

**QUALITY ASSURANCE PROJECT  
PLAN SHORT FORM**

**Project Name: Analysis of Aminocyclopyrachlor in Foliage and Soil Samples for  
Oregon Department of Agriculture**

Project Number: **B19-08**

Date QA Project Plan Completed:

Study Director/ Project Leader: **Lynda Podhorniak** *LP* 11/19/18

Analyst(s): **Lynda Podhorniak**

	<u>Reviewed / Approved by</u>	<u>Date</u>
QA Officer:	<b>Paul Golden</b> <i>Paul Golden</i>	<i>11/19/2018</i>
Branch Chief:	<b>Thuy Nguyen</b> <i>Thuy Nguyen</i>	<i>11/19/2018</i>

	<u>Concurrence by</u>	<u>Date</u>
Data user or requestor:	<b>Kathleen Wickman</b> <i>Kathleen Wickman</i> Laboratory Manager Oregon Department of Agriculture	<i>11/19/18</i>

Date Project Started: 11-13-18

Date Project Completed:

## **1. Purpose of Project:**

Trees from four locations within Central Oregon, adjacent to Rights of Ways, are exhibiting herbicide symptoms characteristic of the herbicide aminocyclopyrachlor. It is suspected that aminocyclopyrachlor negatively impacted the health of the trees. Oregon Department of Agriculture is requesting analytical support for the investigation of the dying trees.

### **A. Brief Summary of the Project:**

Impacted tree foliage samples (mostly pines) and soil samples in the vicinity will be collected by the Oregon Department of Agriculture and shipped to Analytical Chemistry Branch (ACB) to be analyzed for the residue of aminocyclopyrachlor. It is anticipated that 25 foliage samples and 15 soils will be collected. ACB will conduct the analysis for aminocyclopyrachlor using liquid chromatography coupled with tandem mass spectroscopy (LC-MS/MS).

### **B. Requestor(s):**

Kathleen Wickman, Laboratory Manager, Oregon Department of Agriculture, Tel: 503-872-6633 [kwickman@oda.state.or.us](mailto:kwickman@oda.state.or.us)

### **C. Requestor's Intended Use of Data:**

The results will support the investigation of the dying trees, possibly related to the use of herbicides containing aminocyclopyrachlor. Conclusions of the investigation may impact the usage restrictions and future label changes for herbicides containing aminocyclopyrachlor.

### **D. Requestor's Data Quality Objectives (DQO):**

1. Use the sample processing and analysis method provided by the Indiana State Chemist laboratory.
2. The target method Limit of Quantitation (LOQ) is 0.01ppm (or lower), and the target Limit of Detection (LOD) is one-third of the LOQ.
3. Any non-conformance that may impact the accuracy and reliability of the results will be documented and addressed in consultation with the requestor.

**2. Sample Acceptance Criteria** [ISO 17025:2017 Sec. 7.4.3 and section 3.1 of SOP ACB-30]):

A physical description of the samples, including its condition upon receipt, will be recorded and kept with the project file. If sample container is broken, or there is sign of spoilage, or other conditions that may affect the integrity of the samples, the customer will be notified.

**3. Methodology:**

See attachments.

Brief Description of Method:

1. Extraction: acetonitrile/formic acid (soil) and acetonitrile/ammonium acetate (foliage).
2. Clean-up: Enviro-Carb SPE
3. Derivatization: none
4. Determination: Agilent 6460 LC-MS/MS or equivalent.

Any deviation from the method will be documented in notebook.

**4. Quality Assurance/ Quality Control:**

- A. Source of Standards [ISO 17025:2017 Sec. 6.6.1]: Pesticide standard from the National Pesticide Standard Repository (NPSR), Fort Meade, MD.
- B. Source of Control Samples: Control (blank) samples have been requested from Region 7.
- C. Method Validation:

A method validation with fortified control samples at multiple levels will be conducted following ACB SOP-030.

- D. Describe the calibration curve criteria (SOP ACB-030):

1. For analyte quantitation, a minimum of a four-point calibration curve, typically between LOD and 100x LOQ, with linear regression will be used. A five-point minimum will be used for quadratic regression. The target correlation coefficient is 0.98 or above.
2. Quantitation will be within (no greater than 10% outside) the curve minimum and maximum concentrations.

E. Calibration Verification Frequency, Acceptance Criteria [ISO 17025:2017 Sec. 6.4.10]:

1. The instrument used in this project (Agilent 6460 LC-MS/MS) is tuned with reference compounds during the annual preventive maintenance performed by the manufacturer according to the acceptance criteria established by the manufacturers.
2. For each sequence of samples, one calibration verification standard (generally a mid-level calibration standard) will be run within an analytical sequence for every 20 samples or less. The relative percent difference (RPD) of the calibration verification standard against the calibration curve should be less than 20%.

F. Number and Concentration of Procedural Standards, Spikes, Matrix Blanks, Procedural Blanks, or other and their Acceptance Criteria:

At a minimum, one procedural blank, one matrix blank (control) and one fortified matrix blank will be analyzed with each sequence of samples. The fortified matrix blank should be chosen to adequately represent each extraction/detection system fortified at a concentration between 2x- 5x of the LOQ. The recoveries of the fortified compound should be within 70% to 120%. The blanks should be free of target analytes at or above LOD.

G. Confirmation Techniques, Acceptance Criteria (SOP ACB-030):

1. Retention time comparison with standards should be within 0.1 minute.
2. Tandem mass spectrometry analysis with two transitions. The ratios of the two transitions should be within 25% at levels above the LOQ.

H. Corrective Actions:

The above stated criteria or that inferred in the ACB SOPs should be used to evaluate and assess the validity of the results. If any of the criteria was not met, the sources or causes should be investigated. The corrective actions may include re-injection of the sample extracts, recalibration the instrument, repair or perform necessary maintenance of the instrument, or re-extraction and reanalysis of the samples. Whether instrument recalibration or reevaluation of all sample results is necessary, the ACB Branch Chief, team leader and/or QA Officer should be consulted with if other remedies do not resolve the problems.

If all common remedies do not resolve the issues, the data should be flagged. All action(s) should be documented and concurred by ACB QA officer or BC.

I. Critical Environmental Conditions [ISO 17025:2017 Sec. 6.3.1]

Samples will be kept frozen at a nominal temperature of at least -30°C, where they are expected to be stable for at least 1 year, until analysis. All the prepared standards will be stored at -10°C or below, and will expire after 1 year.

J. Critical Measurements [ISO 17025:2017 Sec. 6.4.5] (SOP ACB-006 and ACB-029)

The analyst(s) should ensure that analytical balances and pipettes used are calibrated annually to NIST standards. Accuracy of the pipettes will be checked on the day of quantitative use.

**5. Safety Precautions**

General and standard chemical laboratory safety precautions.

**6. Reporting Requirements:**

1. Typically, amounts (ppm, or mg/kg) at or above LOQ will be reported. Amounts between LOD and LOQ will be reported as BLQ (below limit of quantitation).
2. Standard ACB report with a memorandum and table(s) summarizing the analytical results of the samples to the requestor.
3. The report is subject to the standard ACB QAO and Branch Chief reviews (SOP ACB-004).

**7. Disposition of Samples**

Follow Environmental Science Center (ESC)'s standard hazardous waste disposal guidelines unless otherwise directed by the requestor.

**8. References:**

1. SOP ACB-004/current version "Auditing Laboratory Projects".
2. SOP ACB-006/current version "Maintaining Instrument and Balance Logbooks".
3. SOP ACB-029/current version "Calibration of Laboratory Pipettes".
4. SOP ACB-030/current version "Pesticide Residue Chemistry Sample Analysis".
5. ISO/EIC 17025:2017 Third Edition 2017-11 "General Requirements for the Competence of Testing and Calibration Laboratories".

**9. Attachments:**

Analytical Method: Analysis of Aminocyclopyrachlor in Soil and Vegetation Samples Using LC/MS/MS; Office of Indiana State Chemist; TM PR-001 Rev. 0 (2012/08).

B19-09  
D11-18  
EE 10/14/18

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## Analysis of Aminocyclopyrachlor in Soil and Vegetation Samples Using LC/MS/MS



### 1.0 PURPOSE

This test method is for the determination of Aminocyclopyrachlor residue in vegetation or soil.

### 2.0 EQUIPMENT AND SUPPLIES

- 2.1 Waters Aquity TQD UPLC/MS/MS or Waters Quattro Micro UPLC/MS/MS
- 2.2 Analytical balance
- 2.3 Sonicator
- 2.4 Shaker
- 2.5 Centrifuge
- 2.6 Vortex
- 2.7 Nitrogen evaporator
- 2.8 Vacuum manifold for SPE
- 2.9 Supelco Envi-Carb 12cm/1g SPE cartridges
- 2.10 250mL Nalgene bottles
- 2.11 50mL polypropylene centrifuge tubes
- 2.12 15mL polypropylene centrifuge tubes
- 2.13 10mL K-D tubes
- 2.14 6mL Luer lock syringes
- 2.15 Nylon 0.45µm syringe filters
- 2.16 Mechanical pipettes
- 2.17 Disposable transfer pipettes
- 2.18 Autosampler vials with pre-slit caps

### 3.0 CHEMICALS AND REAGENTS

- 3.1 Aminocyclopyrachlor reference standard, EPA# 01045
- 3.2 Methanol, HPLC grade or better
- 3.3 Acetonitrile, HPLC grade or better
- 3.4 Ammonium acetate, HPLC grade
- 3.5 Formic acid, 88% (concentrated)
- 3.6 Hydrochloric acid, 37% (concentrated)
- 3.7 Milli-Q deionized water

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#### 4.0 SOLUTION PREPARATION

Volume of each solution can be adjusted as needed.

##### 4.1 Solvent solutions

4.1.1 0.5% Formic acid: 500 $\mu$ L of concentrated formic acid to 100mL of Milli-Q water

4.1.2 0.2% Formic acid: 200 $\mu$ L of concentrated formic acid to 100mL of Milli-Q water

4.1.3 0.01% Formic acid: 5mL of 0.2% formic acid solution mixed with 95mL of Milli-Q water

4.1.4 70/30 ACN/0.2% formic acid: 700mL of acetonitrile mixed with 300mL of 0.2% formic acid solution

4.1.5 70/30 ACN/150mM ammonium acetate solution: 700mL of acetonitrile mixed with 300 mL of 150mM ammonium acetate solution (3.47 grams of ammonium acetate in 300mL of Milli-Q water)

4.1.6 10 mM Ammonium acetate in methanol: 0.39 grams of ammonium acetate in 500mL of methanol

##### 4.2 Standard solutions

Stock standard solution and intermediate standard solution (if needed) of aminocyclopyrachlor must be prepared in methanol. Calibration standard solutions should be prepared using matching matrix solvent (veg or soil). A typical range of calibration standard solutions is usually 0.1 ng/mL to 100 ng/mL.

##### 4.3 Sample fortification

Targeted on-column concentration is 5 ppb, e.g. spike 100  $\mu$ L of 1 $\mu$ g/mL aminocyclopyrachlor standard to vegetation or soil blank.

#### 5.0 SOIL SAMPLE PREPARATION PROCEDURE (EXTRACTION ONLY)

5.1 Weigh approximately 10 grams of soil into a 250 mL Nalgene bottle.

5.2 Add 50.0 mL of extraction solution (70/30 acetonitrile/0.2% formic acid) into the bottle.

5.3 Sonicate the sample for 10 minutes.

5.4 Shake the sample for 30 minutes.

5.5 Centrifuge the sample at 4000 rpm for 5 minutes.

5.6 Transfer 10.0 mL of supernatant to a 10 mL K-D tube.

5.7 Reduce the sample volume to approximately 1mL using nitrogen evaporator at 40-45°C.

5.8 Add 1mL of methanol to the sample.

5.9 Bring the final sample volume to 4.0 mL with 0.01% formic acid.

5.10 Vortex the solution briefly.

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5.11 Filter the sample solution through a 0.45  $\mu\text{m}$  nylon syringe filter.

5.12 Fill the filtered sample solution in an autosampler vial.

## 6.0 VEGETATION SAMPLE PREPARATION PROCEDURE (EXTRACTION AND CLEAN-UP)

### 6.1 Extraction Procedure

6.1.1 Weigh approximately 10 grams of vegetation into a 250 mL Nalgene bottle.

6.1.2 Add 50 mL of extraction solution (70/30 acetonitrile/150 mM ammonium acetate) into the bottle.

6.1.3 Sonicate the sample for 10 minutes.

6.1.4 Shake the sample for 30 minutes.

6.1.5 Centrifuge the sample at 4000 rpm for 5 minutes.

6.1.6 Decant the supernatant into a 50 mL centrifuge tube, record actual volume of supernatant obtained, e.g. 42.0 mL.

6.1.7 Add additional extraction solution to bring the volume to 50.0 mL.

6.1.8 Centrifuge the sample at 9500 rpm for 5 minutes.

6.1.9 Transfer 10.0 mL of supernatant into a 15 mL centrifuge tube.

6.1.10 Reduce the sample volume to approximately 3 mL using nitrogen evaporator at 40-45°C.

6.1.11 Add 100  $\mu\text{L}$  of concentrated hydrochloric acid and 5 mL of 0.5% formic acid to the sample tube and mix well.

### 6.2 Clean-up (SPE) Procedure

6.2.1 Condition Envi-Carb SPE cartridge with 2 column volume of methanol and 2 column volume of Milli-Q water (do not let the cartridge dry).

6.2.2 Load sample extract (from 6.1.11) to the cartridge.

6.2.3 Rinse the sample tube with 3 mL of Milli-Q water and pour onto the column (wait until extract has almost completely passed through the column).

6.2.4 Place a 10 mL K-D tube containing 50  $\mu\text{L}$  of 0.2% formic acid in it under the cartridge to collect the eluent.

6.2.5 Elute with 10 mL of 10 mM ammonium acetate in methanol into the K-D tube.

6.2.6 Reduce the sample volume to approximately 1mL using nitrogen evaporator at 40-45°C.

6.2.7 Add 1mL of Milli-Q water and bring the sample volume to 4.0mL with 0.01% of formic acid.

6.2.8 Vortex the solution briefly.

6.2.9 Filter the sample solution through a 0.45 $\mu\text{m}$  nylon syringe filter.

6.2.10 Vial the filtered sample solution and run on UPLC/MS/MS.

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## 7.0 UPLC/MS/MS ANALYSIS

### 7.1 UPLC Parameters

- 7.1.1 Column: Waters Aquity UPLC HSS T3, 100mm x 2.1mm x 1.8 $\mu$ m  
 7.1.2 Mobile phase A: 0.1% formic acid in Milli-Q water  
 7.1.3 Mobile phase B: 0.1% formic acid in methanol  
 7.1.4 Gradient profile

Time (min)	Flow Rate (mL/min)	%A	%B
Initial	0.40	93.0	7.0
3.00	0.40	40.0	60.0
4.00	0.40	10.0	90.0
4.10	0.40	93.0	7.0
5.00	0.40	93.0	7.0

### 7.2 MS Parameters

- 7.2.1 ES(+)  
 7.2.2 Parent ion: 214 (exact mass is dependent on instrument)  
 7.2.3 Product ions: (exact mass is dependent on instrument)  
     214 > 68  
     214 > 168  
     214 > 101

## 8.0 NOTATIONS

A blank matrix sample (vegetation or soil), a method blank sample (without matrix) and two fortified matrix samples (vegetation or soil) should be prepared along with the vegetation or soil samples.

## 9.0 REFERENCES

- 9.1 Minnesota Department of Agriculture, Laboratory Service Division, Document No. EA-WI-003, Original Revision, "Work Instructions for Aminocyclopyrachlor in Soil".  
 9.2 Minnesota Department of Agriculture, Laboratory Service Division, Document No. EA-WI-004, Original Revision, "Work Instructions for Aminocyclopyrachlor in Vegetation".  
 9.3 Du-pont-22582, Supplement No. 1, Revision No. 2, "Analytical Method for the Determination of DPX-KJM44, DPX-MAT28, IN-LXT69, IN-QFH57, and IN-QGC48 in Grass Forage and Grass Hay using LC/MS/MS".

**ATTACHMENT 2 (SOP No. 008, Revision 2.1)**

**QUALITY ASSURANCE PROJECT PLAN  
Permission to start**

Date: 11/13/2018

Project ID and Description: B19-08

QA Project Plan submitted: Currently being drafted.

Analysts:

Lynda Podhomiak Chemist, ACB

Preliminary work (instrument tuning, preliminary linearity studies, standard retention times, and analysis of blanks) can begin on this project while waiting for the final approval of the QAPP.

Paul Golden, Quality Assurance Officer, ACB

*Paul Golden 11/13/2018*