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Fenoxycarb, Pyriproxyfen and Hydramethylnon in Water

Scope: This method is for the determination of Fenoxycarb, Pyriproxyfen and Hydramethylnon in surface and well water. The reporting limit of this method for Fenoxycarb and Pyriproxyfen is 0.1ppb, and for Hydramethylnon is 0.2ppb.

Principle: Fenoxycarb, Pyriproxyfen and Hydrameyhylnon in water are extracted with methylene chloride. After the solvent evaporated, the extract is dissolved in methanol. All three compounds are determined by HPLC using an UV detector.

Reagents:

1. Fenoxycarb, CAS#79127-80-3, 1.0 mg/mL in methanol, obtained from CDFA Standard Repository (Center for analytical Chemistry, California Department of Food and Agriculture).
2. Pyriproxyfen, CAS# 95737-68-1, 1.0 mg/mL in methanol, obtained from CDFA Standard Repository (Center for analytical Chemistry, California Department of Food and Agriculture)
3. Hydramethylnon, CAS#67485-29-4,1.0 mg/mL in methanol, obtained from CDFA Standard Repository (Center for analytical Chemistry, California Department of Food and Agriculture)
4. Methylene chloride, pesticide residue grade,
5. Methanol, pesticide residue grade
6. Water, HPLC grade
7. Acetonitrile, HPLC grade
8. Buffers:

A: In 1000mL	B: In 500mL
90mL HPLC water	471mL HPLC water
900mL Methanol	25mL Methanol
10mL 1M Ammonium formate	4.75mL 1M Ammonium formate
1mL Formic Acid	0.5mL Formic acid

Safety:

1. Most of the reagents used and analyzed for in this method have not been completely characterized. All general laboratory safety rules must be followed.
2. Methylene chloride may consider be a carcinogen, please handle it with extremely caution.

Equipment:

1. Separatory funnels, 1000 mL
2. Boiling flasks, flat-bottomed, 24/40 joints, 500 mL
3. Funnels, glass short stemmed 100 mm diameter

Equipment: (continuous)

4. Rotary evaporator, Büchi-Brinkmann, Model R 110
5. Conical test tubes, graduated, calibrated 15 mL
6. Nitrogen evaporator, Organomation, Model 12
7. Vortex mixer, Thermolyne, Model 37600
8. Acrodisc®, Gelman, 25 mm x 0.2 µm, disposable filter
9. Syringe, Hypodermic, 5 or 10 mL

Instruments:

1. HPLC: Hewlett-Packard 1050 Liquid Chromatographs with a ChemStation and UV detector.
2. Analytical columns: Beckman Ultrasphere 5 µm, 4.6mm, 25 cm in length and Pickering column for carbamate analysis 5µm, 4.6mm, 25cm in length.

Interference:

1. The UV detector has limited specificity. The coleution interference may occur.
2. All positive samples will be confirmed on LC/MS.

Standard Preparation:

1. After received the standard mix containing 1mg/mL of each compound from CDFA Standard Repository, the standard mix is diluted to 10ηg/µl with methanol as a working standard.
2. Dilute the working standard into a series of desired standard set that will be used for instrument calibration and samples 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 arrived and records it in the notebook for temperature.
2. Sign the chain custody and obtain the EMON number from supervisor.
3. Store all samples in the walk-in refrigerator waiting for analysis.

Test Sample Preparation:

1. Remove samples from storage and allow them to come to room temperature (± 5 °C).
2. Shake each sample well and weigh out approximately 500 grams by difference. Place this aliquot into a separatory funnel. Unused sample will be restored in the walk-in refrigerator.
3. Extract samples by adding 100 mL of methylene chloride and shake vigorously for one minute. **Vent frequently to relieve pressure.**
4. After phase separation, drain the methylene chloride through a glass funnel containing 15 grams of Na_2SO_4 and glass wool under. Collect the extract into a boiling flask.
5. Repeat steps 3 and 4 two more times with 80 mL of methylene chloride each.
6. After the final extract drained, rinse the sodium sulfate with 25 mL of methylene chloride.
7. Concentrate the extract to 2 ~ 3 mL on a rotary evaporator using 30 ~ 35 °C water bath and a vacuum of 15 inches Hg.
8. Filter the extract through an Acrodisc® unit and collect the filtrate in a calibrated 1mL conical test tube.
9. Rinse the flask two times with 3 mL of methanol each. Filter through the same Acrodisc® unit and collect the rinse in the same test tube.

Test Sample Preparation: (continuous)

10. Place the extract in a nitrogen evaporator with water bath set at 45 °C and evaporate just to a mark for 1mL. (If below 1mL mark, add methanol to bring up to 1mL)
11. Votex the tube for 15 second and transfer the content into two autosampler vials with inserts for analysis.

Instrument Conditions:

For Fenoxycarb and Pyriproxyfen,

Instrument:	HPLC, Hewlett- Packard Model 1050, controlled by Chemstation
Column:	Beckman Ultrasphere 5 μ, 4.6 mm, 25 cm long
Mobile phase:	Isocratic 20% water and 80% acetonitrile
Flow:	1.0 mL/min.
Injection volume:	20 μL
UV detector:	230 nm
Retention Time:	Fenoxycarb=3.95 ± 0.2 minutes, Pyriproxyfen=7.95±0.2 minutes

For Hydramethylnon,

Instrument:	HPLC, Hewlett- Packard Model 1050, controlled by Chemstation
Column:	Pickering Column for carbamate analysis 5 μ, 4.6 mm, 25 cm long
Mobile Phase:	95% buffer A and 5% buffer B
Flow:	1.0 mL
Injection Volume:	20 μL
UV detector:	290 nm
Retention Time:	5.2±0.2 minutes

* Both instruments operate in ambient temperature. The Retention Time of each compound may shift dramatically if temperature changes too much.

Instrument Calibration:

1. Load a method, set the desired condition for analysis on both instruments.
2. Run 0.1, 0.2 1.0, 5.0 and 10 ηg/μL to check the system linearity for Hydramethylnon.
3. Run 0.05, 0.2, 1.0, 5.0 and 10 ηg/μL to check the system linearity for Fenoxycarb and Pyriproxyfen.

Analysis:

Quality Control:

1. A 5-point calibration curve of 0.1, 0.2, 1.0, 5.0 and 10 ηg/μL for Hydramethylnon and a 5-point calibration curve of 0.05, 0.2, 1.0, 5.0 and 10ηg/μL for Fenoxycarb and Pyriproxyfen 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. 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 together with standards twice more. A sample set is usually comprised of 10 samples, a blank and a spike.

Analysis: (continuous)

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, 500 ± 1g of background surface water each, with 0.2 ppb of Fenoxycarb, Pypripoxyfen and 0.5 ppb of Hydramethylnon and process each 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-1degrees 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: Table 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 for the method is 0.1ppb for fenoxycarb, pyriproxyfen and 0.2 ppb for hydramethylnon.

Recovery Data:

Method validation was made by preparing eight sets of spike samples. Each set contained a blank and three levels of spikes. The background water (American River water) was obtained from Department of Pesticide Regulation. Each set was processed through the entire analytical method. Recoveries of Fenoxycarb, Pyriproxyfen and Hydramethlynon are shown in Appendix 1: Table 2.

Calculations:

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

$$\text{where: response factor } (\eta g) = \frac{\Sigma[\ (\text{std. conc.}_n, \eta g/\mu L)\ (\text{std. vol. injected, } \mu L)/\ (\text{std. peak ht.}_n)]}{n}$$

n = number of standards

Discussion:

1. During our initial method validation process we encountered an extreme problem of low recovery for Hydramethylnon in two spiked samples. We examined every step for a clue to the problem. The finding was inconclusive. We decided to abandon all 5 sets of validation data and re-validated with slight modification of the method. The reported 8 sets of data are generated according to this method. It is our concern that the problem of low recovery may occur again. We will pay attention to this problem very closely.

Discussion: (continuous)

2. MDL and three sets validation for well water were done from 6/22/00 to 6/28/00. The results are in Appendix 2: Table 3 and 4. All three compounds were spiked 0.2 ηg/μL for MDL. Same spike levels for surface water were used for well water validation.

References:

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Appendix 1:

Table1. Fenoxycarb, Pyriproxyfen and Hydramethylnon MDL Results (ppb) for surface water

Spike #	Fenoxycarb	Pyriproxyfen	Hydramethylnon
1	0.174	0.218	0.402
2	0.199	0.206	0.422
3	0.192	0.200	0.367
4	0.190	0.200	0.328
5	0.191	0.189	0.348
6	0.191	0.206	0.387
7	0.203	0.219	0.401
S =	0.0092	0.0107	0.0333
MDL = 3.143 x S	0.0288	0.0337	0.1045

Table 2. Fenoxycarb, Pyriproxyfen and Hydramethylnon Method Validation Results and Recovery for surface water

	Fenoxycarb		**Pyriproxyfen**		**Hydramethylnon**	
Spike Level (ppb)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)
1	0.965	96.5	0.994	99.4	0.705	70.5
	0.960	96.0	0.957	95.7	0.791	79.1
	0.969	96.9	0.973	97.3	0.753	75.3
	0.980	98.0	1.004	100.4	0.745	74.5
	0.958	95.8	1.017	101.7	0.759	75.9
	0.975	97.5	0.980	98.0	0.786	78.6
	0.947	94.7	0.949	94.9	0.753	75.3
	0.983	98.3	1.016	101.6	0.814	81.4
10	11.2	111.8	9.784	97.8	8.84	88.4
	9.69	96.9	9.78	97.8	9.72	97.2
	8.81	88.1	9.05	90.5	7.86	78.6
	11.0	110.0	10.89	108.9	10.75	107.5
	9.60	96.0	9.58	95.8	9.76	97.6
	9.94	99.4	9.82	98.2	10.45	104.5
	9.22	92.2	9.34	93.4	104.9	104.9
	9.79	97.9	9.81	98.1	10.81	108.1
100	90.1	90.1	98.7	98.7	71.3	71.3
	97.8	97.8	98.2	98.2	85.3	85.3
	95.2	95.2	92.8	92.8	66.8	66.8
	102.0	102.0	100.6	100.6	71.5	71.5
	101.4	101.4	100.7	100.7	87.7	87.7
	96.6	96.6	95.3	95.3	77.2	77.2
	97.5	97.5	96.1	96.1	101.9	101.9
	97.9	97.9	97.8	97.8	79.7	79.7

Appendix 2:

Table3. Fenoxycarb, Pyriproxyfen and Hydramethylnon MDL Results (ppb) for well water

Spike #	Fenoxycarb	Pyriproxyfen	Hydramethylnon
1	0.186	0.183	0.205
2	0.188	0.183	0.161
3	0.182	0.169	0.149
4	0.185	0.179	0.130
5	0.183	0.183	0.189
6	0.185	0.178	0.208
7	0.222	0.202	0.209
S =	0.0142	0.0102	0.0320
MDL = 3.143 x S	0.0447	0.0322	0.1006

Table 4. Fenoxycarb, Pyriproxyfen and Hydramethylnon Method Validation Results and Recovery for well water

	Fenoxycarb		**Pyriproxyfen**		**Hydramethylnon**	
Spike Level (ppb)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)
1	0.975	97.5	0.950	95.0	0.887	88.7
	0.708	70.8	0.941	94.1	0.717	71.7
	1.024	102.4	1.013	101.3	0.764	76.4
10	8.368	83.7	8.687	86.9	8.349	83.5
	8.994	89.9	9.280	92.8	7.910	79.1
	12.199	122.0	12.459	124.6	11.472	114.7
100	86.326	86.3	89.143	89.1	80.013	80.0
	93.030	93.0	98.200	98.2	79.531	79.5
	88.623	88.6	89.567	89.6	75.485	75.5