Analysis of Aminopyralid in Groundwater by Liquid Chromatography Triple Quadrupole Mass Spectrometry

1. Scope:

This Section Method provides a stepwise procedure for the analysis of aminopyralid in groundwater. The objective of this standard operating procedure is to quantify the concentration of aminopyralid using liquid chromatography-mass spectroscopy (LC-MS/MS). The reporting limit for Aminopyralid is 0.05 ng/mL.

2. Principle:

Aminopyralid is an acidic pesticide and is analyzed in a groundwater matrix using LC-MS/MS. In this direct analysis, LC-MS/MS provides a separation and quantitation technique for Aminopyralid without derivatization and preparation of complex mobile phases.

3. Safety:

3.1 Read the Safety Data Sheet for all materials before use.

3.2 Aminopyralid is classified as category I [DANGER].

The analyst should avoid all contact with, and inhalation of the materials containing pesticides and wear appropriate protective clothing as well as safety glasses and chemical resistant gloves when handling the samples.

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

3.4 All flammable solvents should be used and handled with care in a ventilated area.

3.5 Special storage, use, handling and disposal procedures are necessary to ensure the safety for using compressed gases.

4. Interference:

There were matrix interferences for aminopyralid when using Colfax background groundwater at the time of method development; however, these interferences were resolved using matrix-matched calibration standards.

5. Apparatus and Equipment:

5.1 A Shimadzu LC system comprising of a system controller, pumps, degasser, autosampler and column oven coupled to an AB Sciex 6500+ QTRAP mass spectrometer with Turbo V-Source, ESI probe, Varian vacuum pump, and Windows 10 Analyst 1.7.2 PC workstation (or equivalent).

5.2 Chromatographic separations: Luna Omega Polar C18 column (3 µm particle size, 100 x 3.00 mm, Phenomenex), Part# 00D-4760-Y0 (or equivalent)

5.3 Vortex-vibrating mixer

5.4 Micropipettes, adjustable, recommended sizes as follows: 10 µL, 200 µL, 1000 µL, and 10 mL

6. Reagents and Supplies:

6.1 Water, Optima LC/MS grade or higher purity

6.2 Methanol, Optima LC/MS grade or higher purity

6.3 Acetonitrile, Optima LC/MS grade or higher purity

6.4 Formic acid, HPLC grade or higher purity

6.5 Syringe filter, 25 mm, with PTFE membrane, 0.45 µm, Pall, Part# AP-4501

6.6 Syringe for single use, 10 mL, Henke-Ject (or equivalent)

7. Standards Preparation:

7.1 Aminopyralid CAS# 150114-71-9

7.2 5-Amino-2-chlorobenzoic acid CAS# 89-54-3

7.3 Aminopyralid is purchased from AccuStandard (or other ISO 17034 accredited source) at a concentration of 100 µg/mL in methanol and diluted to 1.0 µg/mL (intermediate standard solution 1) with methanol. The 1.0 µg/mL standard is used to dilute to 0.01 µg/mL (intermediate standard solution 2), and 0.01 µg/mL is used to dilute to 0.001 µg/mL (intermediate standard solution 3), respectively.

Note: See standard preparation sheet.

7.4 5-Amino-2-chlorobenzoic acid is purchased from Sigma-Aldrich (or other ISO 17034 accredited source) and is used as an internal standard. A concentration of 1.0 mg/ml in methanol is prepared in the Standard Repository Laboratory. The 1.0 mg/mL standard is diluted to 10.0 µg/mL. The 10.0 µg/mL is then diluted to 50.0 ng/mL. A 10 µL internal standard with final concentration of 0.5 ng/mL is used for all calibration points, matrix spike and samples.

Note: See standard preparation sheet.

7.5 Calibration standards (working standard solutions) are prepared at the following concentrations: 5.0, 2.0, 1.0, 0.5, 0.2, 0.1, 0.05 and 0.04 ng/mL in 10:90 ACN:background groundwater. The calibration standards should be prepared freshly every time before use.

Note: See standard preparation sheet.

7.6 The expiration date of each intermediate standard solution is six months from the preparation date.

7.7 Keep all working standard solutions in the freezer at -20°C. After removing from the freezer, allow the working standard solutions to thaw at ambient temperature before further dilutions are made.

Mobile Phase Preparation:

7.8 Aqueous Solution: 0.1% Formic acid in water: For 1000 mL, mix 999 mL water, and 1 mL formic acid.

7.9 Organic Solution: 0.1% Formic acid in acetonitrile: For 1000 mL, mix 999 mL acetonitrile, and 1 mL formic acid.

8. Sample Preservation and Storage:

Store all samples in a refrigerator at 0 – 4°C. The holding time for extraction of groundwater samples is 28 days from the date of sample collection. The holding time is based on a 28-day storage stability study where no degradation for aminopyralid was observed through day 28. See Appendix III.

9. Test Sample Preparation:

9.1 Background Preparation:

The Department of Pesticide Regulations provides a clean background groundwater for matrix blank and spikes.

9. 2 Preparation of Blanks and Spike:

9.2.1 Reagent Blank: Transfer 1000 µL of 10:90 ACN:background groundwater into an autosampler vial for analysis.

9.2.2 Matrix Blank: Filter a 10 mL background groundwater sample through a 0.45 µm Nylon filter into a 10 mL centrifuge tube. Wash the filter with 0.85 mL of acetonitrile and add it directly into the filtered sample. Transfer 1000 µL of the filtered sample into an autosampler vial for analysis.

9.2.3 Matrix Spike: Prepare a 0.5 ng/mL Aminopyralid matrix spike sample in background groundwater. After spiking, shake and filter the sample through a 0.45 µm Nylon filter into a 10 mL centrifuge tube. Wash the filter with 0.85 mL of acetonitrile and add it directly into the filtered sample. Transfer 990 µL of the filtered sample into an autosampler vial and add 10 µL internal standard for a final concentration of 0.5 ng/mL.

9.3 Test Sample Extraction

9.3.1 Remove samples from the refrigerator and allow them to reach ambient temperature.

9.3.2 Shake the sample and then transfer approximately 10 mL into a 10 mL plastic centrifuge tube and filter through a 0.45 µm Nylon filter into another 10 mL centrifuge tube.

9.3.3 Rinse the filter with 0.85 mL of acetonitrile and add directly into the filtered sample.

9.3.4 Add 10 µL internal standard (with final concentration of 0.5 ng/mL) to 990 µL filtered sample into an autosampler vial for analysis by LC-MS/MS.

10. Instrumental Analysis:

10.1 Instrument Calibration

10.1.1 The calibration standard curve consists of a minimum of five levels for a quadratic curve or three levels for a linear curve. The lowest level must be at or below the corresponding reporting limit. The current working standard levels are 5.0, 2.0, 1.0, 0.5, 0.2, 0.1, 0.05 and 0.04 ng/mL.

10.1.2 Calibration is obtained using a quadratic or linear regression with the correlation coefficient (r) equal to or greater than 0.995.

10.2 Sequence Arrangement

The LC-MS/MS needs to be conditioned with standards or sample extracts 2 to 3 times before running the following recommended sequence:

- A set of calibration standards (8 levels)
- Reagent blank
- Matrix blank
- Reagent blank
- Matrix spike
- A set of up to 12 test samples
- A set of 2 reagent blanks; and
- A set of 3 continuing calibration verifications

10.3 Instrument Conditions

10.3.1 LC Separation Conditions

A Shimadzu LC30 liquid chromatograph is equipped with Phenomenex Luna Omega 3µm Polar C-18 100 x 3.0mm column. Samples are eluted using a gradient system at a flow rate of 0.4 mL/min throughout the 7-minute run-time at 40° C with an injection volume of 15 µL. See Table 1.

Table 1. LC Gradient Parameters

Step	Time	Mobile Phase A (%)	Mobile Phase B (%)
0	0.5	98	2
1	3.0	2	98
2	4.0	2	98
3	4.1	90	10
4	7.0	0	0

10.3.2 Mass Spectrometer Conditions

To achieve a mass spectrum, an AB Sciex Triple Quad 6500+ mass spectrometer with an ESI interface is used. See Table 2 for mass spectrometer parameters in positive mode. The mass spectrometer operates in positive scheduled Multiple Reaction Monitoring (MRM) mode as described in Table 3. Quantitation ions are bolded.

Table 2. Mass Spectrometer Operating Parameters

Parameter	Setting
Ion Mode	Positive
Curtain Gas	30
Ion Spray Voltage	5500
Temperature	500
Ion Source Gas 1	60
Ion Source Gas 2	60
Collision Gas	12
Electron Multiplier	1500
MRM Detection Window (sec)	90

Table 3. MRM Parameters for Detection of Aminopyralid and 5-Amino-2-chlorobenzoic Acid

Compound	RT	Precursor Ion	Product Ion[1]	Declustering Potential	Collision Energy	Entrance Potential	Exit Potential
Aminopyralid	3.0	207.1 207.1	**160.9** 134.1	45 45	28 42	10 10	10 10
5-Amino-2-chlorobenzoic acid (Internal Standard)	3.10	172.0	**93.0**	45	47	10	10

[1] Quantitation ions are bolded.

11. Quality Control:

11.1 Method Detection Limit

Method detection limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 background groundwater samples are spiked at 0.10 ng/mL for each analyte along with a blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL using this equation:

$$\mathrm{MDL} = \mathrm{tS}$$

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 replicates used to determine the MDL, t=3.143. An MDL of 0.02 ng/mL was established for this method. Trace will be reported when results fall within this MDL and the Reporting Limit. The results for the standard deviation and MDL are in Appendix I.

11.2 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 client requested RL using this method is 0.05 ng/mL for Aminopyralid.

11.3 Method Validation

The method validation consisted of five sample sets. Each set included five levels of fortification and a matrix blank. All spikes and matrix blanks

were processed through the entire analytical method (Section 9.3). Spike levels and recoveries are shown in Appendix II.

11.4 Control Charts and Limits

A control chart was generated using the data from the method validation for this analyte. The upper and lower control limits are set at ± 3 standard deviations of the percent of recovery, shown in Appendix II.

11.5 Acceptance Criteria

11.5.1 Each set of samples will have one matrix blank sample and one matrix spike sample.

11.5.2 The retention time should be within ± 0.1 minute of that of the standard.

11.5.3 The recovery of the matrix spike shall be within the control limits. See Appendix II.

When spike recoveries fall outside the limits, the analyst must investigate the cause. Samples within any bracket that contains non-conforming matrix spikes are re-analyzed. If the spike fails again, then the bracketed samples must be re-extracted and re-analyzed. If the spike recoveries fall within the limits, then the results from the re-analyzed samples can be reported.

11.5.4 The relative abundances of structurally significant ions used for confirmation must be within ± 30% when compared to a standard injection during the same run.

11.5.5 The sample should be diluted if results fall outside of the calibration curve. Analytical solution concentrations must be less than the highest standard concentration. Dilute with HPLC grade water if necessary; diluting does not change the reporting limit or method detection limit. It also reduces matrix interferences in samples with a high matrix effect.

11.5.6 Analyze a reagent blank to demonstrate that the system is clean and free of interferences. A HPLC grade water free of pesticides is injected to check for carryover.

12. Calculations:

Quantitation is based on an external standard calculation using either the peak area or height. The Linear Ion Trap Quadruple LC-MS/MS software uses

quadratic or linear curve fits. Alternatively, at the chemist's discretion, concentrations may be calculated using the response factor for the standard whose value is < 30% to the level in the sample.

$$\text{ppb} = \frac{(\text{sample peak area or ht})(\text{STD conc})}{(\text{STD peak area or ht})}$$

13. Reporting Procedure:

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

14. Discussion:

14.1 The Department of Pesticide Regulations requested an analytical method for Aminopyralid in groundwater. Aminopyralid is analyzed by LC/MS-MS with no sample preparation.

14.2 The Aminopyralid standard and internal standard are prepared in methanol. Stock solution standards are stored in the refrigerator and working standards are stored in the freezer.

14.3 There were matrix interferences for Aminopyralid using Colfax background groundwater at the time of method development, which was resolved using matrix-matched calibration standards. Adding 5-amino-2-chlorobenzoic acid as an internal standard and acetonitrile as a washing solution normalizes response data for potential matrix effects in real samples with matrix interferences.

14.4 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 in amber glass bottle. Three glass bottles containing background groundwater were spiked and stored in the refrigerator until analyzed on day 0, 2, 3, 8, 14, 20 and 28. Along with the storage spikes, a matrix blank and a method control spike were also extracted. This storage stability study showed no degradation for Aminopyralid until day 28. The results are shown in Appendix III.

14.5 5-amino-2-chlorobenzoic acid is used as an internal standard. The injection procedure and analyte performance are monitored throughout the entire analytical method using a 0.5 ppb internal standard in each sample.

14.6 The segment durations in the mass spectrometer settings determine the retention time windows for each analyte. As the HPLC column performance may change over time because of irreversible contamination,

phase stripping, etc., it may be necessary to adjust these windows before beginning a sequence for the observed retention times of the analytes.

Installation of a new analytical column may also necessitate adjustments of window times. The retention time windows should be verified before each sequence and adjusted as necessary.

15. References:

15.1 Baumhover, N. J., Larabee-Zierath, D., Vargo, J. D., Spak, D. R., Netzband, N, Dai, S. Y., JRS. 2018, 6, 2, 1-7.

15.2 Sack, Ch., Vonderbrink, J., Smoker, M., Smith, R., E, J. Agric. Food chem. J. 2015, 63, 43, 9657-9665.

Appendix I

Calculated MDL for Aminopyralid (ng/mL)

Compound	Spike (ng/mL)	Spike 1	Spike 2	Spike 3	Spike 4	Spike 5	Spike 6	Spike 7	SD	MDL
Aminopyralid	**0.1**	0.105	0.100	0.106	0.0952	0.101	0.107	0.0928	0.00539	0.0169

Appendix II

Method Validation Study for Aminopyralid in Groundwater

Compound	**Spike Level (ng/mL)**						**Control Limit (%)**	
Aminopyralid	**Set #**	**0.1**	**0.25**	**0.5**	**1.25**	**2.5**		
	1	86.3	91.2	98.7	107	107.4	**Mean**	98.5
	2	98.7	103.6	100.2	112.8	115.6	**SD**	9.5
	3	86.0	94.0	105.8	109.9	103.9	**RSD**	9.8
	4	104.8	95.2	80.1	108.3	95.6	**UCL**	126.9
	5	94.7	84.4	95.0	85.6	96.4	**LCL**	70.0

Appendix III

Storage Stability Study for Aminopyralid in Groundwater

Compound	**Recovery (%)**							
Aminopyralid	**Spike (ng/mL)**	**Day 0**	**Day 2**	**Day 3**	**Day 8**	**Day 14**	**Day 20**	**Day 28**
	blk	ND	ND	ND	ND	ND	ND	ND
	QC Spk	104.8	94.7	82.7	108.4	95.9	83.0	99.1
	Spk 1	99.8	99.3	90.0	101.5	97.0	104.3	102.6
	Spk 2	105.8	89.3	96.6	95.6	96.4	101.8	101.7
	Spk 3	105.5	96.9	102.0	99.8	101.0	98.9	102.3

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