

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

### **1. Scope:**

This Section Method provides stepwise procedures for the analysis of S-metolachlor, metolachlor ESA, and metolachlor OXA analysis in well water. It is followed by all authorized Environmental Analysis Section personnel.

### **2. Principle:**

A 50 mL aliquot of well water is passed through an Oasis HLB solid phase extraction (SPE) cartridge (0.2 g). The column is 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 liquid chromatography tandem mass spectrometry (LC/MS/MS).

### **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 for the compounds at the time of method development.

### **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, Oasis HLB 0.2 g 6cc)
- 6.10 Recommended UPLC analytical column:
  - 6.10.1 Waters Acquity BEH C18 1.7  $\mu$ m, 2.1 x 100 mm column or equivalent
- 6.11 Aqueous Solution: For 500 mL, mix 470  $\pm$  2 mL water, 25  $\pm$  0.5 mL methanol, 4.50  $\pm$  0.25 mL 1 M ammonium formate and 0.5  $\pm$  0.05 mL formic acid.
- 6.12 Organic Solution: For 500 mL, mix 450  $\pm$  2 mL methanol and 45  $\pm$  0.5 mL water with 4.50  $\pm$  0.25 mL 1 M ammonium formate and 0.5  $\pm$  0.05 mL formic acid.

## **7. Standards Preparation:**

- 7.1 Stock mixes of 0.1 mg/mL are obtained from an ISO 17034 accredited commercial supplier (such as Restek).
- 7.2 The stock mixes are diluted to 10 µg/mL with methanol. The combination standard at 10 µg/mL is serially diluted with methanol to produce the following concentrations: 0.00125, 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 (less than 5 °C).

## **9. Test Sample Preparation:**

### 9.1 Background Preparation:

The Department of Pesticide Regulation provides the background control well water for the blank and matrix spike samples.

#### 9.1.1 Blank

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

#### 9.1.2 Matrix Spike

Thoroughly mix background well water 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 distributed. Allow

the spiked background to sit for 15 minutes before proceeding to step 9.2.2 of section 9.2.

9.2 Test Sample Extraction:

- 9.2.1 Remove sample(s) from the refrigerator.
- 9.2.2 Measure  $50 \pm 0.1$  grams of the sample into a beaker.
- 9.2.3 Set up a 24-channel solid phase extraction (SPE) vacuum manifold.
- 9.2.4 Connect an Oasis HLB 0.2 g 6cc cartridge 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.
  - 9.2.4.1 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.
  - 9.2.4.2 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 inches of 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.
- 9.2.5 Transfer the lines from the individual SPE cartridge to each sample, blank and matrix spike sample. The flow should maintain the same as for the water.
- 9.2.6 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.
- 9.2.7 Remove the sampling tube. Apply vacuum (15 inches of Hg) for 5 minutes to allow excess water to be removed.
- 9.2.8 Elute the columns with 10 mL methanol and collect into a 15 mL graduated conical test tube.

- 9.2.8.1 Evaporate the eluant in a water bath at  $40 \pm 2$  °C with a gentle stream of nitrogen to approximately 0.4 mL.
- 9.2.8.2 The volume of extract is adjusted to 0.5 mL with methanol, and then 0.5 mL of water is added making the final extract volume 1.0 mL.
- 9.2.9 Transfer the entire content to a Waters total recovery autosampler vial.
- 9.2.10 If necessary, dilute the final extract of sample 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.
- 10.3 Calibration is obtained using a linear or quadratic regression 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 analyzing the following sequence:

- 11.1.1 Standard Curve
- 11.1.2 Solvent Blank
- 11.1.3 Matrix Blank
- 11.1.4 Matrix Spike
- 11.1.5 Test Samples (maximum of 10 – 12)
- 11.1.6 Standard Curve
- 11.1.7 Repeat the order for the second injection.

## 11.2 LC/MS/MS System

### 11.2.1 LC/MS/MS Instrument:

11.2.1.1 LC Controller: Shimadzu CBM20A

11.2.1.2 LC Pumps: Shimadzu LC30AD

11.2.1.3 Autosampler: Shimadzu SIL30AC

11.2.1.4 Column Oven: Shimadzu CTO30A

11.2.1.5 Column: Waters Acquity BEH C18 1.7  $\mu\text{m}$ , 2.1 x 100 mm

11.2.1.6 Column Temperature: 40  $^{\circ}\text{C}$

11.2.1.7 Mobile Phase:

11.2.1.7.1 Solvent 1: Aqueous Solution

11.2.1.7.2 Solvent 2: Organic Solution

11.2.1.8 Gradient:

**Table 1 – LC/MS/MS Mobile Phase Gradient Flow Rate**

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

11.2.1.9 Injection Volume: 3.0  $\mu\text{L}$

### 11.2.2 Mass Spectrometer and Operating Parameters:

11.2.2.1 Model: ABSciex QTRAP 6500

11.2.2.2 Ionization: Electrospray Ionization (ESI)

11.2.2.3 Polarity: Positive

11.2.2.4 Curtain Gas: 20

11.2.2.5 Ion Spray Voltage: 5500

11.2.2.6 Source Temperature: 500

11.2.2.7 Ion Source Gas 1: 50

11.2.2.8 Ion Source Gas 2: 50

11.2.2.9 Collision Gas: Medium

11.2.2.10 Electron Multiplier: 2300 V

11.2.2.11 Scheduled MRM: No

**Table 2 – LC/MS/MS Instrument Conditions**

Compound	RT	Precursor Ion	Product Ion <sup>1</sup>	De-Clustering Potential	Collision Energy	Entrance Potential	Exit Potential
S-Metolachlor	6.76	284	<b>252</b>	41	19	10	30
		284	176	41	33	10	22
Metolachlor ESA	5.72	330	<b>298</b>	71	19	10	10
		330	202	71	37	10	16
Metolachlor OXA	5.88	280	<b>248</b>	21	19	10	8
		280	90	21	65	10	10

<sup>1</sup> Quantitation transition is in bold

## 12. Quality Control:

### 12.1 SPE Cartridge Verification

SPE cartridges are verified per lot number to ensure they meet the manufacturer specifications.

### 12.2 Method Detection Limit (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of each analyte that a method can detect reliably. 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:

$$\text{MDL} = tS$$

Where  $t$  is the Student  $t$  test value for the 99% confidence level with

$n - 1$  degrees of freedom ( $n - 1$ ,  $1 - \alpha = 0.99$ ) 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 I.

### 12.3 Reporting Limit

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 considering matrix effects.

### 12.4 Method Validation Recovery Data

The method validation consisted of 5 sample sets. Each set included five levels of fortification (0.1, 0.25, 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 II.

### 12.5 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  $\pm 2$  and  $3$  standard deviations of the % recovery, respectively, shown in Appendix II.

### 12.6 Acceptance Criteria

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



- 12.6.2 The retention time should be within  $\pm 0.1$  minute of that of the standards.
- 12.6.3 The recoveries of the matrix spikes shall be within the control limits.
- 12.6.3.1 When spike recoveries fall outside the control limits, the analyst must investigate the cause. The entire extraction set of samples is re-analyzed. If the spike recoveries fall within the limit, then the results from the re-analyzed samples shall be reported.
- 12.6.3.2 If the spike recoveries still fall outside the control limits, the client will be notified. The backup samples will be re-extracted for analysis.
- 12.6.4 The sample shall be diluted if results exceed the calibration curve.
- 12.6.5 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:
- $$\% \text{ Change in Response} = \frac{(\text{absolute value of } [\text{slope of STD curve before} - \text{STD curve after}])}{(\text{STD curve before})} * 100$$
- 12.6.6 The  $R^2$  of each calibration curve shall be greater than 0.990.
- 12.6.7 The relative abundance or ratio of selective ions shall be within  $\pm 30\%$  of the standard.
- 12.6.8 When the above criteria are met, the analyst may report the average of the two injections.

### 13. Calculations:

- 13.1 Quantitation is based on external standard (ESTD) calculation using either the peak area or height. The LC/MS/MS software uses quadratic curve fit with all levels weighted  $1/x$ . Alternatively, at the analyst's discretion, concentrations may be calculated using the response factor for the standard whose value is closest to the level in the sample.

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

$$\text{ppb} = \frac{(\text{sample peak area or ht})(\text{STD conc})(\text{STD vol. injected})(\text{final vol. of sample})(1000)}{(\text{STD peak area or ht})(\text{sample vol. injected})(\text{sample weight } g)}$$

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

## Appendix I

### Method Detection Limit Data and Reporting Limits

Compound	Blank	Set 1 (ppb)	Set 2 (ppb)	Set 3 (ppb)	Set 4 (ppb)	Set 5 (ppb)	Set 6 (ppb)	Set 7 (ppb)	SD	MDL (ppb)	RL (ppb)
S-Metolachlor	ND	0.093	0.090	0.087	0.096	0.094	0.096	0.097	0.00364	0.0114	0.05
Metolachlor ESA	ND	0.107	0.088	0.085	0.084	0.109	0.091	0.088	0.0104	0.0327	0.05
Metolachlor OXA	ND	0.091	0.076	0.070	0.070	0.098	0.085	0.082	0.0106	0.0321	0.05

#### **Definitions**

**MDL** = Method Detection Limit

**ND** = Not detected

**ppb** = Part per billion

**RL** = Reporting limit

**SD** = Standard deviation

## Appendix II

### Method Validation Data and Control Limits

Compound	Spike Level	0.1 ppb	0.25 ppb	0.5 ppb	1.0 ppb	2.0 ppb	Control Limits (%)	
	MV Run	Avg. Rec. (%)	Avg. Rec. (%)	Avg. Rec. (%)	Avg. Rec. (%)	Avg. Rec. (%)		
S-Metolachlor	Day 1	79.8	68.6	71.1	80.4	81.7	Mean:	72.3
	Day 2	64.8	58.3	60.9	48.0	54.4	SD:	10.3
	Day 3	75.3	65.2	79.3	80.3	81.9	UCL:	103
	Day 4	89.4	84.5	81.0	75.1	80.3	UWL:	92.8
	Day 5	76.6	65.8	71.6	63.5	68.6	LWL:	51.7
							LCL:	41.5
Metolachlor ESA	Day 1	79.6	84.2	83.7	85.6	88.9	Mean:	82.9
	Day 2	78.3	82.1	73.8	74.2	80.9	SD:	6.00
	Day 3	80.2	81.1	88.2	85.5	80.5	UCL:	101
	Day 4	89.0	88.1	92.5	87.0	86.0	UWL:	94.9
	Day 5	64.0	87.9	83.1	83.8	85.3	LWL:	70.9
							LCL:	64.9
Metolachlor OXA	Day 1	71.8	70.5	74.8	74.6	80.0	Mean:	72.9
	Day 2	67.4	69.2	66.3	64.9	72.1	SD:	6.68
	Day 3	70.8	71.2	79.0	75.0	72.4	UCL:	92.9
	Day 4	80.8	81.3	84.0	81.4	79.5	UWL:	86.3
	Day 5	54.9	71.5	76.7	65.8	66.3	LWL:	59.5
							LCL:	52.8

**Definitions**

**Avg. Rec.** = Average recovery

**LCL** = Lower control limit

**LWL** = Lower warning limit

**MV** = Method validation

**ppb** = Part per billion

**%** = percent

**SD** = Standard deviation

**UCL** = Upper control limit

**UWL** = Upper warning limit

**Revised By:**



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**Revision Log:**

Date	What was revised? Why?
2/4/2021	Re-validated the method using Waters HLB cartridges
	9.2.3 Switched from Sep-Pak Vac 6cc 1-gram cartridges to Waters HLB cartridges for ease of use and higher recoveries.
	9.2.7 Volume of extract adjusted to 0.5 mL with methanol and 0.5 mL of water is added for a final volume of 1 mL (MeOH/H2O 1:1) to match the solvent of the standards and diluent.
6/30/2022	Reformatted the entire document in accordance with web accessibility requirements
	Made editorial revisions throughout the document for improved readability
	Corrected grammatical errors
	7.1 The procedures for standards preparation were revised to reflect the current practice of obtaining stock mixes from ISO 17034 accredited suppliers.
	12.1 Added SPE cartridge verification
	12.6.3 Added procedures to follow when spike recoveries fall outside the control limits
	12.6.7 Added qualifier ion quality control criteria