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Determination of Hexazinone and Diuron in Alfalfa by LC/MS

Scope:

This method is for the determination of Hexazinone and Diuron in Alfalfa. The reporting limit for this method is 0.100 ppm. CAS registered numbers for Hexazinone and Diuron are 51235-04-2 and 330-54-1 respectively.

Principle:

The plant materials are cut into small pieces and homogenized with dry ice using a Cuisinart. A portion of homogeneous sample is extracted with acetonitrile. The acetonitrile extract is evaporated to almost dryness on a steambath under nitrogen and cleaned up by passing through an aminopropyl Bond Elut. Then Hexazinone and Diuron are eluted with a mixture of methanol and methylene chloride. The final extract is 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. Alfalfa may contain high levels of pesticides, and should be handled with gloves at all times. 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 1mg/mL standard solutions (obtained from the CAC Standard Repository) with methanol into a set of mixed standards of the desired concentrations for spiking, instrument calibration and sample calculation. 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 shelf life of each prepared standard is six months unless a shorter expiration date is specified by the Standard Repository.

Reagents:

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

Equipment:

1. Mason jar: One quart, narrow mouth with cap
2. Blender: Omni-Mixer
3. Vacuum manifold: Vac Elut SPS 24. Varian
4. Bond Elut: Aminopropyl Bond Elut 10 cc/ 500 mg. Varian®
5. Filter paper: Whatman # 4
6. Acrodisc®, 0.2 µm filter. Gehman Sciences

Instrumentation:

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

Procedure:

1. Cut the entire sample into small pieces (less than one inch in length). Homogenize the sample with dry ice using a Cuisinart until obtaining the smallest particles possible. Store the sample in a freezer overnight loosely capped to allow the CO₂ to dissipate.
2. Bring the sample to room temperature. Weigh out 25.0 grams of the homogenous sample-into a narrow mouth, one pint mason jar. Add 100 mL of acetonitrile and blend the sample for 5 minutes using an Omni-Mixer set at medium speed.
3. Decant the liquid layer through a funnel containing a filter paper and 20 g of sodium sulfate into a 100 mL graduated cylinder.
4. Pipette 10 mL of the acetonitrile extract into a 50 mL beaker. Evaporate to about 0.5 mL under nitrogen on a steambath set at 60 °C. Add 5 mL of 10 % methanol in methylene chloride to the beaker and sonicate the beaker for 2 minutes.
Note: When evaporating and sonicating the sample, do not let water get into the beaker.
5. Set the vacuum manifold *at the discard position*. Condition an aminopropyl Bond Elut by adding 10 mL of the 10 % methanol in methylene chloride solution to the Bond Elut.
6. Set the flow to about 3 mL per minute by adjusting the vacuum pressure. Turn off vacuum and switch the vacuum manifold to *collect position* just before the Bond Eluts go to dryness. Immediately transfer the sample *from* the beaker to the conditioned Bond Elut and collect the eluant in a 15 mL test tube. Add 10 mL more of the 10% solution to the beaker, sonicate for 1 minutes and then transfer to the bond elut (wait until the first 5 mL just goes through the bond elut before adding). Collect the eluant in the 15 mL test tube calibrated for 2.0 mL
7. Evaporate the eluant to ~0.1 mL using a N-EVAP set at 40 °C. Bring to a final volume of 2 mL with methanol. Mix well for 20 seconds. Filter through a 0.2 µm acrodisc HPLC filter into two autosampler vials.
8. Analyzed by APCI-LC/MSMS .

Instrument Conditions:

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

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.

Calculations:

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

For this method, aliquot final volume is 2 mL, aliquot sample weight is 2.5 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 -10 °C. Thaw the samples in a refrigerator overnight before grinding.
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.
7. To avoid cross- contamination, all glassware and grinding equipments shall be rinsed with water several times followed by an acetone or methanol rinse before grinding the next sample.

Method Detection Limit:

The Method Detection Limit (MDL) refers to the lowest concentration of an analyte that a method can detect reliably. For each matrix, to determine the MDL, 7 replicated background samples were fortified 0.200 ppm. The standard deviation derived from the fortified samples was used to calculate the MDL using the following equation:

$$\text{MDL} = t s$$

where:

t is the Student 't' value for the 99% confidence level with n-1 degrees of freedom (n-1, 1 - a = 0.99) which is 3.143 for n=7

n represents the number of replicates which is 7

S denotes the standard deviation obtained from replicate analyses.

The results for the standard deviations and MDL are in Appendix 1.

Recovery data:

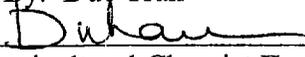
The analytical method was validated by preparing five sets of fortified samples. Each set contained three levels of fortification and a blank. All fortified and blank samples were processed through the entire analytical method and the results are in Appendix 2.

Reporting Limit:

The Reporting Limit (RL) refers to the level which reliable quantitative results may be obtained, usually 1-5 times the MDL. The reporting limit for Hexazinone and Diuron is 0.100 ppm .

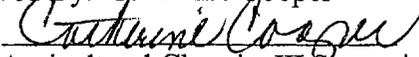
The clean-up procedures in this method were developed based on the provided backgrounds. Samples may be different from the background because they may be collected at a different season, and for this reason samples may produce unexpected interferences.

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Appendix 1:

MDL Results (ppm) for Hexazinone and Diuron in Alfalfa:

Spike # (0.300 ppm)	Hexazinone (ppm)	Diuron (ppm)
MDL 1	0.138	0.184
MDL 2	0.144	0.164
MDL 3	0.149	0.162
MDL 4	0.151	0.159
MDL 5	0.141	0.164
MDL 6	0.141	0.159
MDL 7	0.131	0.157
S=	0.006744	0.009155
MDL=3:143 x S	0.020	0.028
RL=	0.100	0.100

Appendix 2:

Method Validation Results (ppm) for Hexazinone and Diuron in Alfalfa:

Spike level (ppm)	Hexazinone results (ppm)	Hexazinone recovery (%)	Diuron results (ppm)	Diuron recovery (%)
0.300	0.232	77.3	0.232	77.3
	0.205	68.3	0.221	73.7
	0.199	66.3	0.210	70.0
	0.209	69.7	0.211	70.3
	0.206	68.7	0.236	78.7
3.000	2.934	97.8	2.898	96.6
	2.640	88	2.631	87.7
	2.514	83.8	2.670	89
	2.607	86.9	2.571	85.7
	2.772	92.4	2.814	93.8
30.00	20.460	68.2	26.190	87.3
	26.100	87	24.660	82.2
	24.360	81.2	27.330	91.1
	24.030	80.1	24.150	80.5
	24.660	82.2	24.180	80.6