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Determination of Linuron, Isoxaben, Mefenoxam, Metalaxyl, Methomyl and Propyzamide in Well Water by Ultra Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry

1. Scope

   This section method (SM) is for the analysis of Linuron, Isoxaben, Mefenoxam, Metalaxyl, Methomyl and Propyzamide in well water. It is to be followed by all authorized section personnel. The reporting limit is 0.05 ppb for all compounds.

2. Principle:

   Linuron, isoxaben, mefenoxam, metalaxyl, methomyl and propyzamide are extracted by passing a 500 mL well water sample through a solid phase extraction HLB cartridge.
   The compounds are eluted from the solid phase cartridge with acetonitrile. The extract is concentrated to just dryness with nitrogen in a heated water bath, and then adjusted to a 0.5 mL volume with methanol. The extract is analysis on Ultra Performance Liquid Chromatography (UPLC) coupled to a positive electrospray ionization triple quadrupole mass spectrometry (ES-LC/MS/MS). Mefenoxam and metalaxyl are stereo isomers. These compounds cannot be differentiated from each other in this method. The two compounds have the same retention time and have the same product ions and product ion ratios. These two compounds will be reported as a total amount of either or both of mefenoxam and metalaxyl.

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:

   There were no matrix interferences for linuron, isoxaben, mefenoxam, methomyl and propyzamide at the time of method development.

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 UPLC equipped with a triple quadrupole mass spectrometry and ES ion source
    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:

    6.1 Linuron CAS#330-55-2
    6.2 Isoxaben CAS#82558-50-7
    6.3 Mefenoxam CAS#70630-17-0
    6.4 Methomyl CAS#16752-77-5
    6.5 Propyzamide CAS#23950-58-5
    6.6 Formic acid, HPLC grade (Fisher #A35-500 or equivalent)
    6.7 Acetonitrile, (Burdick &Jackson, UV grade, High Purity)
    6.8 Methanol, (Burdick &Jackson, High Purity)
    6.9 Water, (HPLC grade, Burdick & Jackson Cat #AH365-4 or equivalent)
    6.10 Analytical column: Waters Acquity BEH C18 1.7µm, 2.1 x 100 mm
    6.11 SPE cartridge: Waters Oasis® HLB (Hydrophilic-Lipophilic-Balanced) cartridge, 500 mg, 6 cc.
    6.12 Ammonium formate, 1.0 M
    6.13 Aqueous Solution: For 500 mL, mix 470 ± 2mL water, 25 ± 0.5 mL methanol, 4.50 ± 0.25 mL 1 M ammonium formate and 0.5 ± 0.05 mL formic acid.
    6.14 Organic Solution: For 500mL, mix 450 ± 2mL methanol and 45 ± 0.5 mL water with 4.50 ± 0.25 mL 1 M ammonium formate and 0.5 ± 0.05 mL formic acid

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.

7.2 Prepare a combination standard of 10 µg/mL of Linuron, isoxaben, mefenoxam, methomyl and propyzamide. The combination working standard was diluted to the following concentrations: 0.025, 0.05, 0.1, 0.25, 0.5, and 1.0 µg/mL.

7.3 Keep all standards in the designated refrigerator for storage.

7.4 The expiration date of each standard is six months from the preparation date.

8. Sample Preservation and Storage:

Store all samples waiting for extraction in a separate refrigerator (4 ± 3 °C).

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulations (DPR) provides the background water for matrix blank and spikes.

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, mix well and let it stand for 1 minute. Follow the test sample extraction procedure.

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 HLB cartridge to the solid phase extraction manifold. Condition the cartridges with a total ~10 mL of acetonitrile 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. Fill the cartridge with D.I. water and attach the sample delivery tube to the cartridge. Place weighted tube end of the sample delivery tube into a beaker of D.I. water. Turn on the vacuum source and adjust the vacuum flow rate to ~8 mL per minute then transfer the weighted sample tube to the samples.

9.3.5 After the water sample has passed through the cartridge, increase the vacuum to ~20 psi for 2 minutes or until no more water is dripping from the cartridge. Detach the sample delivery tube from the HLB cartridge. Shake out any excess water in the cartridge reservoir and place back on the vacuum manifold.

9.3.6 Lift the extraction manifold top and insert a 15 mL graduated conical tube into the vacuum chamber. Elute the cartridge with 10 mL acetonitrile and collect the eluant into conical tube with flow rate of approximate ~8 mL/min.

9.3.7 Concentrate the extract to dryness under a gentle stream of nitrogen in a heated water bath (40 ± 2 °C) to remove all the water/acetonitrle mix. Adjust to a final volume 0.5 mL with methanol.

9.3.8 Transfer the extract to an autosampler vial. Submit extract for LC-MS analysis.

10. Instrument Calibration:

10.1 The calibration standard curve consists of a minimum of three levels. The lowest level must be at or below the corresponding reporting limits. The current working standard levels are 0.025, 0.05, 0.1, 0.25, and 0.5 µg/mL.

10.2 Calibration curve or overlay calibration curves are obtained using a linear or quadratic regression with the correlation coefficient (r) equal to or greater than 0.995, with all levels weighted 1/x.

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 Instrumentation UPLC-MS/MS

11.2.1 UPLC Instrument: Waters Acquity Ultra Performance LC
Column: Waters Acquity BEH C18 1.7µm, 2.1 x 100 mm
Column Temperature: 60 °C
Mobile Phase: Gradient
Solvent 1: Aqueous Solution
Solvent 2: Organic Solution
Gradient:

Time(min)	Flow rate (mL/min)	Solvent 1	Solvent 2
0	0.60	90.0	10.0
0.5	0.60	90.0	10.0
7.00	0.60	10.0	90.0
7.80	0.60	10.0	90.0
8.00	0.60	90.0	10.0
8.50	0.60	90.0	10.0

Injection Volume: 1.0 µL

11.2.3 Mass Spectrometry and Operating Parameters
Model: Waters Xevo Triple Quadrupole
Ion ProbeType: Electrospray Ionization (ES)
Ion Mode: ES+
Source Temp: 150 °C

Compound	Retention Time (min)	Precursor ion	Product Ion	Dwell (s)	Cone(V)	Collision Energy/-ev
Methomyl	1.57	163.01 163.01	**87.99** 106.02	0.128 0.128	10 10	8.00 10.0
Mefenoxam	4.97	280.18 280.18	**192.17** 220.12	0.061 0.061	6.00 6.00	18.0 14.0
Linuron	5.27	249.04 249.04	**159.98** 182.03	0.039 0.039	32.0 32.0	18.0 16.0
Propyzamide	5.58	256.05 256.05	**172.95** 190.00	0.039 0.039	20.0 20.0	22.0 14.0
Isoxaben	5.73	333.21 333.21	**149.98** 165.00	0.039 0.039	8.00 8.00	40.0 18.0

Quantitation ions are in bold.

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 0.1 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 1

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 5 levels of fortification (0.1, 0.25, 0. 5, 1.0 and 5.0 ppb) and a method blank. All spikes and method blanks were processed through the entire analytical method. Spike levels and recoveries for the analytes are shown in Appendix 2.

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 percent recovery, respectively, shown in Appendix 2

12.5 Acceptance Criteria

12.5.1.1 Each set of samples will have a matrix blank and a spiked matrix sample.

12.5.1.2 The matrix blank shall be free of target compounds above the MDL.

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

12.5.1.3 The retention time between standards, QC, spikes and unknowns shall be within ± 2 percent of that of the standard. If there is a known reason of retention time shifting, an explanation memo shall be included.

12.5.1.4 Peak response shall be within the calibration range. The sample extract shall be diluted if the results fall outside of the calibration curve.

13. Calculations:

Quantitation is based on an external standard (ESTD) calculation using either the peak area or height. The triple quadrupole LCMS software used a linear curve fit,

with all levels weighted 1/x. Alternatively, at the chemist's discretion, sample results may be calculated using the response factor for the standard.

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

14. Reporting Procedure:

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

15. Discussion:

15.1 A storage stability study was done with this project. The storage stability study consisted of a 1.0 ppb spike level and 3 replicates over a 28 day period. Twelve liters of background well water were spiked and then transferred to twelve of the one liter amber bottles. These spiked samples were stored in the refrigerator until analyzed on 0, 2, 4, 7, 15, 21 and 28 days. Along with the storage spikes, a blank and method control spike were also extracted. This storage study showed no significant degradation for these compounds within 28 days. Results for the storage study are shown in Appendix 3.

15.2 In the early stage of method development, Waters Oasis® MCX 500 mg, 6cc, solid phase cartridge was tried. The cartridge yielded ~60% recovery for all compounds for a 500 mL sample size. The HLB cartridge gave overall better recoveries.

16. References:

16.1 Cullum, Neil; Stephens, Paul, and Evans, Stan "Determination of Acidic Herbicides in Groundwater and Potable Water by LC/MSD Using Selective Ion Monitoring" Application, 2002

16.2 "Crop Protection Handbook, 2010", MeisterPro Executive Office 27722 Euclid Ave., Willoughby, OH

Appendix 1

Method Detection Limit (MDL) Data

Lab #	Spk\Analyte	Isoxaben	Linuron	Mefenoxam	Methomyl	Propyzamide
2011-0661	blk	nd	nd	nd	nd	nd
2011-0662	0.1 ppb spk 1	0.087	0.082	0.085	0.086	0.074
2011-0663	0.1 ppb spk 2	0.097	0.094	0.098	0.100	0.086
2011-0664	0.1 ppb spk 3	0.089	0.085	0.088	0.086	0.078
2011-0665	0.1 ppb spk 4	0.100	0.091	0.098	0.095	0.082
2011-0666	0.1 ppb spk 5	0.092	0.085	0.092	0.092	0.075
2011-0667	0.1 ppb spk 6	0.095	0.086	0.091	0.090	0.073
2011-0668	0.1 ppb spk 7	0.097	0.089	0.094	0.093	0.079
	SD	0.00471	0.00412	0.00486	0.00499	0.00467
Reported	**MDL**	**0.0148**	**0.0129**	**0.0152**	**0.0157**	**0.0147**
	RL	**0.05**	**0.05**	**0.05**	**0.05**	**0.05**

Lab #	Spike/analyte	Metalaxyl
2012-2382	0.1 ppb spk 1	0.085
2012-2383	MDL Spike 1	0.087
2012-2384	MDL Spike 2	0.091
2012-2385	MDL Spike 3	0.086
2012-2386	MDL Spike 4	0.084
2012-2387	MDL Spike 5	0.091
2012-2388	MDL Spike 6	0.088

Reported	SD	0.00276
	MDL	0.0087
	RL	0.05

All concentrations are expressed in ppb.

Appendix 2

Method Validation Data

Analyte	Spike ppb	Recovery (%) Set1	Set2	Set3	Set4	Set5		%
Isoxaben	0.1	93.0	91.0	97.0	91.0	88.0	Mean:	92.9
	0.25	90.8	99.6	91.2	95.2	95.2	SD:	5.35
	0.5	95.4	102	88.6	95.0	87.8	UCL:	109
	1	86.7	98.4	89.1	99.6	88.4	UWL:	104
	5	96.8	95.0	99.4	91.2	77.8	LWL:	82.2
							LCL:	76.9
Linuron	0.1	87.0	81.0	86.0	89.0	84.0	Mean:	87.5
	0.25	84.0	92.8	86.8	91.2	92.0	SD:	5.42
	0.5	89.8	98.2	83.2	91.8	83.4	UCL:	104
	1	76.2	90.9	81.2	93.0	86.4	UWL:	98
	5	90.6	89.0	95.0	87.4	76.6	LWL:	76.6
							LCL:	71.2
Mefenoxam	0.1	91.0	89.0	96.0	93.0	88.0	Mean:	92.7
	0.25	88.8	98.8	92.0	97.2	97.2	SD:	5.73
	0.5	92.2	103	87.8	94.8	87.6	UCL:	110
	1	85.3	96.6	87.8	98.8	88.7	UWL:	104
	5	96.0	97.4	101	91.4	77.8	LWL:	81.2
							LCL:	75.5
Methomyl	0.1	90.0	90.0	94.0	93.0	87.0	Mean:	91.8
	0.25	88.8	95.2	90.0	96.4	97.6	SD:	5.56
	0.5	91.4	105	86.2	94.4	88.2	UCL:	108
	1	84.4	94.9	86.8	96.5	87.6	UWL:	103
	5	94.2	97.0	98.8	89.2	78.0	LWL:	80.7
							LCL:	75.1
Propyzamide	0.1	77.0	71.0	78.0	77.0	73.0	Mean:	78.3
	0.25	70.0	84.8	77.6	87.6	85.6	SD:	7.16
	0.5	80.0	95.4	76.2	81.4	69.8	UCL:	100
	1	60.2	80.1	74.8	82.4	75.4	UWL:	92.7
	5	81.2	79.6	88.4	79.2	72.6	LWL:	64.0
							LCL:	56.8

Analyte	Spike ppb	Recovery(%) Set 1	Set 2	Set 3		
					Mean	89.0
Metalaxyl	0.10	92.0	93.0	88.0	SD	4.1
	0.25	92.4	93.2	85.2	UCL	101.4
	0.5	85.6	87.2	85.2	UWL	97.3
	1.0	93.6	94.3	86.5	LWL	80.7
	5.0	89.4	90.0	79.8	LCL	76.6

Appendix 3

Storage Stability Study

Analyte\Recovery %		Day 0	Day 2	Day 4	Day 7	Day 15	Day 21	Day 28
Isoxaben	blk							
	QC spk		93.0	82.5	81.3	91.8	84.0	89.4
	Spike 1	88.2	86.6	84.5	87.2	93.6	82.9	97.8
	Spike 2	85.4	85.1	84.4	81.2	87.9	89.0	90.8
	Spike 3	90.3	99.4	86.5	86.8	85.8	86.7	91.9
Linuron	blank							
	QC spk		86.2	78.2	74.8	86.7	84.6	83.2
	Spike 1	81.6	82.4	80.9	73.9	85.7	78.4	90.7
	Spike 2	80.7	78.9	80.4	73.4	81.7	79.1	81.4
	Spike 3	86.4	89.2	80.1	77.7	76.4	80.7	84.6
Mefenoxam	blank							
	QC spk		88.8	79.9	79.9	92.2	90.4	86.9
	Spike 1	87.2	84.8	82.7	82.9	90.2	83.6	95.7
	Spike 2	83.8	84.4	85.0	80.2	85.4	86.8	86.9
	Spike 3	87.9	96.1	84.3	81.9	81.2	85.6	90.8
Methomyl	blank							
	QC spk		84.2	78.8	77.8	90.6	90.2	85.2
	Spike 1	84.3	83.1	82.0	77.3	89.2	81.2	91.4
	Spike 2	83.4	82.7	82.5	78	82.8	86.0	83.1
	Spike 3	84.6	90.9	83.6	78.7	77.6	84.8	88.2
Propyzamide	blank							
	QC spk		74.4	65.9	62.7	75.8	73.3	74.3
	Spike 1	68.1	73.2	69.0	54.2	71.1	68.3	79.4
	Spike 2	67.9	70.1	66.8	59.4	69.6	63.8	72.6
	Spike 3	71.5	77.3	66.3	60.3	65.6	69.1	71.9

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Date	What was revised? Why?
2/14/13	Added in validation for Metalaxyl and comments in section 2.