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Determination of Fenamiphos, Fenamiphos Sulfoxide, and Fenamiphos Sulfone in Well Water By Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry

Scope: This method is applicable to the analysis of Fenamiphos, Fenamiphos Sulfoxide, and Fenamiphos Sulfone in well water using APCI/LC/MS/MS. The reporting limit for all three analytes is 0.05 µg/L.

Principle: Two conditioned Waters Oasis®HLB and C18 Cartridges connected in tandem are used to retain the analytes from well water samples. The cartridges are placed under vacuum to eliminate any remaining water. The chemicals are eluted with methanol. The eluant is then filtered, concentrated, and analyzed by APCI/LC/MS/MS.

Reagents, Equipment and Instruments:

Reagents:

1. Methanol, LCQ grade. Burdick & Jackson 230-4.
2. Distilled water, LCQ grade. Burdick & Jackson 365-4. Burdick & Jackson solvents are available from VWR and other suppliers.
3. Acetic acid. Glacial.
   Note: The highest available purity reagents (1,2,3) should be specified when ordering.
4. Mobile phase C: 0.2% acetic acid in water.
5. Mobile phase D: 0.2% acetic acid in methanol.

Equipment:

1. In-house vacuum manifold.
2. Solid-phase extraction cartridges: Waters Oasis® HLB 6 cc (500 mg), C18 6cc (1000 mg) cartridges, Waters Division of Millipore Corporation.
3. Nylon Acrodisc®, 0.2 micron, Gelman Sciences.
4. Vac-Elut SPS 24, Varian Analytical.
5. N-EVAP, Meyers Organomation Associates Incorporated-Model 112.
6. Vibrating or vortex mixer.
7. Syringe and plunger for filtration, 10 mL.
8. Graduated test tube, 15 mL (calibrated at 0.5 mL with methanol)
9. Cartridge connector. J.T.Baker

Instruments:

1. LCQ™DECA LC/MS^n System. ThermoQuest/Finnigan Corporation
2. Waters 2690 HPLC system with autosampler.

Analysis:

1. Allow each sample to come to room temperature. Pour the sample from the 1 L amber glass bottle into a 400 mL beaker. Record the sample weight in grams (g) by weighing the bottle before and after transfer. Sample weight should be close to 250.0 g.
2. A Waters Oasis® HLB cartridge (top) is connected to a C18 cartridge (bottom) by a cartridge connector and connected to the house vacuum using the manifold as shown in Diagram # 1.
3. Condition the cartridges at a flow rate of about 10 mL/minute with about 15 mL of methanol followed by about 15 mL of purified water by applying vacuum.
   Do not let the cartridges go to dryness. Turn off the vacuum when the purified water has just passed through the cartridges. Detach the cartridges from the vacuum line and fill up the cartridges with purified water.

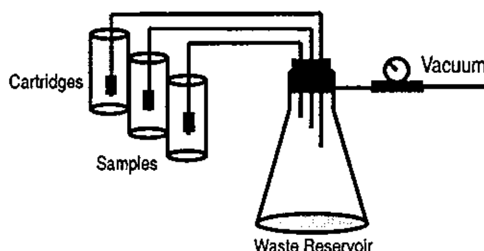

Diagram # 1

4. Reattach the conditioned cartridges to the vacuum line and transfer to the 400 mL beaker containing the water sample. Allow the sample to pass through the conditioned cartridges by applying vacuum. Adjust the flow rate to about 3 mL/minute to 5 mL/minute.
5. After all of the water sample has passed through the cartridges, remove the cartridges and insert them into the inlets of the Vac-Elut SPS 24 at the "waste position". Turn the vacuum on (~5 psi) to dry the cartridges separately for 2 minutes. Turn the vacuum off and reverse the order of the cartridge positions (Waters Oasis® HLB cartridge now is at the bottom and C18 cartridge is at the top). Add 5 mL of methanol to each cartridge. Switch the Vac-Elut SPS 24 to the "collect position" and turn the vacuum on. Elute all chemicals with a total of 12 mL methanol at a flow rate of about 5 mL/minute. Collect the eluant in a 15 mL graduated test tube.
6. Filter the eluant through a 0.2 µm Acrodisc into a 15 mL graduated test tube which has been calibrated at 0.5 mL. Concentrate the eluant to 0.5 mL in a 40 °C waterbath under a stream of nitrogen. Vortex for 30 seconds. Transfer the eluant into two autosampler vials with inserts. Analyze by APCI/LC/MS/MS.

Instrument Conditions:

APCI Source Settings:

Vaporizer Temp (°C): 550
Sheath Gas Flow Rate (arb): 80
Aux Gas Flow Rate (arb): 6
Discharge Current (μA): 5
Discharge Voltage (kV): 5
Capillary Temp (°C): 200
Capillary Voltage (V): 5

HPLC Settings:

Analytical column: *HPLC column: Phenomenex® Luna 3 u C8 50 x 3.0 mm x 3 um*
HPLC guard cartridge: Phenomenex® C8 (MOS,Octyl) 4.0 mm x 2.0 mm
HPLC gradient program:

Time (min.)	C%	D %	*Flow (ml/min.)*
0.00	90	10	0.4
4.00	90	10	0.4
10.00	10	90	0.4
12.00	90	10	0.4
15.00	90	10	0.4

Solvent A: 0.2% acetic acid in water
Solvent B: 0.2% acetic acid in methanol
Injection volume: 20 uL
Column temperature: 40 °C

MS Detector Settings:

MS run time (min.): 12.00
Divert valve (min.): 0.00 to waste.
8.00 to source.
Segment: 1
Duration time (min.): 8
Number of scan events: 1
Tune method: Fenamiphos and metabolites
Scan event details:

1. MS: [100-500]

Segment: 2
Duration time (min.): 2.00
Number of scan events: 2
Tune method: Fenamiphos and metabolites
Scan event details:

1. Pos [336]]⇒ [90-400] : Fenamiphos Sulfone
   Ms/Ms: Amp: 26% Q: 0.250 Time (msec.): 30 IsoW: 4.0
   Quan. Ions: 336, 308, 294
   Rt: 9.47 min.

MS Detector Settings: (cont.)

Segment: 2 (cont.)

2. Pos [320]⇒ [85-400] : Fenamiphos Sulfoxide
   Ms/Ms: Amp: 29% Q: 0.250 Time (msec.): 30 IsoW: 4.0
   Quan. Ions: 320, 292, 278
   Rt: 9.42 min

Segment: 3
Duration time (min.): 2.00
Number of scan events: 1
Tune method: Fenamiphos and metabolites
Scan event details:

1. Pos [304]⇒ [80-400] : Fenamiphos
   Ms/Ms: Amp: 28% Q: 0.250 Time (msec.): 30 IsoW: 5.0
   Quan. Ions: 304, 276, 262

Calculations:

The results are reported in μg/L:

$$\mu g/L = \frac{\mu g/mL \text{ (from standard curve) x final volume (mL)}}{\text{Sample weight (g)}} \text{ x } 1000 \text{ g/L}$$

Method Performance:

Quality Control:

1. Sample storage: All field samples shall be kept refrigerated at 4 °C ± 2 until extracted.
2. Sample extraction: All extracts shall be kept refrigerated at 4 °C ± 2 until analyzed.
3. For each set of samples, at least one matrix blank and one matrix spike shall be included.

Each set of samples shall not contain more than twelve samples including quality control samples.

Recovery data:

This analytical method was validated by preparing five sets of samples using the provided background well water. Each set contained three different levels of spike and a matrix blank. Each set was processed through the entire analytical method on a different day. The results are shown in Appendix II.

Method detection limit (MDL):

Method Detection Limit (MDL) refers to the lowest concentration of analytes that a method can detect reliably. To determine the MDL, 7 replicate background samples were spiked at 0.200 μg/L. The standard deviation from the spiked samples was used to calculate the MDL using the following equation:

$$MDL = tS$$

where:

t is the Student t 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. See Appendix I for recovery data from the determination of the Method Detection Limits.

Method detection limit (MDL): (cont.)

The Reporting Limit (RL) refers to the level at which quantitative results may be obtained. By convention, the RL is chosen in a range 1-5 times the MDL. The Reporting Limit for this method is 0.05 μg/L for all analytes.

Discussion:

A standard curve consisting of four levels was used for every twelve injections. At the analyst' discretion, a five or six-level calibration may be run. Each sample was injected twice back to back. The external standard technique with the average of all standard levels from the beginning of a sequence to the end of a sequence was used to quantify samples. The response of a same level standard before and after samples should not differ by more than 25%. If the standard responses are not in acceptable range, a root analysis should be done to identify the causes. Aceptable recoveries of all analytes are Mean ± 2 standard deviations.

After each sequence is completed, the column should be rinsed with high organic mobile phase (≥ 80%) solution for few hours and stored in that condition. Before starting a sequence, test standards should be run first to ensure that the column is fully equilibrated. Test standards will not be used in quantitation.

References:

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Appendix I: Recovery data for determination of method limits

Sample name	Spike level (µg/L)	Fenamiphos recovery (µg/L)	F.Sulfoxide recovery (µg/L)	F. Sulfone recovery (µg/L)
BLK	0.0	0.0	0.0	0.0
SPIKE 1	0.2	0.164	0.180	0.174
SPIKE 2	0.2	0.148	0.168	0.150
SPIKE 3	0.2	0.136	0.154	0.154
SPIKE 4	0.2	0.128	0.160	0.140
SPIKE 5	0.2	0.152	0.204	0.170
SPIKE 6	0.2	0.162	0.214	0.186
SPIKE 7	0.2	0.156	0.176	0.172
MDL		0.022	0.034	0.025
RL		0.050	0.050	0.050

Appendix II: Recovery data for method validation

Spike level (µg/L)	Fenamiphos recovery (%)	F.Sulfoxide recovery (%)	F. Sulfone recovery (%)
0.200	67.0	88.0	81.0
0.200	86.0	99.0	98.0
0.200	87.0	109.0	106.0
0.200	91.0	110.0	103.0
0.200	92.0	102.0	109.0
2.000	81.0	110.0	97.0
2.000	109.0	103.0	110.0
2.000	97.0	114.0	120.0
2.000	118.0	116.0	109.0
2.000	111.0	100.0	113.0
5.000	95.2	118.4	105.6
5.000	114.4	112.0	117.6
5.000	90.4	118.0	102.0
5.000	112.0	112.8	115.2
5.000	108.8	105.6	114.4