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Revised:

Determination of Diuron and Hexazinone in Soil by Liquid Chromatography-Mass Spectrometry

Scope: This method is for the determination of Diuron and Hexazinone in soil using Liquid Chromatography-Mass Spectrometry (LC-MS). The reporting limit is 8 µg/kg for both compounds.

Principle: Diuron and Hexazinone are extracted from soil with a mixture of hexane and acetone (1:1). After solvent evaporation, the residue is transferred with hexane and acetone (4:1) to a Florisil Bond Elut. The analytes are eluted with hexane and acetone (4:1). The eluant is concentrated to approximate 0.1 mL and diluted to 5 ml with methanol. An aliquot of the sample is filtered through a 0.2 µm Acrodisc into autosampler vial for analysis. The analytes are isolated with C-18 column and detected with Mass Spectrometry.

Reagents:

Use residue grade solvent for sample extraction and ultra pure grade solvent and reagent for HPLC elution and Mass Spectrometry detection.

1. Diuron, CAS #330-54-1, 1.0 mg/mL, provide by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture.
2. Hexazinone, CAS #51235-04-2, 1.0 mg/mL, provide by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture.
3. Monuron, CAS #150-68-5, 1.0 mg/mL, provide by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture.
4. Methanol, pesticide residue grade and ultra pure grade
5. Hexane, pesticide residue grade
6. Acetone, pesticide residue grade
7. Water, ultra pure grade, Burdick & Jackson
8. Acetic acid, HPLC grade (Fisher #A35-500 or equivalent)
9. Acrodisc® 0.2 µm, Gelman Laboratory, Cat # 09730191.
10. Florisil Mega Bond Elut®, Varian Part Number 1225-6014.
11. Dry ice
12. Sodium sulfate, anhydrous

Safety:

No known carcinogens were used in this method. However, for precaution, a general laboratory safety procedure must be followed (e.g. wear safety glasses, gloves, use ventilation hood, etc...)

Equipment:

1. Blender, a quart size, stainless container, with varic speed control
2. Aluminum weighing dish (57 mm) for determining moisture.
3. Balances, analytical and top load
4. Mason jar, quart size
5. Oven, 105 °C
6. Desiccator
7. Graduated cylinders,
8. Funnel
9. Glass wool
10. Boiling flask, flat bottomed, 24/24 joints, 250 mL
11. Rotary evaporator, Buchi, Model RE 111
12. Nylon Acrodisc, 0.2 um, Gelman
13. Graduated conical test tube, 15 mL, 5.0 mL calibrated
14. Nitrogen evaporator, Organomation, Model 112
15. Vortex mixer, Fisher Scientific, Model Vortex-Genie 2

Instrument: (see detail in operating parameters)

1. HPLC with autosampler and column oven
2. Mass spectrometer
3. Computer
4. C-18 HPLC column

Interference:

The detection of diuron and hexazinone were specific. Multiple factors were used to eliminate possible interferences. The factors were retention time, specific parent mass (M+1) and specific daughter ions,

	Parent Mass	Daughter ions	Retention Time
diuron	234	72	10.3
hexazinone	254	171	9.8
monuron	199	72	9.3

Although diuron and monuron have a common daughter ion 72, they can be easily distinguished by retention times and parent mass. Interference to each other was unlikely to occur.

Standard Preparation:

Individual stock standards were obtained from the Standards Repository, CAC, CDFA. The concentrations were 1.00 mg/mL in methanol. They were in ampules and stored in a freezer (less than -10°C). A 50 ng/µL mixed standard of diuron and hexazinone were prepared by mixing equal volumes of the individual stock standards and 18 volumes of methanol. This

mixed standard was stored in a refrigerator (less than 5°C) and was used for spiking. Working standards were prepared by diluting the mixed standard with methanol by volume and ratio. During this study we did not observe any problem of analytes stability in the standard solutions.

Sample Preservation and storage:

Check sample temperature upon arrival. Store all samples in a locked designated area in the walk-in freezer (less than -10 °C). Transport samples to a refrigerator (less than 5 °C) the night before sample preparation or extraction. Return to the freezer for storage immediately after subsample is taken.

Sample Preparation:

This procedure is for homogenizing the sample. Thaw the sample in a refrigerator overnight. Transfer a partial (about 200-300 grams) soil sample into a stainless steel blender jar. Add approximate 100-200 grams crushed dry ice. Blend the content at moderate speed until the sample appears sandy. Gradually add the remaining soil (usually about 600 grams) and add more dry ice to maintain the sandy texture. Make sure the sample is uniformly blended. Transfer the sample back to the original container, cover with aluminum foil and cap with lid loosely. Store the sample in a freezer over night to allow the dry ice to sublime. Remember to tightly cover the lid the following day. Use these prepared samples for moisture determination and sample extraction.

Moisture Determination:

1. Transport samples from freezer to refrigerator and allow them to thaw overnight. Prior to moisture determination and sample extraction, take the samples out from refrigerator and allow them to come to room temperature.
2. Weigh approximately 15 g of the homogenized sub sample into a preweighed aluminum weighing dish and record the wet weight. Clearly indicate whether the dish weight is included or not.
3. Place the weighing dish with sample into an oven at 105 °C for at least six hours. Remove the dish from oven and allow cooling in a desiccator. Weigh the dried sample and record the Weight.

Sample Extraction:

1. Weigh out 25 g. of the homogenized sample into a one-pint wide mouth Mason jar. (For QC samples, spike at this step, mix and set for 30 minutes before continuing)).
2. Add 100 mL 50:50 hexane in acetone. Sonicate for 45 minutes. Swirl the sample every 15 minutes. Decant the organic solvent through a funnel containing glass wool and 20 g. of anhydrous sodium sulfate into a 500 mL flat bottomed boiling flask. Use a minimum amount of glass wool. It is just for supporting the sodium sulfate.
3. Repeat step 2.
4. Rinse with 40 mL 50:50 hexane in acetone through the sodium sulfate.
5. Evaporate the extract to about 2 mL on a rotary evaporator at approx. 40 °C water bath and 15 mm Hg vacuum
6. Condition a Florisil Bond Elute cartridge with 8 mL of 20% hexane in acetone. Switch the vacuum manifold to collect position. Transfer the residue from the flask to the conditioned cartridge. Wash the flask with 5 mL 20% hexane in acetone and transfer to the conditioned cartridge. Repeat the wash two more time.

7. Evaporate the combined eluant to about 0.1 mL using a N-evap at 40 °C. Bring to a final volume of 5.0 mL with methanol. Mix well and filter through a 0.2 μ Acrodisc into two autosampler vials.
8. Measure 300 μL of the extract into an insert in an autosampler vial. Add 10 μL of 10 ng/μL monuron as an internal standard and mix well with a pasture pipet.
9. Analyze the sample extract on a HPLC-MS system.

Equipment Conditions:

HPLC System and Operating Parameters

Instrument: Waters model 2690 HPLC, gradient pump, autosampler, column heater and remote control through Finnigan Xcalibur system
Detector: Finnigan LCQ Deca Mass spectrometer
Column: Phennomex C18 Luna 3μ, 50 mm x 3 mm x 3 μ
Precolumn: Phennomex C-18 5 mm L x 2.0 mm ID cartridge (AJO-4286)
Column Temperature: 40 °C
Solvent: Gradient Program,
Solvent A: 0.2% acetic acid in methanol
Solvent B: 0.2% acetic acid in ultra pure water

Time (min)	Flow (ml/min)	A (%)	B (%)
0.00	0.3	10	90
1.00	0.3	10	90
15.00	0.3	100	0
15.10	0.3	100	0
20.0	0.3	10	90
23.0	0.3	10	90

Total run time 23 minutes

Flow rate: 0.3 mL / min
Injection volume: 20 μL
Retention time: Monuron 9.3 min
Diuron 10.3 min
Hexazinone 9.8 min

Note: An alternative C-18 column will probably work. You may also vary the mobile phase percentage at your convenience.

Mass Spectrometry System and Operating Parameters:

Instrumentation:

Finnigan LCQ Deca, ion trap mass spectrometer
Instrument control and data handling: Gateway computer model E-4200
Software: Xcalibur Version 1 SR1

MS run time (min): 11.5

Divert valve: in use during run

Divert Time (min)	Valve State
0	To waste
7.92	To source
11.4	To waste

Contact Closure: not used during run
MS Detector Settings:
Acquisition start Delay (min7.00
Segment 1 Information
Duration (min): 9.80
Number of Scan Events: 2
Tune Method: APCI diuron233 3-20-00 HAC (Noted in detail in tune method section)
Scan Event Details:
1: Pos (199.0)- > (50.0-250.0)
MS/MS: Amp. 30.5% Q 0.250 Time(µsec) 30.000 IsoW 5.0
2: Pos (253.0)- >(65.0-200.0)
MS/MS: Amp. 25.0% Q 0.250 Time(µsec) 30.000 IsoW 5.0
Segment 2 Information
Duration (min): 1.70
Number of Scan Events: 1
Tune Method: APCI diuron233 3-20-00 HAC
Scan Event Details:
1: Pos (234.0)- >(60.0-250.0)
MS/MS: Amp. 30.5% Q 0.250 Time(µsec) 30.000 IsoW 5.0

Tune method: APCI diuron233 3-20-00 HAC.LUQTune

APCI Source Setting

Vaporizer Temp (°C)	500
Sheath Gas Flow Rate (arb)	80
Aux Gas Flow Rate (arb)	0
Discharge Current (µA)	
Discharge Voltage (kV)	0.01
Capillary Temp (°C)	150
Capillary Voltage (V)	6.0
Tube Lens Offset (V)	55

Ion Optics Setting

Octapole 1 Offset (V)	-7.50
Lens Voltage (V)	-18.00
Octapole 2 Offset (V)	-9.00
Octapole RF Amplitude (V p-p)	400.00
Entrance Lens (V)	-40.00

Automatic Gain Control: on

Full Mass Target	5 x e7
SIM	2 x e7
MSn Target	2 x e7
Zoom Target	2 x e7
Inject Waveform	off
Total microscans	1
Maximum inject time(μsecond)	300

Instrument Calibration:

A 5 level standards were run before and after each sample set. The concentration of working standards were 0.05, 0.1, 0.2, 0.4 and 1.0 ng/μL. The standard curve had correlation coefficiencent of 0.990 or better.

Analysis:

Build up a sequence; inject the first standard at least twice to condition the instrument. Log-in the correct dilution factors. The sequence is in the order of standards, blank, spikes, 10 samples and standards, then repeat.

Calculations:

Report the percent moisture on a **dry weight** basis.

% moisture = (Wet weight – Dry weight)/Dry weight X 100

Calculate the concentration of chemical(s) of a sample as follows:

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

Using the program of LCQuan software in Xcalibur performed actual calculation
In general, std vol. Injected = sample vol injected.
final volume =5.0 mL
sample wt.= 25 g.

Method Performance:

Method Detection Limit:

Method Detection Limit (MDL) refers to the lowest concentration of analytes that a method can detect reliably in either a sample or blank. To determine the MDL, each of the 7 samples containing 25.0 g of background soil obtained for Study 187 (matrix blank) were spiked separately with 0.5 μg of diuron and hexazinone. These spiked samples along with a blank were analyzed using the described method. The standard deviation derived from the 7 spiked samples was used to calculate the MDL using the following equation:

$$\mathbf{MDL} = t\,S$$

where:

t is the Student 't' value for the 99% confidence level with n-1 degrees of freedom (n-1, 1 - $\alpha = 0.99$). **n** represents the number of replicates.
S denotes the standard deviation obtained from replicate analyses.

Reporting Limit:

Report Limit (RL) refers to the level above which quantitative results may be obtained usually 1-5 times the MDL

Spiking solution and spiking volume:

MDL, method validation and QC spikes are made by spiking 25.0 g of background soil obtained for Study 187 with diuron and hexazinone mixed solutions.
The concentration of mixed standard for spiking is 50.0 ng/μL for both diuron and hexazinone.
The volumes spiked are as in the following table.

	Sample Size (grams)	Volume Added (μL)	Analyte Spiked (ng)	Equivalent to (ppm)
MDL	25	10	500	0.02
Validation level 1	25	10	500	0.02
Validation level 2	25	250	12,500	0.50
Validation level 3	25	1000	50,000	2.00
Set QC	25	20	1000	0.04

MDL Data

Appendix 1

Method Validation Data:

Appendix 2

Acceptance Criteria:

1. All samples were injected at least two times. The standard curves at the beginning and end of each sample set should not have a percent change greater than 20%. The % change in response was calculated as follows:

% Change in response = absolute value of [slope of (STD curve before - STD curve after)/ STD curve before] x 100

2. The response of internal standard may be used to evaluate the quality of the data.
3. The sample results were calculated based on the average of two adjacent calibration curve using Xcalibur software. When the difference between the two injections was less than 15%, either result can be reported. Additional injections were required if the differences were greater than 15%.

Discussion:

This method provides good accuracy and precision, as measured by the average recovery at all spiking levels for both diuron and hexazinone

Residues were detected in matrix blank for diuron at levels between 0.006 ppm to 0.014 ppm. This is near the reporting level. The blanks were sampled prior to the current application. We were told, diuron and hexazinone used in previous year. Therefore, the finding were true residue from previous applications, not false positive.

Duplicated sample were extracted and analyzed for several sets. The results were in agreement. This is highly due to the homogeneous sample treatment of blending with dry ice.

ESI ion source was used in the beginning of the method development. We encountered the problem of drastic sensitivity change. The slopes of standard curves increased drastically after the sample set. The cause of changing sensivity was not determined. Use of an internal standard did not resolve this problem. In this method, we changed to APCI ion source, suggested by Dr. Jack Cunnif of Finnigan, and 0.2 % acetic acid in methanol and in water as the mobile phase, the problem was corrected.

Over all, this is a very reliable method for determination of diuron and hexazinone.

Reference:

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Paul Lee

Title: Agricultural Chemist III

Approved By: Catherine Cooper

Catherine Cooper

Title: Agricultural Chemist III
Supervisor

Appendix 1:

Diuron and Hexazinone MDL Results (ppm) for soil

Spike #	Spike level (ppm)	Diuron found (ppm)	Hexazinone found (ppm)
Blank 1	0	0.012	0.001
Blank 2	0	0.012	0.001
Blank 3	0	0.012	0.001
1	0.02	0.028	0.021
2	0.02	0.028	0.020
3	0.02	0.027	0.020
4	0.02	0.029	0.020
5	0.02	0.027	0.019
6	0.02	0.028	0.021
7	0.02	0.027	0.020
S=		0.0024	0.0022
MDL = 3.143 x S		0.008	0.007
Reporting Limit		0.008	0.008

Appendix: 2

Diuron and Hexazinone Method Validation Results and Recovery on background soil

	Diuron		Hexazinone	
Spike Level (ppm)	Result (ppm)	Recovery (%)	Result (ppm)	Recovery(%)
0.02	0.019	95.0	0.015	75.0
	0.018	90.0	0.016	80.0
	0.023	115	0.017	85.0
	0.017	85.0	0.016	80.0
	0.017	85.0	0.021	105
0.5	0.477	95.4	0.426	85.2
	0.513	102.6	0.378	75.6
	0.516	103.2	0.391	78.2
	0.438	87.6	0.425	85.0
	0.460	92.0	0.419	83.8
2.0	2.00	100	1.82	91.0
	1.89	94.5	1.55	77.5
	1.98	99.0	1.47	73.5
	1.86	93.0	1.77	88.5
	1.80	90.0	1.69	84.5