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Method #: EM 29.6
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Determination of Hexazinone and Diuron in Sediment by LC/MS

Scope:

This method is for the determination of Hexazinone and Diuron in sediment. The reporting limit for this method is 0.010 ppm. CAS registered numbers for Hexazinone and Diuron are 51235-04-2 and 330-54-1 respectively.

Principle:

Diuron and Hexazinone are extracted from sediment by acetonitrile, the extract is concentrated and analyzed by APCI-LC/ MSMS.

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 from supervisor.
3. Store all samples waiting for extraction in a freezer.

Safety:

All general laboratory safety rules for sample preparation and analysis shall be followed. Samples shall be prepared in a fume hood. Proper disposal procedures must be followed.

Interference:

No interferences have been observed for this method.

Standard Preparation:

1. Dilute the 1mg/mL standard solutions (obtained from CAC Standard Repository) with methanol into a set of mixed standards of the desired concentrations for spiking, instrument calibration and sample calculation. The concentration for spiking is 1 ng/ul of each analyte. A set of five calibration standards shall be prepared to cover the linear range from 0.04 ng/ul to 1.0 ng/ul. Levels of 0.04, 0.1, 0.2, 0.4, and 1.0 ng/ul were used for method validation, and these levels are recommended.
2. Keep all prepared standards in the designated refrigerator for storage while not in use.
3. The shell life of each prepared standard is six months unless a shorter expiration date is specified by the Standard Repository.

Reagents:

1. Methanol, LC/MS grade
2. Acetonitrile, LC/MS grade
3 Sodium Sulfate, granular, anhydrous
4 Water, LC/MS grade
5. Acetic acid, glacial

Equipment:

1. 50 mL test tube.
2. Filter paper: Whatman # 4.
3. Acrodisc®, 0.2 µm filter. Gelman Science.
4. 10 mL syringe with plunger.

Instrumentation:

1. HPLC Waters 2690 with auto sampler
2. LCQ DECA ® Finnigan

Procedure:

1. Remove sediment samples from freezer and allow them to come to room temperature.
2. Weigh 20.0 g of sample into a 50 mL test tube.
3. Add 10 g of sodium chloride.
4. Add 25 mL of Acetonitrile to the test tube using 25 mL pipet.
5. Shake the tube for 2 minutes by hand.
6. Let the tube stand for 10 minutes.
7. Pipet 5 mL of the top layer into a 10 mL syringe and filter it through a 0.2 micron acrodisc into a 15 mL test tube calibrated for 1.0 mL.
8. Concentrate the filtrate to 1 mL using a nitrogen evaporator set at 45 °C and transfer into two autosampler vials with inserts.
9. Analyze by APCI-LC/MSMS.

Instrument Condition:

HPLC column: Phenomenex® Luna C8 50 x 3.0 mm x 3 um
HPLC guard cartridge: Phenomenex® C8 4.0 mm x 2.0 mm
HPLC gradient program:

Time (min.)	*A%*	*B %*	*Flow (ml/min.)*
0.00	80	20	0.4
14.00	10	90	0.4
15.00	10	90	0.4
16.00	80	20	0.4
18.00	80	20	0.4

Solvent A: 0.2% acetic acid in water
Solvent B: 0.2% acetic acid in methanol
Injection volume: 25 uL
Column temperature: 40 °C

Instrument Condition: (cont.)

Mass Detector Settings:

MS run time (min.): 10
Divert valve: 5.0 minutes to source
Segment 1:
Duration time (min.): 5
Number of scan events: 1
Tune method: 1-400 (hexazinone)
Scan event details:
1. Pos [60.0-250.0]

Segment 2
Duration time (min.): 4
Number of scan events: 2
Tune method: 1-400 (hexazinone)
Scan event details:
1. Pos [253.0]=>[65.0-275.0]
   MS/MS: Amp: 29.0%. Q: 0.250. Time 30.00. IsoW: 5.0
2. Pos [234]=>[60.0-275.0]
   MS/MS: Amp: 31.0%. Q: 0.250. Time 30.00. IsoW: 5

Note: This method was validated on the LCQ Deca with Waters HPLC 2960 system and column listed above. A "mini-validation" using a protocol approved by the project leader and the section supervisor may be run if it is necessary to use a different instrument or column.

Calculation:

$$\text{ppm } (\mu g/g) = \frac{\mu g/mL \text{ (from the standard curve) X aliquot final volume (mL)}}{\text{Aliquot sample weight (g)}}$$

For this method, aliquot final volume is 1 mL, aliquot sample weight is 4.0 g

Analysis:

Quality Control:

1. A 4 - point calibration curve shall be run 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, dilute the sample. Reinject the diluted sample with standards as directed above.
3. Sample storage: All field samples shall be kept frozen at until extracted. Samples shall be brought to a room temperature before extracted.
4. Sample extracts: All extracts shall be kept frozen at -10 °C until analyzed.
5. Freezer, refrigerator and oven temperatures shall be monitored and recorded daily.
6. 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.

Analysis: (cont.)

Method Detection Limit:

The Method Detection Limit (MDL) refers to the lowest concentration of an analyte that a method can detect reliably in either a sample or a blank. To determine the MDL, spike 7 samples with 0.02 ppm of hexazinone and diuron 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 n=7), 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):

The RL refers to level above which reliable quantitative results may be obtained. The MDL was used as a guide to determine the RL. The reporting limit for Hexazinone and Diuron is 0.010 ppm.

Recovery Data:

The analytical method was validated using five sets of spiked samples. Each set contained a blank and four levels of spikes. Each set was processed through the entire analytical method. Recoveries of hexazinone and diuron are in Appendix 2.

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Appendix 1:

Hexazinone and Diuron MDL Results (ppm) for sediment

Spike #	Hexazinone	Diuron
1	0.0158	0.0247
2	0.0160	0.0.247
3	0.0158	0.0258
4	0.0159	0.0189
5	0.0158	0.0217
6	0.0148	0.0202
7	0.0180	0.0260
S= MDL = 3.143 x S	0.0009 0.0028	0.0032 0.010
RL	0.010	0.010

Appendix 2:

Hexazinone and Diuron Method Validation Results and Recovery for Sediment

	Hexazinone		Diuron	
Spike Level (µg/kg)	Result (µg/kg)	Recovery (%)	Result (µg/kg)	Recovery (%)
50	42.5	85.0	49.5	99
	45.0	90.0	51.3	102.6
	41.3	82.5	53.8	107.6
	43.3	86.6	49.8	99.6
	36.0	72.0	57.3	114.6
100	93.8	93.8	106.3	106.3
	86.3	86.3	105.3	105.3
	80.5	80.5	92.5	92.5
	90.8	90.8	108.5	108.5
	100.8	100.8	114.5	114.5
500	475.0	95.0	472.5	94.5
	470.0	94.0	460.0	92.0
	457.0	91.5	415.0	83.0
	492.0	98.4	489.5	97.9
	494.5	98.9	501.5	100.3
2000	1860.0	93.0	1776.0	88.8
	2616.0	130.8	2510.0	125.5
	2200.0	110.0	1970.0	98.5
	2418.0	120.9	2464.0	123.2
	2088.0	101.4	2028.0	101.4