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Determination of Diuron and Hexazinone on Kimbies® using Liquid Chromatography/Mass Spectrometry

Scope: This method is for the determination of diuron and hexazinone pesticides on Kimbies®. The reporting limit (RL) of the method is 1 µg/sample.

Principle: Diuron and hexazinone are extracted from Kimbies® by shaking with methanol. The extract concentration is adjusted as required for analysis by Liquid Chromatography/Mass Spectrometry (LC/MS) with Atmospheric Pressure Chemical Ionization (APCI).

Safety: All general laboratory safety rules for sample preparation and analysis shall be followed. Kimbies® often contain high levels of pesticides, and should be handled with gloves at all times. Samples shall be prepared in a fume hood designated for high-level samples to avoid contamination of other areas of the laboratory. Proper disposal procedures must be followed.

Interferences: No interferences have been observed for this method.

Equipment, Reagents, and Instruments:

Equipment:

1. Mechanical shaker (G 10 Gyrotory Shaker, New Brunswick Scientific, or equivalent)
2. Mason jars: One quart, with caps
3. Nitrogen evaporator, (Meyer N-EVAP Organomation Model # 112 or equivalent)
4. Acrodisc®, 0.2 µm filter. Gelman Sciences
5. Vortex-vibrating mixer
6. Phenomenex Luna C18 LC column: 2.0 x 30 mm 5 µ (p/n 00A-4249-B0) with SecurityGuard™ cartridge holder kit (p/n KJ0-4282) with 2.0 mm C18 cartridge (p/n AJ0-4286).
7. Bond Elut®: Aminopropyl Bond Elut® 10 cc/500 mg. Varian
8. Vacuum manifold: Vac Elut SPS24. Varian
9. Conical test tube with glass stopper, 15 mL, graduated
10. Funnel, short stem, 60°, 100 mm
11. Disposable Pasteur pipettes, 5.75 inches
12. Balance, Mettler PC 4400

Equipment, Reagents, and Instruments: continued

Equipment (continued):

13. Aluminum foil
14. Brown bottle: 8 oz with cap

Reagents:

1. Methanol, HPLC - Residue and Pesticide Analysis
2. Methanol, LC/MS – Burdick and Jackson or equivalent
3. Water, LC/MS – Burdick and Jackson or equivalent
4. Sodium Chloride, granular, anhydrous
5. Diuron CAS# 330-54-1, 1 mg/mL in methanol, stock standard solution from the Standard Repository, Center for Analytical Chemistry.
6. Hexazinone CAS# 51235-04-2, 1 mg/mL in methanol, stock standard solution from the Standard Repository, Center for Analytical Chemistry.
7. Ammonium formate, LC/MS purity.
8. Formic acid, LC/MS purity.

Instrument:

Thermoquest LCQ Classic Ion Trap Liquid Chromatograph/Mass Spectrometer with Atmospheric Pressure Chemical Ionization interface.
ThermoSeparations LC system consisting of an SCM 1000 membrane degasser, a P4000 pump and an AS3000 autosampler.

Note: This method was validated on the LCQ Classic 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.

Standard Preparation:

1. Dilute the 1 mg/mL standard solutions with methanol into a set of desired mixed standards of the desired concentrations for spiking, instrument calibration and sample calculation. The concentration for spiking is 1 µg/ml 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 shelf life of each prepared standard is six months unless the Standard Repository specifies an earlier expiration date.

Sample Preservation and Storage:

1. Check and record the temperature of ten percent of the samples upon arrival.
2. Sign the sample chain of custody and obtain the EMON number from supervisor.
3. Store all samples waiting for extraction in a freezer.
4. Store all sample extracts waiting for analysis in a refrigerator.

Analysis:

Sample Extraction:

1. If samples are frozen, allow them to come to room temperature. Wearing appropriate gloves, remove the foil-wrapped Kimbie® from the sample envelope, and unwrap the Kimbie®. Because of the possibility of high analyte concentrations, all sample packaging must be disposed of as hazardous waste.
2. Handling the Kimbie® with tongs, place the loosely folded Kimbie® in a labeled 1 qt Mason jar.
3. Add 500 ml HPLC grade methanol to the jar.
4. Use a disposal glass pipet to push the Kimbie® beneath the methanol level, and to hold it there when the jar is capped. Use aluminum foil to line the cap.
5. When all jars are prepared, load the G 10 Mechanical shaker and shake for 1 hour at 200 rpm. Check periodically to ensure that the jars are secure in the shaker.

Preparation of blanks and spikes

Blank: Loosely fold a fresh Kimbie® matching the sample Kimbies®, and place in a 1 qt Mason jar. Add 500 ml methanol and cap as above. Prepare one blank for each set of up to 12 samples.

Spike: Loosely fold a fresh Kimbie® matching the sample Kimbies®, and place in a 1 qt Mason jar. Spike with 1 ml of the 1 µg/ml mixed spiking solution with a pipette. Add 500 ml methanol and cap as above. Prepare one or two spikes as called for in the study protocol.

Analysis:

1. Filter a 1 ml aliquot of each sample through an Acrodisc® 0.2 µm filter and divide into two autosampler vials.
2. Run a sequence consisting of an instrument blank, a set of five calibration mixtures, the method blank and spikes, and samples. The method blank and spikes, and the samples should be injected twice. Calibration sets should bracket each group of 15 injections. This number may be adjusted according to the sample set size.
3. For samples for which no analyte is detected, or a response below the reporting level is observed, concentrate an aliquot of 150 ml of the original methanol sample extract in a 500 ml round-bottom flask on a rotary evaporator set to 55 °C. The extract should be evaporated just to dryness and quantitatively transferred to a graduated test tube with 12 ml of methanol. Concentrate to 3 ml on the N-EVAP, and filter a 1 ml aliquot through an Acrodisc® 0.2 µm filter and divide into two autosampler vials.
4. For samples that have analytes detected above the linear range of the method (extract concentration equivalent to = 115% * 1.0 ng/ul), dilute a filtered aliquot of the original extract to obtain a final concentration of around 0.15 ng/ul. This target concentration will help account for response enhancement effects at high levels in obtaining a diluted sample within the linear range of the method.
5. Run a sequence as above with the concentrated and diluted samples.

6. If the study protocol requires the extract to be retained, transfer the required amount of original extract (minimum 50 ml) to an amber bottle for freezer storage. A list of stored samples should be retained indicating the concentration reported, and the dilution or concentration factors required for each. The remaining original extract shall be disposed of following laboratory procedures for handling hazardous waste. All Mason jars shall be thoroughly rinsed with three portions of methanol (regular HPLC grade) before washing to ensure that contamination from high residue levels does not occur.

Instrument Conditions

Instrument: Thermoquest LCQ Classic Ion Trap with ThermoSeparations LC system consisting of an SCM 1000 membrane degasser, a P4000 pump and an AS3000 autosampler.

LCQ Method: HEXAZ DIUR 2 MM C18. A summary of the instrument method is given in Appendix C.

LCQ Tune File: 2 mm APCI hexaz 1-750 12-21-99 (Note: This tune file was used after trap maintenance. The tune file should be updated using an infusion of 10 ng/ul hexazinone standard at 5 µl/min into a 50% A: 50% B column flow at 0.6 ml/min if more than one month has passed since running the method, or trap maintenance has been performed. The tune file parameters are listed in Appendix C.

Column: Phenomenex Luna® C18 LC column: 2.0 x 30 mm 5 µ (p/n 00A-4249-B0) with SecurityGaurd™ cartridge holder kit (p/n KJ0-4282) with 2.0 mm C18 cartridge (p/n AJ0-4286).

Mobile phase A: To prepare 1 L of mobile phase A ("DACT AQ Phase") mix:

1) 942 ml ± 5 ml Burdick and Jackson LC/MS water
2) 50 ml ±2 ml Burdick and Jackson LC/MS Methanol
3) 9.5 ± 0.25 ml 0.1 M ammonium formate solution
4) 1.0 ml ±0.02 ml LC/MS grade formic acid

Mobile phase B: To prepare 1L of mobile phase A ("DACT MEOH Phase") mix:

5) 900 ml ± 5 ml Burdick and Jackson LC/MS water
6) 90 ml ±2 ml Burdick and Jackson LC/MS Methanol
7) 10.0 ± 0.25 ml 0.1 M ammonium formate solution
8) 1.0 ml ±0.05 ml LC/MS grade formic acid

Mobile phase gradient:

1) time 0.00 min: 20% A, 80% B
2) time 1.00 min: 20% A, 80% B
3) time 5.00 min: 100% A, 0% B
4) time 10.00 min: 10% A, 0% B
5) time 11.00 min: 20% A, 80% B
6) time 15.00 min: 20% A, 80% B

Flow: 0.3 mL/min

Column oven temperature: OFF (Note: This method was validated without using the column heater. If fluctuating ambient temperatures cause instrument instability, setting the column heater to 35 °C is recommended. This will cause a slight shift in the analyte retention times, but should not have any other effect on the analysis.)

Divert valve:
1) time 0.00 min to waste
2) time 3.00 min to source
3) time 8.00 min to waste

Hexazinone retention time: 5.7 min
Diuron retention time: 6.5 min

Method Performance:

Quality Control:

1. Sample response must be linear over the calibration range. Curvature in the calibration in this range indicates that instrument maintenance is needed or that the LC column is contaminated.
2. Each sample shall be injected two times to insure reliability of the analysis. If replicate injections do not agree within 15% (25% for extract concentrations below 0.1 ng/ul), the sample must be re-analyzed.
3. Sample storage: All samples shall be kept frozen at -10 °C if they are not to be extracted immediately after receipt. Frozen samples must come to room temperature before being opened.
4. Sample extracts: All extracts shall be kept in a refrigerator at 5 °C until analyzed.
5. Freezer and refrigerators temperatures shall be monitored and recorded daily.
6. Each sample set is comprised of 1-12 samples, along with a method blank, and spikes as specified in the study protocol. One 1 µg/sample spike shall be used if not otherwise specified.

Method Validation Recovery Data and Control Limits:

This method was validated with five samples sets. Each set contained three levels of fortification (20, 40, and 50 mg/sample) and a method blank. All spiked and method blank samples were processed through the entire analytical method. Each spike extract was concentrated as stated above (150 ml extract reduced to 3 ml), and then diluted to the desired final concentration. In this way, the validation spikes were treated as high samples would be. Spike extracts were diluted in two steps to minimize solvent consumption and waste. The first step was a dilution of 25 µl of extract (measured with a syringe) to 10 ml. In the second step, the diluted 20 mg spike extracts were diluted a further 10-fold, 1 ml to 10 ml, and the diluted 40 and 50 mg spikes were diluted a further 25-fold, 1 ml to 25 ml. In this way, dilution factors of 1×10^4 for the 20 mg spike and 2.5×10^4 for the 40 and 50 mg spikes were obtained.

Method warning and control limits for the QC spike recovery are calculated based on the validation recovery data tabulated in Appendix A. Upper and lower warning and control limits are set at ± 2 and 3 standard deviations of the average % recovery, respectively.
Method validation results and control limits are tabulated in Appendix A.

Method Detection Limits (MDLS) and Reporting Limits (RLS):

The method Detection Limit (MDL) refers to the lowest concentration of analyte that a method can detect reliably. To determine the MDL, 7 replicate Kimbies® were spiked at 0.100 μg per towel. The standard deviation from the spiked sample recoveries was used to calculate the MDL for each analyte 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.

The Reporting Limit (RL) refers to the level at which reliable quantitative results may be obtained. By convention, the RL is chosen in a range 1-5 times the MDL. The RL for this method is 1 ug/sample, approximately 5 times the MDL for diuron and 10 times the MDL for hexazinone. The continuing QC spike level was set at the RL for both analytes. MDL data and the reporting limits are tabulated in Appendix B.

Calculations:

Analysis of the data may be performed using either the LCQuan or Quan Browser software for the LC/MS system. An external standard quantitation based on peak area is used. The software uses a linear least-squares curve fitting to calculate an average response for the calibration curve, with a 1/X weighting ignoring the origin. Normally, a set of 12 samples will be analyzed using a beginning, middle and ending calibration set. These three calibration sets are normally combined in the calculation. When small sample sets are analyzed, a calculation can be based on two bracketing calibration sets. When instrument drift is encountered (for example, due to ambient temperature fluctuations), two bracketing calibration sets in succession may be used for calculations for a sample set.

Acceptance Criteria:

1. Bracketing standard curves should have a percent change less than 20 % for 0.1, 0.2 and 0.4 ng/ul levels, and 25 % for the 0.04 and 1.0 levels. The % change in response was calculated as follows:

$$\textbf{\% change in response} = \frac{\text{Absolute value of response (std before - std after)}}{\text{Std before}} \times 100$$

2. The samples were calculated base on the calibration curve before the samples using the instrument software. If the results between the two injections differ less than 10 % either result can be reported. A change greater than 10 % with no known reason requires a second analysis.

Reference:

1. Duc Tran. *Method EM-29.0, Diuron and Simazine on Kimbies®, March 25, 1993.*

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APPENDIX A

HEXAZINONE IN KIMBIES® CONTROL LIMITS (expressed as % recovery)	DIURON IN KIMBIES® CONTROL LIMITS (expressed as % recovery)
84.8	79.3
113.8	101.1
86.4	82.6
92.0	93.3
85.0	79.3
93.9	92.7
75.3	79.3
78.1	81.6
82.3	88.4
96.0	91.5
107.6	115.0
84.8	82.4
75.7	86.1
99.1	105.0
84.2	88.1
89.3% Average	**89.7% Average**
11.191% Std	**10.489% Std**
111.7% av+2std UWL	**110.7% av+2std UWL**
66.9% av-2std LWL	**68.7% av-2std LWL**
122.8% av+3std UCL	**121.2% av+3std UCL**
55.7% av-3std LCL	**58.23% av-3std LCL**

APPENDIX B

HEXAZINONE AND DIURON IN KIMBIES® MDLS AND RLS
(expressed as ug/towel)

HEXAZINONE		DIURON	
KIMBIE MDL METHOD BLANK 12-21	0.001	KIMBIE MDL METHOD BLANK 12-21	0.005
KIMBIE MDL 1 12-21	0.095	KIMBIE MDL 1 12-21	0.103
KIMBIE MDL 2 12-21	0.096	KIMBIE MDL 2 12-21	0.115
KIMBIE MDL 3 12-21	0.107	KIMBIE MDL 3 12-21	0.119
KIMBIE MDL 4 12-21	0.103	KIMBIE MDL 4 12-21	0.118
KIMBIE MDL 5 12-21	0.094	KIMBIE MDL 5 12-21	0.112
KIMBIE MDL 6 12-21	0.096	KIMBIE MDL 6 12-21	0.096
KIMBIE MDL 7 12-21	0.098	KIMBIE MDL 7 12-21	0.105
AVERAGE:	0.098	AVERAGE:	0.110
STD DEV:	0.005	STD DEV:	0.009
MDL=	0.108	MDL=	0.187
3*MDL=	0.325	3*MDL=	0.562
5*MDL=	0.542	5*MDL=	0.936
10*MDL=	1.085	10*MDL=	1.872
REPORTING LEVEL (RL) =	1.00	REPORTING LEVEL (RL) =	1.00

APPENDIX C
INSTRUMENT METHOD AND TUNE FILES

METHOD:
MS Run Time (min): 8.25

Divert Valve: in use during run

Divert Time (min)	Valve State
0.00	To Waste
3.00	To Source
8.00	To Waste

PRECURSOR AND PRODUCT IONS

MSMS EVENTS:

	PRECURSOR IONS	QUANT PRODUCT IONS
HEXAZINONE	253	171
DIURON	233/235/237	72

MS Detector Settings:

Segment 1 Information

Duration (min): 8.25
Number of Scan Events: 2
Tune Method: 2 mm apci hexaz 1-750 12-21-99

Scan Event Details:
1: Pos ·(253.0)->o(80.0-280.0)
MS/MS: Amp. 25.0% Q 0.300 Time 30.000 IsoW 1.5

2: Pos ·(235.0)->o(55.0-250.0)
MS/MS: Amp. 30.0% Q 0.230 Time 30.000 IsoW 6.0

TUNE:
Capillary Temp (C): 210.00
Ion Time (ms): 5.00
Sheath Gas Flow: 26.00
Injection Waveforms: Off
APCI Vaporizer Temp (C): 400.00
Source Type: APCI
Aux Gas Flow: 0.00
AGC: On

POSITIVE POLARITY
Source Voltage (kV): 4.50
Capillary Voltage (V): 17.00
Octapole RF Amplifier (Vp-p): 530.0
Source Current (uA): 5.00
Tube Lens Offset (V): 5.00

APPENDIX C (CONT.):

TUNE (CONT):

Octapole 1 Offset (V): -2.50
Octapole 2 Offset (V): -6.00
InterOctapole Lens Voltage (V): -18.00
Trap DC Offset Voltage (V): -10.00
Zoom Micro Scans: 5
Zoom AGC Target: 10000000.00
Zoom Max Ion Time (ms): 50.00
Full Micro Scans: 3
Full AGC Target: 50000000.00
Full Max Ion Time (ms): 200.00
SIM Micro Scans: 5
SIM AGC Target: 20000000.00
SIM Max Ion Time (ms): 200.00
MSn Micro Scans: 1
MSn AGC Target: 20000000.00
MSn Max Ion Time (ms): 750.00

NEGATIVE POLARITY
Source Voltage (kV): 4.50
Source Current (uA): 100.00
Capillary Voltage (V): -10.00
Tube Lens Offset (V): -50.00
Octapole RF Amplifier (Vp-p): 400.00
Octapole 1 Offset (V): 3.00
Octapole 2 Offset (V): 7.00
InterOctapole Lens Voltage (V): 16.00
Trap DC Offset Voltage (V): 10.00
Zoom Micro Scans: 5
Zoom AGC Target: 10000000.00
Zoom Max Ion Time (ms): 50.00
Full Micro Scans: 3
Full AGC Target: 10000000.00
Full Max Ion Time (ms): 50.00
SIM Micro Scans: 5
SIM AGC Target: 20000000.00
SIM Max Ion Time (ms): 200.00
MSn Micro Scans: 3
MSn AGC Target: 20000000.00
MSn Max Ion Time (ms): 200.00