Analysis of S- Metolachlor, Metolachlor ESA, and Metolachlor OXA in Well Water

1. Scope:

This section method (SM) documents the preparation and analysis of S-metolachlor, metolachlor ESA, and metolachlor OXA analysis in well water and is to be followed by all authorized EMON personnel.

2. Principle:

The SM describes the method for determination of s-metolachlor and its metabolites (metolachlor ESA and metolachlor OXA) in well water. A 50 mL aliquot of ground water is passed through a C18 solid phase extraction (SPE) cartridge (1 g). The column are rinsed with water and the analytes are eluted with methanol. The methanol is evaporated at 45 °C with a gentle stream of nitrogen to approximately 0.4 mL. The volume of extract is adjusted to 0.5 mL with water, and then 0.5 mL of methanol is added making the final extract volume 1.0 mL. The sample extract is then analyzed by LC/MS/MS, AB Sciex Qtrap 6500 mass spectrometer coupled to Shimadzu HPLC system.

3. Safety:

3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.

3.2 Methanol is a flammable and toxic solvent. It should be handled with care in a ventilated area.

4. Interferences:

There were no matrix interferences that caused quantitative problems during method development and validation.

5. Apparatus and Equipment:

5.1 Balance, (Mettler PC 4400 or equivalent)
5.2 Visiprep Solid Phase Extraction Vacuum Manifold (Supelco, Cat# 913-0445)
5.3 Visiprep Large Volume Sampler (Supelco, Cat# 57275)
5.4 Graduated conical test tubes, 15 mL, calibrated for 1 mL

5.5 Nitrogen Evaporator (Organomation, Model 112)
5.6 Vortex mixer (Fisher Scientific, Model Vortex-Genie 2)
5.7 Liquid Chromatography system with autosampler and column oven (Shimadzu)

6. Reagents and Supplies:

6.1 S-Metolachlor CAS# 87392-12-9
6.2 Metolachlor ESA CAS# 171118-09-5
6.3 Metolachlor OXA CAS# 152019-73-3
6.4 Methanol, nanograde or equivalent pesticide grade
6.5 Water, MS grade, Burdick & Jackson or equivalent
6.6 Formic acid, HPLC grade
6.7 Ammonium formate 1.0 M
6.8 Disposable Pasteur pipettes, and other laboratory ware as needed
6.9 Solid Phase Extraction Cartridge (Waters, Sep-Pak Vac 6 cc (1 g) C18)
6.9 Recommended UPLC analytical column:
Waters Acquity BEH C18 1.7 µm, 2.1 x 100 mm column or equivalent
6:10 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.
6.11 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 Individual stock standards of 1.0 mg/mL were obtained from the CDFA/CAC Standards Repository. They obtained the neat standards from either the manufactures or from commercial suppliers of standards.

The standards were diluted to 10 µg/mL with methanol. A combination standard of 10 µg/mL was prepared from the individual mg/mL standards in methanol.

The combination standard was also used to dilute to the following concentrations: 00125, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.10, 0.25, 0.5 and 1.0 µg/mL in 1:1 water/methanol for instrument calibration.

7.2 Keep all standards in the designated refrigerator for storage.

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

8. Sample Preservation and Storage:

8.1 Check and record sample temperature upon arrival.

8.2 Store all samples in locked designated area in the walk-in refrigerator (less than 5 °C).

8.3 Return samples to the refrigerator immediately after subsample is taken.

8.4 Sample extracts shall be stored in the refrigerator (32-40 °F).

9. Test Sample Preparation:

9.1 Background Preparation

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

9.1.1 Blank

Mix background sample well before weighing out 50±0.1 grams into a beaker. Proceed to step 9.2.2 of section 9.2.

9.1.2 Spike

Mix background sample well before weighing out 50±0.1 grams into a beaker. Fortify at the level requested by client and mix well to ensure that the pesticides are well distributed. The spiked background was allowed to sit for 15 minutes before proceeding to step 9.2.2 of section 9.2.

9.2 Test Sample Extraction

9.2.1 Measure 50±0.1 gram of the sample into a beaker.

9.2.2 Set up a 24 channels solid phase extraction vacuum manifold (Supelco).

9.2.3 Connect a Waters Sep-Pak Vac 6 cc (1 g) C18 SPE columns to each channel. Turn off the unused channels of the manifold. Pre-condition the SPE columns by passing 10 mL of methanol, by gravity followed by 20 mL of D.I. water. For the addition of water, use the SPE large volume sampler (SPE tube adapter and lines with stainless steel weight) after initially filling the SPE reservoir with water. Attach the SPE adapter to each cartridge and adjust the vacuum, so the water is eluting at a rate of 5-10 mL per minute. The typical operating pressure is about 10-15 inch Hg. Maintain at least 1 cm water level in the column until all sample has passed through the cartridge. Do not allow the columns to go dry.

9.2.4 Transfer the lines from the individual SPE cartridge to each sample, blank and spike sample. The flow should maintain the same as for the water.

9.2.5 As soon as the sample has passed through the column, rinse the beaker with 10 mL of D.I. water and continue the extraction until all the rinse has passed through the columns. Make sure all the columns are properly labeled before disconnecting them.

9.2.6 Remove the sampling tube. Apply 15 inches vacuum for 5 minutes to allow excess water to be removed.

9.2.7 Elute the columns with 10 mL methanol and collect into a 15 mL graduated conical test tube. Evaporate the eluent in a water bath at 40±2°C with a gentle stream of nitrogen to approximately 0.4 mL. The volume of extract is adjusted to 0.5 mL with water, and then 0.5 mL of methanol is added making the final extract volume 1.0 mL.

9.2.8 Transfer the entire content to a Waters total recovery autosampler vial.

9.2.9 If necessary, dilute the final extract of samples with 1:1 MeOH: water to be within the calibration curve range.

10. Instrument Calibration:

10.1 The calibration standard curve consists of a minimum of three levels. The recommended concentration levels of standards are 0.00125, 0.0025, 0.005, 0.01, 0.025, 0.05 and 0.1 ug/mL.

10.2 The calibration standard curve is analyzed before and after each sample set.

10.2 The calibration curve uses either a linear regression or a quadratic fit with a correlation coefficient (r) equal to or greater than 0.995.

11. Analysis:

11.1 Injection Scheme

The instrument may need to be conditioned with a matrix blank or old sample prior to the analyzing the following sequence of Standard Curve, Solvent, Matrix Blank, Matrix Spike, Test Samples (maximum of 10 – 12) and Standard Curve, then repeat the order for the second injection.

11.2 LC/MS/MS System

11.2.1 HPLC Instrument:

LC Controller: Shimadzu CBM20A
LC Pumps: Shimadzu LC30AD
Autosampler: Shimadzu SIL30AC
Column Oven: Shimadzu CTO30A
HPLC Column: Waters Acquity BEH C18 1.7 µm, 2.1 x 100 mm
Column Temperature: 40 °C

Mobile Phase:

Solvent 1: Aqueous Solution
Solvent 2: Organic Solution

HPLC Gradient:

Time (min)	Flow rate (mL/min)	Solvent 1	Solvent 2
0.0	0.4	95	5
0.50	0.4	90	10
1.0	0.4	90.	10
5.0	0.4	10	90
8.50	0.4	10	90
8.60	0.4	90	10

Injection Volume: 3.0 µL

11.2.2 Mass Spectrometer and Operating Parameters

Model:	ABSciex QTRAP 6500
Ion ProbeType:	Electrospray Ionization (ESI)
Ion Mode:	Positive
Curtain Gas:	20.00
Ion Spray Voltage:	5500.0
Temp:	500.0
Ion Source Gas 1	50.0
Ion Source Gas 2	50.0
Collision Gas:	Medium
Electron Multiplier:	2300.0
Scheduled MRM:	No

Compound	RT	Precursor Ion	Product Ion	Declustering Potential	Collision Energy	Entrance Potential	Exit Potential
S-metolachlor	6.76	284	**252**	41	19	10	30
		284	176	41	33	10	22
Metolachlor ESA	5.72	330	**298**	71	19	10	10
		330	202	71	37	10	16
Metolachlor OXA	5.88	280	**248**	21	19	10	8
		280	90	21	65	10	10

12. Quality Control:

12.1 Method Detection Limits (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of each analyte that a method can detect reliably in either a sample of blank. To determine the MDL, 7 control well water samples were spiked at 0.1 ppb and processed through the entire method along with a blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL for metolachlor and its metabolites using the following equation:

MDL = tS

Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom (n-1, 1 - α = 0.99). n represents the number of replicates 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 results for the standard deviations and MDL are in Appendix 1.

12.2 Reporting Limit (RL)

Reporting limit (RL) refers to a level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. The reporting limits for metolachlor and its metabolites are 0.05 ppb. This reporting limit was chosen after taking into account matrix effects and various sample background that could be encountered.

12.3 Method Validation

The method validation consisted of 5 sample sets. Each set included five levels of fortification (0.1, 0.2, 0.5, 1 and 2 ppb) and a method blank. All spikes and method blanks were processed through the entire analytical method. Recoveries for metolachlor and its metabolites are tabulated in Appendix 2.

12.4 Control Charts and Limits

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

12.5 Acceptance Criteria

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

12.5.2 The retention time should be within ± 2 per cent of that of the standards.

12.5.3 The recoveries of the matrix spikes shall be within the control limits.

12.5.4 The sample shall be diluted if results exceed the calibration curve.

12.5.5 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 is calculated as follows:

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

12.5.6 The R^2 of each calibration curve shall be larger than 0.990.

12.5.7 When the above criteria meet, the chemist may report the average of the two injections.

13. Calculations:

Quantitation is based on external standard (ESTD) calculation using either the peak area or height. The data system uses quadratic fit with all levels weighted none. Alternatively, at chemist discretion, concentrations may be calculated using the response factor for the standard whose value is closest to the level in the sample. Calculate the concentration of the analyte(s) of a sample as follows:

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

14. Reporting Procedure:

Sample results are reported accordance with the client's analytical laboratory specification sheets.

15. Discussion:

15.1 This method was adapted from the method listed in the references below.

16. References:

16.1 Vryzas Z', Tsaboula A, Papadopoulou-Mourkidou E.J. *Determination of alachlor, metolachlor, and their acidic metabolites in soils by microwave-assisted extraction (MAE) combined with solid phase extraction (SPE) coupled with GC_MS and HPLC-UV analysis.,*

16.2 Y.H. Long, R.T. Li, X.M. Wu, *Degradation of S-metolachlor in soil as affected by environmental factors,* Journal of soil science and plant nutrition, version On-line ISSN 0718-9516.

16.3 Method of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group-Update and Additions to the Determination of Chloroacetanilide Herbicide Degradation Compounds in Water Using High-Performance Liquid Chromatography/Mass Spectrometry.

16.4 E.A. Lee, J.L. Kish, L.R. Zimmerman, and E.M. Thurman, U.S. Department of the Interior, U.S. Geological Survey. Open-File report 01-10.

16.5 Determination of Metolachlor (CGA-24705)and CGA-77102, and their Degradates CGA-50212, CGA-354743, CGA-380168, CGA-37735, CGA-67125, and CGA-41638 in Water by High Performance Liquid Chromatography with Mass Spectrometric Detection Including Validation Data.

Method Number: AG-682 of Novartis Crop Protection, Inc. Environmental Safety Department, Environmental Residue Studies.

Appendix 1

The Determination of Method Detection Limit (MDL) and Reporting Limit (RL)

Spk\Analyte	Metolachlor ppm	Metolachlor ESA ppm	Metolachlor OXA ppm
blk	nd	nd	nd
0.1 ppb spk 1	0.093	0.107	0.091
0.1 ppb spk 2	0.090	0.088	0.076
0.1 ppb spk 3	0.087	0.085	0.070
0.1 ppb spk 4	0.096	0.084	0.070
0.1 ppb spk 5	0.094	0.109	0.098
0.1 ppb spk 6	0.096	0.091	0.085
0.1 ppb spk 7	0.097	0.088	0.082
SD	**0.00364**	**0.0104**	**0.0106**
MDL	**0.0114**	**0.0327**	**0.0321**
RL	**0.05**	**0.05**	**0.05**

The data above was generated in the original validation,

Summary of Method Validation for S-Metolachlor, Metolachlor ESA, and Metolachlor OXA in Well Water

Analyte	Spike ppb	Recovery (%) Set1	Set2	Set3	Control Limits	
s-Metolachlor	0.1	94.1	88.5	86	Mean:	89.3
					SD:	2.83
	0.5	87.6	86.4	87.2	UCL:	
					UWL:	
	2	90.5	91.5	92	LWL:	
					LCL:	
Metolachlor ESA	0.1	111	103	101	Mean:	103
					SD:	5.48
	0.5	97.8	95	99.4	UCL:	
					UWL:	
	2	111	103	105	LWL:	
					LCL:	
Metolachlor OXA	0.1	103	107	96.5	Mean:	101
					SD:	7.04
	0.5	91.6	95.2	94	UCL:	
					UWL:	
	2	107	110	108	LWL:	
					LCL:	

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Revision Log:

Date	What was revised? Why?
8/28/2015	Modified LC/MS instrument conditions