Title: Determination of Napropamide and Oryzalin in Ground Water by High Performance Liquid Chromatography with Ion Trap Mass Spectrometry

1. Scope

This section method (SM) is for the analysis of napropamide and oryzalin in ground water. It is to be followed by all authorized section personnel. The reporting limit is 0.05 ppb for both napropamide and oryzalin.

2. Principle:

Residues of the fore mentioned pesticide are extracted from sample using C-18 solid phase cartridge. The extracts on cartridge are eluted off with methanol. Both napropamide and oryzalin are determined by the injection of sample extract into an HPLC equipped with a C-18 column and a mass spectrometer (LC-MS). The confirmation of compound identity on LC-MS is achieved simultaneously with collision-induced dissociation to produce a product ion.

3. Safety:

3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.
3.2 All solvents should be handled with care in a ventilated area.

4. Interferences:

The chance of interference for napropamide and oryzalin are unlikely at reporting level, because the collision induced fragment ions are used for the determination.

5. Apparatus and Equipment:

5.1 Nitrogen evaporator (Meyer N-EVAP Organomation Model # 112 or equivalent)
5.2 Vortex-vibrating mixer
5.3 Conical tube with glass stopper, 15-mL graduated
5.4 Disposable Pasteur pipettes, and other laboratory ware as needed
5.5 Liquid chromatograph (Thermo Finnigan Surveyor Model xxx HPLC) equipped with a Thermo Finnigan TSQ Quantum Mass detector.
5.6 Solid phase extraction manifold, Supelco Visiprep™24 or equivalent

5.7 Solid phase extraction manifold accessories which consist of vacuum source, vacuum chamber, vacuum control, cartridge fittings (tube adapters) and connectors, sample delivery tubing with stainless steel weight, sample collection tubes and sample collection rack.

6. Reagents and Supplies: (All reagents shall meet the minimum requirement in HPLC, residue and pesticide analysis)

6.1 Formic acid, HPLC grade (Fisher #A35-500 or equivalent)
6.2 Methanol, (Burdick &Jackson, MS grade, or equivalent)
6.3 Nitrogen, refrigerated liquid or nitrogen generator with capacity of delivering 20 liters per minute.
6.4 Standards: The individual 1.0 mg/mL stock standards of each compound were obtained from the CDFA/CAC Standard Repository.

Napropamide	CAS Number	15299-99-7
Oryzalin	CAS Number	19044-88-3
Propazine(surrogate)	CAS Number	139-40-2

6.5 Water, (HPLC grade, Burdick & Jackson Cat #AH365-4 or equivalent)
6.6 Analytical column: Waters SymmetryShield RP_{18} 5 μm, 3.9 x 150 mm column (part number, 186000108).
6.7 Guard column: Waters Symmetryshield RP 18 5 μm, 3.9 x 20 mm cartridge (part number, 186000107).
6.8 Guard column cartridge holder: Waters Sentry guard holder universal (P/N wat064610)
6.9 Waters Sep-pak cartridge C_{18}, 1 gram
6.10 Ammonium formate, 1.0 M

7. Standards Preparation:

7.1 Dilute the 1.0 mg/mL standards, obtained from the CDFA/CAC Standards Repository, with methanol. The concentration of each diluted individual standard is 10 µg/mL. The propazine standard was diluted to 1.0 µg/mL in methanol for spiking as a surrogate.

7.2 Prepare a combination standard of 1 µg/mL of napropamide, oryzalin and propazine. The combination working standard was diluted to the following concentrations: 0.05, 0.10, 0.25, 0.50 and 1.0 µg/mL.

8. Sample Preservation and Storage:

All samples and sample extracts shall be stored in the refrigerator (4±3ºC).

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the ground water for background to be used in method validation and QC.

9.2 Preparation of blank and spike

Matrix blank: Weigh out 500 g of background water and follow the test sample extraction procedure.

Matrix spike: Weigh out 500 g of background water. Spike a client requested amount of herbicides into the background water and let it stand for 1 minute. Follow the test sample extraction procedure.

Propazine is used as a surrogate. Add 0.1 µg of propazine to each sample and processed through the entire analytical method. This allows the extraction steps to be monitored.

9.3 Sample Preparation

9.3.1 Remove samples from the refrigerator and allow them to reach ambient temperature.

9.3.2 Weigh 500 ± 0.5 g of water sample into a 600 mL beaker.

9.3.3 Connect C18 cartridge to the solid phase extraction manifold. Attach the sample delivery tubes to the cartridge. Condition the cartridges with a total ~10 mL of methanol at a flow rate ~ 8 mL/minutes followed by ~ 10 mL of D.I. water by applying vacuum.

9.3.4 Turn off the vacuum when the D.I. water has just passed through the cartridges. ***Do not let the C18 cartridge go to dryness***. Place weighted tube end of the sample delivery tube into water sample. Start the extraction by turning on the vacuum source and adjust the vacuum flow rate to 5-10 mL per minute.

9.3.5 After all samples have passed through the cartridges, turn the vacuum to full flow for 1 minute to dry off the water in the cartridges.

9.3.6 Elute the cartridge with 10 mL methanol and collect the eluant to a 15 mL graduated conical tube in the vacuum chamber with flow at a rate of approximate 2-3 mL/min.

9.3.7 Evaporate on the nitrogen evaporator at about 35°C water bath under a gentle stream of nitrogen and adjust to 0.5 mL with methanol.

9.3.8 Transfer the extract to two auto sampler vials with inserts.

10 Instrument Calibration:

10.1 The calibration standard curves consist of a minimum of five levels. The lowest level must be at or below the corresponding reporting limits. The current working standard levels are 0.05, 0.1, 0.2, 0.5, and 1.0 µg/mL.

10.2 The calibration curves for the LC-MS are generally obtained using linear regression.

11 Analysis:

11.1 Injection Scheme

The LC-MS needs to be conditioned with standard or a sample extract 2 to 5 runs before running the following sequence: A set of calibration standards, a matrix blank, a matrix spike, a set of up to 12 test samples, then a set of standards, etc.

11.2 HPLC-MS Instrumentation

11.2.1 Waters model 2695 HPLC and auto-sampler with column heater and remote control through Thermo Finnigan Xcalibur system.

11.2.2 Column: Waters SymmetryShield $RP_{18,}$ 5 µm, 3.9 x 150 mm column (part no. 186000108).

11.2.3 Guard column cartridge holder and cartridge: Waters Sentry guard holder universal (P/N wat064610); Waters SymmetryShield $RP_{18,}$ 5 μm, 3.9 x 20 mm.

11.2.4 Mobile Phase:
Solvent A: 3762 mL water, 200 mL methanol, 38 mL 1 M ammonium formate and 4.0 mL formic acid
Solvent B: 3600 mL methanol, 360 mL water, 36 mL 1.0 M ammonium formate, 4 mL formic acid
Flow rate: 0.75 mL/min

Mobile Phase: Gradient

Time(min)	Flow rate	Mobile Phase A	Mobile Phase B
0	0.75	85.0	15.0
3.0	0.75	85.0	15.0
4.0	0.75	50.0	50.0
10.0	0.75	50.0	50.0
14.0	0.75	40.0	60.0
16.0	0.75	5.0	95.0
22.0	0.75	5.0	95.0
24.5	0.75	85.0	15.0
27.0	0.75	85.0	15.0

Injection Volume: 20 μL

11.2.5 Liquid Chromatograph Mass spectrometer (LC-MS) and Operating Parameters

Model:	Finnigan Model DECA ion trap MS
Ion Source Type:	Atmospheric pressure Ionization (APCI)
Source Polarity:	Positive
APCI Vaporizer Temp:	500°C
Capillary Temperature:	200°C
Sheath Gas	44
Auxiliary Gas:	3
Mode of Operation:	MS/MS
Column Temperature:	40 °C

Compound Name	Retention Time (min.)	Molecular Ion	Collision Energy	Ion Filter Range	Product Ions
Napropamide	18.6	272	34	271-273	199
Oryzalin	19.0	347	30	346-348	247,288,305
Propazine	17.2	231	40	230-232	188,190

Note: The column condition, temperature, mobile phase, etc. may slightly shift retention time.

11.2.6 Detail Operating parameters and Tune method are listed in Appendix 1 and Appendix 2

12. Quality Control:

12.1 Method Detection Limits (MDL)

The method detection limit refers to the lowest concentration of analyte that a method can detect reliably. To determine the MDL, 7 ground water samples are spiked at 0.2 ppb and processed through the entire method along with a blank. The standard deviation from the spiked sample recoveries are used to calculate the MDL for each analyte using the follow equation:

$$MDL = tS$$

Where t is the Student t test 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 replicate used to determine the MDL, t=3.143.

The results for the standard deviations and MDL are in Appendix 3

12.2 Reporting limit (RL):

The reporting limit (RL) refers to the level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. In general, the RL is chosen in a range 1-5 times the MDL, as per client agreement. The reporting limit for this method is 0.05 ppb.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included 4 levels of fortification (0.2, 0.5, 0.75 and 1.0 ppb) and a method blank. All spikes and method blanks were processed through the entire analytical method. Percent recoveries for napropamide and oryzalin are shown in Appendix 4

12.4 Control Charts and Limits

Control charts were generated using data from the method validation for each analyte. The upper and lower warning and control limits are set at ± 2 and 3 standard deviations of the average % recovery, respectively, shown in Appendix 4

12.5 Acceptance Criteria

12.5.1.1 Each set of samples shall have a matrix blank and minimum of one matrix spike sample. Each set contains up to 12 samples.

12.5.1.2 The matrix blank shall be free of target compounds.

12.5.1.3 The recoveries of the matrix spike should be within the control limits.

12.5.1.4 The retention time shall be within ± 20 seconds of that of the standard.

12.5.1.5 The sample extract shall be diluted if results fall outside the linear range of the standard curve.

13. Calculations:

13.1 The quantification is based on the area counts of the product ion of the compound analyzed. The calculation is based on external standard (ESTD).

13.2 The software LCQuan in the Xcalibur system is used to establish the standard curve and to calculate the analyte in the samples. The correlation coefficient, slope, intercept of the linear regression line are calculated once the calibration standards are defined. The equation for calculating analytes is as follows:

$$y = mx+b$$

Where: y = peak response
m = slope
b = intercept
x = concentration of compound

When the unit and the dilution factor are entered correctly in the analysis sequence, the software will then correctly generate the results.

13.3 Results can be manually calculated by a single point standard. This calculation is to verify the results derived from the software.

$$ppb = \frac{(\text{sample peak area or ht}) \times (\text{std conc}) \times (\text{std vol. Injected}) \times (\text{final vol of sample})(1000\ \mu L/mL)}{(\text{std.peak area or ht}) \times (\text{sample vol injected}) \times (\text{sample wt (g)})}$$

14. Reporting Procedure:

Sample results are reported according to the client's analytical laboratory specification sheets.

15. References:

15.1 "Farm Chemicals Handbook 2001", Meister Publishing Company, 37733 Euclid Avenue, Willoughby, OH 44094-5992.

15.2 "The Agrochemicals Handbook, Third Edition", The Royal Society of Chemictry, Thomas Graham House, Science Park, Milton Road Cambridge, CB4 4WF England

APPENDIX I Operating parameters

Creator: Administrator
Last modified: 1/18/2007 by Administrator

MS Run Time (min): 22.00

Sequence override of method parameters not enabled.

Divert Valve: in use during run

Divert Time (min)	Valve State
0.00	To Waste
4.00	To Source
21.00	To Waste

Contact Closure: not used during run

MS Detector Settings:

Acquisition Start Delay (min): 2.00
Real-time modifications to method disabled

Segment 1 Information

Duration (min): 10.20
Number of Scan Events: 2
Tune Method: Tune 261 6-20-03 high flow

Scan Event Details:
1: + c norm oS(245.0-247.0)
2: + c norm ·(290.0)->o(95.0-350.0)
MS/MS: Amp. 33.5% Q 0.250 Time 30.000 IsoW 2.0

Segment 2 Information

Duration (min): 3.97
Number of Scan Events: 4
Tune Method: Tune 261 6-20-03 high flow

Scan Event Details:
1: + c norm ·(365.0)->o(120.0-400.0)
MS/MS: Amp. 25.0% Q 0.300 Time 30.000 IsoW 2.0
2: + c norm ·(229.0)->o(75.0-350.0)
MS/MS: Amp. 32.0% Q 0.300 Time 30.000 IsoW 3.0
3: + c norm oS(149.0-151.0)
4: + c norm oS(123.0-125.0)

Segment 3 Information

Duration (min): 7.83
Number of Scan Events: 3
Tune Method: Napropamide HIGH flow

Scan Event Details:
1: + c norm ·(272.0)->o(85.0-350.0)
MS/MS: Amp. 34.0% Q 0.300 Time 30.000 IsoW 2.0
2: + c norm ·(347.0)->o(95.0-400.0)
MS/MS: Amp. 30.0% Q 0.250 Time 30.000 IsoW 2.0
3: + c norm ·(231.0)->o(75.0-300.0)
MS/MS: Amp. 40.0% Q 0.300 Time 30.000 IsoW 3.0

APPENDIX 2 Tune Method

10valid3-4
Segment: 1

Capillary Temp (C):	220.00
APCI Vaporizer Temp (C):	450.00
AGC:	On
AGC Off Ion Time (ms):	5.000
Sheath Gas Flow ():	80.00
Aux Gas Flow ():	10.00
Source Type:	APCI
Injection Waveforms:	Off

POSITIVE POLARITY

Source Voltage (kV):	4.50
Source Current (uA):	5.00
Capillary Voltage (V):	46.00
Tube Lens Offset (V):	35.00
Multipole RF Amplifier (Vp-p):	400.00
Multipole 1 Offset (V):	-4.25
Multipole 2 Offset (V):	-8.50
InterMultipole Lens Voltage (V):	-32.00
Entrance Lens (V):	-64.00
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):	50.00
SIM Micro Scans:	5
SIM AGC Target:	20000000.00

Appendix 3

Method Detection Limit (MDL) data

Spk\Analyte	Napropamide ppb	Oryzalin ppb	Propazine ppb
0.2 ppb spk 1	0.179	0.19	0.179
0.2 ppb spk 2	0.166	0.178	0.166
0.2 ppb spk 3	0.165	0.168	0.165
0.2 ppb spk 4	0.151	0.191	0.151
0.2 ppb spk 5	0.157	0.183	0.157
0.2 ppb spk 6	0.142	0.167	0.142
0.2 ppb spk 7	0.157	0.196	0.157
Average	0.160	0.182	0.160
STDEV	0.012	0.011	0.012
MDL	0.037	0.036	0.037
RL	0.05	0.05	0.05

Appendix 4
Method Validation Data

Analyte	Spike ppb	% Recovery Set 1	Set 2	Set 3	Set 4	Set 5		%
							Mean:	86.5
Napropamide	0.2	80.5	80.0	80.5	86	83.5	SD:	7.15
	0.5	91.6	80.4	83.2	96.6	79.4	UCL:	107.9
	0.75	82.9	85.1	78.8	102.4	92.5	UWL:	100.8
	1.0	86.8	82.2	83.6	94.1	99.7	LWL:	72.2
							LCL:	65.0
							Mean:	91.7
Oryzalin	0.2	92.0	88.5	89.5	91.5	93.5	SD:	7.84
	0.5	96.4	93.6	84.8	110.6	73.2	UCL:	115.2
	0.75	81.7	88.7	90.0	97.2	93.9	UWL:	107.3
	1.0	89.3	83.2	96.5	98.1	101	LWL:	76.0
							LCL:	68.2

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Date	**What was revised? Why?**
9/8/10	**Section 15 discussion removed**