8 Fipronil Sulfide CAS#
6.9 Fipronil Sulfone CAS# 120068-36-2
6.10 Phenanthrene-d10 CAS# 1517-22-2
6.11 Conical tube with glass stopper, 15-mL graduated, 0.1mL subdivision
6.12 Separatory funnel, 1 L
6.13 Boiling flask, 500mL
6.14 Funnel, long stem, 10 mm diameter
6.15 Disposable Pasteur pipettes, and other laboratory ware as needed
6.16 Recommended analytical columns:

For MSD - 5% phenyl Methyl silicone (HP-5ms or equivalent) fused silica column, 30 m x 0.25 mm x 0.25 μm film thickness.

7. Standards Preparation:

7.1 Dilute the 1 mg/mL Fipronil standards obtained from the CDFA/CAC Environmental Analysis Standards Repository with acetone to make up a series of mixed working standards (see 10.2). These standards shall be prepared to cover the linear range from 0.025 ηg/μL to 1.0 ηg/μL.

7.2 Prepare the Phenanthrene-d10 internal standard at 20μg/mL from the stock standard.

7.3 The calibration standards are diluted with matrix blank extracts (9.1.2.1) to correct for matrix background interference.

7.4 Store standards according to manufacturing requirement. Keep all standards in designated refrigerator for storage.

7.5 The expiration date of each mixed working standard is six months from the preparation date or same as stock standards, if sooner.

8. Sample Preservation and Storage:

All water samples and sample extracts shall be stored in the refrigerator (4 ± 3 °C).

9. Test Sample Preparation:

9.1 Sample Preparation

9.1.1 Remove samples from refrigerator and allow samples to come to room temperature before extraction.

9.1.2 Preparation of matrix blank and matrix spike:

The Department of Pesticide Regulations (DPR) provides the background water for matrix blank and spikes.

9.1.2.1 Matrix blank: Weigh out approximately 500 g of background water and follow the test sample extraction procedure.

9.1.2.2 Matrix spike: Weigh out approximate 500 g of background water. Spike a client requested amount of Fipronil pesticides into the background water and let it stand for 1 minute. Follow the test sample extraction procedure.

9.2 Test Sample Extraction

9.2.1 Shake sample bottle before making sample aliquot. Measure 500mL of sample into a graduated cylinder then transfer sample into a separatory funnel. Add 5 ± 1 grams sodium chloride and shake to dissolve.

9.2.2 Shake with 60 ± 5mL of methylene chloride for 2 minutes. Vent frequently to relieve pressure.

9.2.3 After phases have separated, drain lower methylene chloride layer through 20 ± 4 g of anhydrous sodium sulfate and glass wool, into a 500mL boiling flask.

9.2.4 Repeat steps 9.2.2 & 9.2.3 two more times using 60 ± 5 mL of methylene chloride each time. Combine the extracts in the same boiling flask.

9.2.5 After draining the final extraction, rinse the sodium sulfate with 25 ± 5 mL of methylene chloride.

9.2.6 Evaporate the sample extract to 2 - 4 mL on a rotary evaporator using a water bath at 35 ± 2 °C and 15 - 20 inch Hg vacuum. Transfer the extract to a calibrated 15 mL graduated test tube.

9.2.7 Rinse flask 3 times with 2 - 4 mL of acetone and transfer each rinse to the same test tube.

9.2.8 Evaporate the extract to a volume slightly less than 0.4 mL in a water bath at 35 ± 4 °C under a gentle stream of nitrogen. Then bring to a final volume of 0.5mL with acetone. Add 10µL of the 20.0µg/mL Phenanthrene-d10 standard, mix well and transfer into auto sampler vials.

9.2.9 Submit extract for GC/MSD analysis.

10. Instrument Calibration:

10.1 The calibration standards are added to a matrix blank extract to correct for matrix background interference.

10.2 A calibration standard curve consists of minimum of three levels. Standard concentrations of 0.025, 0.10, 0.25, 0.50, 1.0µg/mL are recommended. Calibration is obtained using a linear or quadratic regression with the correlation coefficient (r) equal to or greater than 0.995. All standards, samples and quality control samples have the internal standard, Phenanthrene-d10, added at 1.0µg/mL final concentration.

11. Analysis:

11.1 Injection Scheme

Recommended injection scheme: Calibration standards, Solvent, Matrix Bank, Matrix Spike, Test Samples (maximum of 10-12 samples) and Calibration standards. Injection of an old sample or matrix blank before the sequence analysis to condition the instrument is recommended.

11.2 GC/MSD Instrumentation

11.2.1 Recommended instrument (GC/MSD) parameters: Injector 230 °C; MSD transfer line heater 280 °C; initial oven temperature 50 °C, hold 2 min., ramp @ 25 °C/min. to 200 °C hold 1 min. and then ramp @ 5 °C/min. to 275 °C, hold 8 min; Injection volume 2 or 3 µL.

Ions Selected for SIM Acquisition: (in retention time order)

Phenanthrene-d10	**188**	Group 1
Desulfinyl Fipronil	333, 369, **388**, 390	Group 1
Fipronil Sulfide	255, **351**, 353, 420	Group 2
Fipronil	213, **367**, 369	Group 2
Desulfinyl Fipronil amide	308, 390, **406**	Group 3
Fipronil Sulfone	213, 365, **383**	Group 3
Fipronil amide	255, 368, **385**, 387	Group 4

(Quantitation ions are in bold)

12. Quality Control:

12.1 Each set of samples shall have a matrix blank and minimum of one matrix spike sample.

12.2 The matrix blank should be free of target compounds above the MDL.

12.3 The recoveries of the matrix spike shall be within the control limits.

12.3.1 When spike recoveries fall outside the control limits, the chemist must investigate the cause. The entire extraction set of samples may be re-analyzed. If the spike recoveries fall within the limit, then the results from the re-analyzed samples shall be reported.

12.3.2 If the spike recoveries still fall outside the control limits, the client will be notified. The backup samples may need to be re-extracted for analysis.

12.4 The retention time should be within ± 2 percent of that of the standard.

12.5 The sample must be diluted if results fall outside the linear range of the standard curve.

12.6 Bracketing standard should have a percent change less than 25%.

12.7 Method Detection Limits (MDL)

The method detection limit refers to the lowest concentration of analyte that a method can detect reliably. To determine the MDL, 7 replicate water samples are spiked at 0.025 ppb for Fipronil and metabolites. The standard deviation from the spiked sample recoveries are used to calculate the MDL for each analyte using the follow 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 replicate used to determine the MDL, t=3.143.

12.8 Reporting limit (RL):

The reporting limit (RL) refers to the level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. Per client agreement, the RL is chosen in a range 1-5 times the MDL.

MDL data and the RL are tabulated in Appendix IA and IB.

12.9 Method Validation Recovery Data and Control Limits:

12.9.1 The method validation consisted of three sample sets. Each set included 5 levels of fortification (0.025, 0.05, 0.10, 0.2, and 1.0 ppb) and a method blank. All spikes and method blank samples were processed through the entire analytical method.

12.9.2 Upper and lower warning and control limits are set at $\pm$ 2 and $\pm$ 3 standard deviations of the average % recovery, respectively.

12.10 Estimated Measurement Uncertainty:
Total uncertainty for this method is 13.9% based on the method validation.

12.11 Trend Identification

12.11.1 All matrix spike recoveries for this analysis will be put into control charts and monitored for trends. Three trend characteristics will be evaluated at least bi-yearly by the supervisor or designee.
2 of 3 points above or below 2/3 of the UCL or LCL.
7 continuous points above or below the center line (CL)
14 points alternating above and below the CL.

12.11.2 When results indicate an out of control situation the supervisor or designee will indicate this on the control chart and take appropriate corrective action, which may include monitoring the results more closely to initiating a formal corrective action with root cause investigation.

13. Calculations:

Quantitation is based on external standard (ESTD) calculation using either the peak area or height. The software uses a linear or quadratic 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.

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

14. Reporting Procedure:

14.1 Identification of Analyte

For responses within calibration range, compare the retention time of the peaks with the retention time of standards. For positive results retention times shall not vary from the standards more than 2 percent.

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

15. Discussion and References:

15.1 Sample response and quantitation vary depending on matrix background in the samples. The calibration standards are diluted with a matrix blank extract to correct for matrix background interference.

16. References:

16.1 *EPA Method 507, Pesticides, Capillary Column*. EPA Test Method for Drinking Water and Raw Source Water, 1987.

16.2 Hsu, J. and Hernandez J. *Determination of Organophosphate Pesticides in Surface Water using Gas Chromatography*, 1997, Environmental Monitoring Method, Center for Analytical Chemistry, CDFA.

APPENDIX IA

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

Seven replicates of a 0.025µg/mL spike (25.00 µg/L)

Sample #	Desulfinyl Fipronil	Fipronil sulfide	Fipronil	Desulfinyl fipronil amide	Fipronil sulfone	Fipronil amide	
	ng/L	**ng/L**	**ng/L**	**ng/L**	**ng/L**	**ng/L**	
1	26.39	26.94	28.44	28.40	26.68	24.63	
2	25.30	26.24	29.20	28.53	26.14	22.40	
3	24.79	25.73	31.52	30.22	27.26	25.61	
4	26.45	26.67	30.94	31.32	29.32	25.84	
5	24.84	25.28	29.06	28.13	24.47	23.95	
6	24.30	25.23	29.09	27.10	25.26	23.09	
7	25.93	26.13	29.25	28.40	26.17	23.30	
Std. Dev	0.84	0.66	1.13	1.42	1.55	1.30	
3.14*SD	2.65	2.06	3.55	4.45	4.87	4.09	
MDL **(ng/L)**	2.650	2.060	3.550	4.450	4.870	4.090	MDL in ng/L
MDL (µg/L)	**0.003**	**0.003**	**0.004**	**0.005**	**0.005**	**0.005**	**MDL in ppb**

Spiked water sample with 0.5mL of a 0.025µg/mL fipronil and metabolites standard.
The extraction is 1:1000 concentration.
The MDL then is spiked at 0.025µg/L (ppb)
The results are reported in ng/L, which is 1000 times the ppb value.
The MDL is reported in µg/L (ppb)

APPENDIX IB

Method Validation and Control Limit

Compound	Mean	Std. Dev.	Control limit
Desulfinyl Fipronil	100.4	13.9	UCL: 142.2 UWL: 128.3 LWL: 72.6 LCL: 58.5
Fipronil Sulfide	97.3	16.8	UCL: 147.6 UWL: 130.8 LWL: 63.8 LCL: 46.9
Fipronil	103.3	12.0	UCL: 139.4 UWL: 127.4 LWL: 79.2 LCL: 67.1
Desulfinyl Fipronil amide	110.7	16.2	UCL: 159.2 UWL: 143.0 LWL: 78.3 LCL: 62.2
Fipronil Sulfone	106.1	15.0	UCL: 151.2 UWL: 136.2 LWL: 76.1 LCL: 60.9
Fipronil amide	92.4	9.1	UCL: 119.6 UWL: 110.5 LWL: 74.2 LCL: 65.1

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