

Analysis of Imidacloprid, S-Metolachlor, Metolachlor ESA, and Metolachlor OXA in Soil

1. Scope:

This section method (SM) documents Imidacloprid, s-metolachlor, metolachlor ESA, and metolachlor OXA analysis in soil and is followed by all authorized EMON personnel.

2. Principle:

The SM describes the method for determination of Imidacloprid, s-metolachlor and its metabolites in soil. The sample is homogenized and extracted with 1:1 methanol/water by shaking on an orbital shaker. The extract is then allowed to settle before filtering through 0.45 µm nylon acrodisc. The sample extract is then analyzed by Liquid Chromatography coupled to a Linear Ion Trap Quadrupole 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 fume hood.

4. Interferences:

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

5. Apparatus and Equipment:

- 5.1 Shaker, (Lab-Line Force Orbital Air Shaker or equivalent)
- 5.2 Balance, (Mettler PC 4400 or equivalent)
- 5.6 Liquid Chromatography equipped with a linear ion trap quadrupole (MS/MS)

6. Reagents and Supplies:

- 6.1 Imidacloprid CAS# 138261-41-3
- 6.2 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 Mason jars, pint size with lids
 - 6.7 Nylon Acrodisc®, 0.45 micron, Gelman Sciences or equivalent
 - 6.8 Syringe and plunger for filtration, 10mL
 - 6.9 Formic acid, HPLC grade
 - 6.10 Ammonium formate 1.0 M
 - 6.11 Disposable Pasteur pipettes, and other laboratory ware as needed
 - 6.12 Recommended UPLC analytical column:
Waters Acquity BEH C18 1.7 µm, 2.1 x 100 mm column or equivalent
 - 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 Individual stock standards of 1.0 mg/mL were obtained from the CDFA/CAC Standards Repository. Compounds are obtained from an ISO 17034 accredited supplier.

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/water 1:1. The combination standard was also used to dilute to the following concentrations: 0.0025, 0.005, 0.01, 0.025, 0.05, 0.10, 0.25, 0.5. The standards were prepared in matrix using the solvent from the extracted soil blank.
 - 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:
- Store all samples waiting for extraction in a freezer. If samples are to be extracted the next day, they may be stored in the refrigerator. Sample extracts shall be stored in the refrigerator (4 ± 3 ° C).
9. Test Sample Preparation:
- 9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the soil for background to be used in method validation. The soil used for this study was a dry sandy soil.

9.1.1 Blank

Mix background sample well before weighing out 25 ± 0.5 g. Proceed to step 9.2.2 of section 9.2.

9.1.2 Spike

Mix background sample well before weighing out 25 ± 0.5 g. 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.

9.1.3 Moistures

9.1.3.1 Thaw soil sample and homogenize using a spatula. Remove any debris.

9.1.3.2 Weigh out a 15 – 20 g sub-sample into a pre-weighed aluminum weighing pan.

9.1.3.3 Dry the pan with soil for at least 6 hours in a $\sim 105^{\circ}\text{C}$ oven.

9.1.3.4 Reweigh soil after cooling in a dessicator.

9.1.3.5 Report the wet and dry weights on Chain of Custody sample sheets.

9.2 Test Sample Extraction

9.2.1 Thaw soil sample and homogenize using a spatula. Remove any debris (e.g., gravel, sticks). Weigh out a 25 ± 0.5 g sub-sample into a pint size mason jar.

9.2.2 Add 50 mL mixture of methanol/water 1:1 to the mason jar, cover with foil and cap. Place on shaker and shake for 30 min. at 200rpm.

9.2.3 Allow sample to settle for 30 min. before filtering through 0.45 µm nylon acrodisc into an autosampler vial. Analyze using LC/MS/MS.

10. Instrument Calibration:

10.1 The calibration standard curve consists of a minimum of five levels.

The recommended concentration levels of standards are 0.0025, 0.005, 0.01, 0.025, 0.05 and 0.01 µg/mL

10.2 The Linear Ion Trap Quadrupole used 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 before running the following sequence of Standard Curve, Solvent, Matrix Blank, Matrix Spike, Test Samples (maximum of 10 – 12) and Standard Curve.

11.2 Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer

11.2.1 LC Instrument: Shimadzu LC30

Column: Waters Acquity BEH C18 1.7 µm, 2.1 x 100 mm column

Column Temperature: 40 °C

Mobile Phase: Gradient

Solvent 1: Aqueous Solution

Solvent 2: Organic Solution

| <u>Time (min)</u> | <u>Flow rate (mL/min)</u> | <u>Solvent 1</u> | <u>Solvent 2</u> |
|-------------------|---------------------------|------------------|------------------|
| 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: 2.0 µL

11.2.2 Mass Spectrometer and Operating Parameters

Model: ABSciex QTRAP 6500

Ion Probe Type: Electropray 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: 1700.0
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 |
| Imidacloprid | 3.80 | 256 | 209 | 30 | 21 | 10 | 26 |
| | | 256 | 175 | 30 | 25 | 10 | 16 |

12. Quality Control:

12.1 Method Detection Limits (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 soil samples are spiked at 0.01 ppm 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 single tailed 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 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 limit for metolachlor and its metabolites is 0.005 ppm. This reporting limit was determined after taking into account matrix effects.

12.3 Method Validation

The method validation consisted of 5 sample sets. Each set included five levels of fortification (0.02, 0.05, 0.1, 0.2 and 1 ppm) 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 average % 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 For positive results the retention time shall not vary from standards more than 0.1 minute.

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.
dilutions shall be made using blank soil matrix

12.5.5 Continuing calibration verification is within 20%.

12.5.6 Ion ratios will be within 30% of those obtained from the standards used with the sequence.

13. Calculations:

Quantitation is based on external standard (ESTD) calculation using either the peak area or height. The software 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.

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

14. Reporting Procedure:

Sample results are reported out according to the client's analytical laboratory specifications.

15. Discussion:

15.1 A storage stability study was done with this project. The storage stability study consisted of a 0.1 ppm spike level and 3 replicates over a 28 day period. Twenty one pint size mason jars contain 25g of background soil were spiked and then capped and stored in the freezer. The spiked samples were then analyzed on 0, 2, 4, 7, 14, 22 and 28 days by LCMSMS. Along with the storage spikes a blank and method control spike were also extracted. Results for the storage study are shown in Appendix 3.

15.2 This method was adapted from the methods 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

Appendix 1

Method Detection Limit (MDL)

| S-Metolachlor | |
|--------------------------|----------|
| Summary | |
| Sample | Result |
| Blank | ND |
| 0.005µg Spike 1 | 0.00561 |
| 0.005µg Spike 2 | 0.00510 |
| 0.005µg Spike 3 | 0.00458 |
| 0.005µg Spike 4 | 0.00455 |
| 0.005µg Spike 5 | 0.00408 |
| 0.005µg Spike 6 | 0.00414 |
| 0.005µg Spike 7 | 0.00467 |
| Standard Deviation (SD) | 0.000535 |
| MDL (3.14*SD) µg/sample | 0.00168 |

| Metolachlor ESA | |
|--------------------------|----------|
| Summary | |
| Sample | Result |
| Blank | ND |
| 0.005µg Spike 1 | 0.00496 |
| 0.005µg Spike 2 | 0.00466 |
| 0.005µg Spike 3 | 0.00508 |
| 0.005µg Spike 4 | 0.00506 |
| 0.005µg Spike 5 | 0.00442 |
| 0.005µg Spike 6 | 0.00441 |
| 0.005µg Spike 7 | 0.00578 |
| Standard Deviation (SD) | 0.000477 |
| MDL (3.14*SD) µg/sample | 0.00150 |

| Metolachlor OXA | |
|--------------------------|----------|
| Summary | |
| Sample | Result |
| Blank | ND |
| 0.005µg Spike 1 | 0.00419 |
| 0.005µg Spike 2 | 0.00380 |
| 0.005µg Spike 3 | 0.00342 |
| 0.005µg Spike 4 | 0.00345 |
| 0.005µg Spike 5 | 0.00260 |
| 0.005µg Spike 6 | 0.00265 |
| 0.005µg Spike 7 | 0.00280 |
| Standard Deviation (SD) | 0.000611 |
| MDL (3.14*SD) µg/sample | 0.00192 |

| Imidacloprid | |
|--------------------------|----------|
| Summary | |
| Sample | Result |
| Blank | ND |
| 0.005µg Spike 1 | 0.00482 |
| 0.005µg Spike 2 | 0.00444 |
| 0.005µg Spike 3 | 0.00416 |
| 0.005µg Spike 4 | 0.00418 |
| 0.005µg Spike 5 | 0.00410 |
| 0.005µg Spike 6 | 0.00400 |
| 0.005µg Spike 7 | 0.00461 |
| Standard Deviation (SD) | 0.000301 |
| MDL (3.14*SD) µg/sample | 0.00095 |

The reporting level for all compounds is 0.005 ppm.

Appendix 2

Method Validation

| S-Metolachlor | | | | | | | | |
|---------------|-------|-------|-------|-------|-------|------|---------|------|
| Summary | | | | | | | | |
| Sample | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | AVE | | |
| Blank | ND | ND | ND | ND | ND | ND | Average | 92.0 |
| 0.01 | 90.1 | 94.3 | 88.3 | 90.9 | 86.8 | 90.1 | SD | 2.96 |
| 0.05 | 95.8 | 96.5 | 88.5 | 92.2 | 90.9 | 92.8 | UCL | 101 |
| 0.10 | 95.8 | 92.3 | 89.9 | 92.4 | 90.1 | 92.1 | UWL | 97.9 |
| 0.20 | 92.3 | 90.0 | 94.3 | 88.0 | 87.8 | 90.5 | LWL | 86.1 |
| 1.0 | 92.0 | 95.3 | 95.3 | 96.2 | 94.4 | 94.6 | LCL | 83.1 |

| Metolachlor ESA | | | | | | | | |
|-----------------|-------|-------|-------|-------|-------|------|---------|------|
| Summary | | | | | | | | |
| Sample | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | AVE | | |
| Blank | ND | ND | ND | ND | ND | ND | Average | 102 |
| 0.01 | 96.7 | 98.5 | 104 | 103 | 105 | 95.1 | SD | 11.7 |
| 0.05 | 98.2 | 102 | 97.9 | 99.4 | 100 | 99.5 | UCL | 137 |
| 0.10 | 98.7 | 101 | 97.2 | 98.7 | 99.5 | 90.9 | UWL | 125 |
| 0.20 | 96.0 | 94.5 | 142 | 94.0 | 97.0 | 93.4 | LWL | 78.6 |
| 1.0 | 93.6 | 95.9 | 137 | 98.3 | 101 | 91.3 | LCL | 66.9 |

| Metolachlor OXA | | | | | | | | |
|-----------------|-------|-------|-------|-------|-------|------|---------|------|
| Summary | | | | | | | | |
| Sample | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | AVE | | |
| Blank | ND | ND | ND | ND | ND | ND | Average | 72.0 |
| 0.01 | 65.2 | 68.8 | 62.8 | 60.7 | 67.4 | 65.0 | SD | 3.29 |
| 0.05 | 65.5 | 70.5 | 68.6 | 70.3 | 69.7 | 91.5 | UCL | 81.9 |
| 0.10 | 68.1 | 69.1 | 69.4 | 69.8 | 69.0 | 91.8 | UWL | 78.6 |
| 0.20 | 64.0 | 65.0 | 68.8 | 63.3 | 66.3 | 65.5 | LWL | 65.4 |
| 1.0 | 68.3 | 72.9 | 71.4 | 71.0 | 74.9 | 92.6 | LCL | 62.1 |

| Imidacloprid | | | | | | | | |
|--------------|-------|-------|-------|-------|-------|------|---------|------|
| Summary | | | | | | | | |
| Sample | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | AVE | | |
| Blank | ND | ND | ND | ND | ND | ND | Average | 87.0 |
| 0.01 | 80.1 | 82.6 | 83.1 | 84.3 | 84.6 | 82.9 | SD | 8.85 |
| 0.05 | 85.1 | 87.1 | 82.1 | 85.5 | 84.6 | 91.5 | UCL | 114 |
| 0.10 | 86.3 | 86.6 | 83.5 | 86.8 | 85.0 | 91.8 | UWL | 105 |
| 0.20 | 84.3 | 82.8 | 86.3 | 82.5 | 111 | 89.4 | LWL | 69.3 |
| 1.0 | 82.8 | 87.2 | 85.2 | 86.6 | 120 | 92.6 | LCL | 60.5 |

Appendix 3

Storage Study Summary for S-Metolachlor, Metolachlor ESA and Metolachlor OXA in Soil

| Analyte | Sample | Recovery (%) | | | | | | |
|-----------------|---------------|---------------------|--------------|--------------|--------------|---------------|---------------|---------------|
| | | Day 0 | Day 2 | Day 4 | Day 7 | Day 14 | Day 22 | Day 28 |
| s-Metolachlor | Blank | ND | ND | ND | ND | ND | ND | ND |
| | QC Spike | | 90.4 | 83.6 | 83.4 | 103.0 | 99.2 | 98.9 |
| | Spike 1 | 80.2 | 84.3 | 81.3 | 79.6 | 90.4 | 89.6 | 90.7 |
| | Spike 2 | 78.3 | 82.9 | 78.3 | 86.8 | 86.2 | 90.1 | 93.4 |
| | Spike 3 | 81.1 | 88.3 | 77.3 | 71.4 | 91.4 | 89.3 | 93.4 |
| Metolachlor ESA | Blank | ND | ND | ND | ND | ND | ND | ND |
| | QC Spike | | 94.7 | 88.6 | 90.0 | 104 | 104 | 103 |
| | Spike 1 | 89.2 | 86.4 | 86.8 | 83.9 | 94.9 | 95.0 | 95.9 |
| | Spike 2 | 85 | 87.9 | 86.5 | 93.0 | 91.1 | 96.6 | 97.0 |
| | Spike 3 | 90.4 | 91.7 | 80.3 | 72.9 | 95.4 | 94.3 | 95.5 |
| Metolachlor OXA | Blank | ND | ND | ND | ND | ND | ND | ND |
| | QC Spike | | 93.2 | 87.2 | 88.0 | 104 | 102 | 102 |
| | Spike 1 | 89.2 | 80.1 | 83.6 | 82.4 | 92.7 | 93.7 | 94.9 |
| | Spike 2 | 85.8 | 84.9 | 82.5 | 89.8 | 90.8 | 96.1 | 97.0 |
| | Spike 3 | 92.5 | 90.2 | 75.0 | 68.2 | 93.3 | 93.9 | 95.2 |

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