

Analytical method for aminopyralid in water by liquid chromatography with tandem mass spectrometry detection (Method GRM 01.32)

Reports: ECM: MRID 46235601. Hastings, M. (2003) Method Validation Report for the Determination of Residues of Aminopyralid in Water by Liquid Chromatography with Tandem Mass Spectrometry Detection Using Dow AgroSciences Method GRM 01.32. Project Number: 011159. Unpublished study prepared by Dow AgroSciences LLC. 48 p.

ILV: MRID 46235713. Reed, R (2004) Independent Laboratory Validation of Dow AgroSciences Method GRM 01.32 - Determination of Residues of Aminopyralid in Water by Liquid Chromatography with Tandem Mass Spectrometry. Project Number: 030039, ML03/1111/DOW. Unpublished study prepared by Morse Laboratories. 113 p

Document No.: MRIDs 46235601 & 46235713

Guideline: 850.6100 Section 8.2.2.3 water

Statements: Method validations were conducted in compliance with FIFRA GLP standards. Signed and dated Data Confidentiality, GLP Compliance, Quality Assurance, and Authenticity Certification statements were provided for the method and its ILV.

Classification: This analytical method is classified as **acceptable**. This method may be used to measure aminopyralid in water with an LOQ of 0.05 µg/L and an LOD of 0.01 µg/L.

PC Code: 005100

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Executive Summary

This analytical method, MRID 46235601, is designed for the quantitative determination of aminopyralid in tap, surface, and ground water using liquid chromatograph (LC) with tandem mass spectrometry (MS/MS) detection (see **Table 1**). The method was validated for a concentration range of 0.05 to 5.0 µg/L. The limit of quantitation (LOQ) is 0.05 µg/L. The LOQ is less than the lowest toxicological level of concern in water.¹ There were no major issues with the Independent Laboratory Validation (ILV).

¹ Target LOQ for irrigation water in mg/L = Toxicity Endpoint for terrestrial plant lb a.i./Acre × (453,592 mg/lb) × (Acre-in/102,790 L water). The lowest terrestrial plant toxicity endpoint reported is a No Observable Adverse Effect Concentration of 0.0004 lb a.i./A for soybean(*Glycine max*) (MRID 462358-25).

Table 1. Analytical Method Summary

| Analyte(s) | MRID | | EPA Review | Matrix | Method Date | Registrant | Analysis | Limit of Quantitation (LOQ) |
|--------------|----------|----------|------------|--------|-------------|-----------------|----------|-----------------------------|
| | ECM | ILV | | | | | | |
| Aminopyralid | 46235601 | 46235713 | | Water | 12/9/2003 | Dow Agrosiences | LC/MS/MS | 0.05 µg/L |

ECM= Environmental Chemistry Method; ILV= Independent Laboratory Validation

I. Principle of the Method

Fifty µL of sulfuric acid is added to a 10 mL water sample. Next a Phenomenex Strata X SPE cartridge is prepared by adding 3-mL methanol, followed by 3- mL water/concentrated sulfuric acid (99.5:0.5). Then the cartridge is dried under full vacuum. Finally, the water sample is pulled through the cartridge at approximately 2 mL/min. The vial is rinsed with 2-mL of water/methanol/formic acid (90:10:1) and pulled through the cartridge. The cartridge is dried with vacuum. Finally, aminopyralid is eluted from the SPE cartridge at approximately 1 mL/min with three 2.0-mL aliquots of methyl *tert*-butyl ether/methanol (90:10). An aliquot of an aminopyralid stable isotope solution is added to the sample and the eluate is evaporated to dryness. The residue is reconstituted in an acetonitrile:pyridine:butanol solution (22:2:1) and the sample and standards are derivatized with butyl chloroformate to aminopyralid 1-butyl ester. The sample and standards are then diluted with a methanol:water: acetic acid mobile phase (50:50:0.1) and analyzed by high performance liquid chromatography (HPLC) with positive-ion electrospray (ESI) tandem mass spectrometry (MS/MS). The method measures the amount of aminopyralid 1-butyl ester.

II. Recovery Findings

Mean recoveries and relative standard deviations (RSD) were within guideline requirements in the method validation and the ILV (mean 70-120%; RSD ≤20%), except for one sample where 121% recovery was obtained. The method was quantitative for aminopyralid and the LOQ and LOD appropriate.

Table 2. Initial Validation Method Recoveries for Analytes in Aminopyralid

| Matrix | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---------------|----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| Tap Water | 0.05 | 6 | 98-106 | 101 | 2.8 | 2.8 |
| | 0.50 | 6 | 101-106 | 103 | 1.9 | 1.8 |
| | 5.00 | 6 | 93-96 | 95 | 1.0 | 1.1 |
| Groundwater | 0.05 | 6 | 101-106 | 104 | 1.8 | 1.8 |
| | 0.50 | 6 | 102-106 | 104 | 1.7 | 1.6 |
| | 5.00 | 6 | 95-101 | 97 | 2.4 | 2.5 |
| Surface Water | 0.05 | 6 | 98-101 | 100 | 1.2 | 1.2 |
| | 0.50 | 6 | 102-104 | 103 | 0.9 | 0.9 |
| | 5.00 | 6 | 92-94 | 93 | 0.6 | 0.7 |

Table 3. Independent Validation Method Recoveries for Analytes in River Water

| Analyte | Fortification Level (units) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|--------------|-----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| Aminopyralid | 0.05 | 5 | 109-121 | 113 | 9.4 | 8.3 |
| | 0.50 | 5 | 110-113 | 112 | 2.7 | 2.4 |

III. Method Characteristics

The LOQ was calculated using the standard deviation from the 0.05 µg/L recovery results. The LOQ was calculated as ten times the standard deviation and the limit of detection (LOD) was calculated as three times the standard deviation of the results of the analysis of six samples. The report provided calculated LOQ and LOD (*i.e.*, those based on the standard calculations stated above) and an overall method LOQ and LOD (the method to get the overall method LOQ and LOD was not reported). The calculated LOQs ranged from 0.0087 to 0.0137 µg/L and are all lower than the overall method reported LOQ of 0.05 µg/L. The calculated LOD ranged from 0.0017 to 0.0041, which support an LOD of 0.01 µg/L. The percent recoveries results and calculated LOD and LOQ reported are consistent with the overall method LOD and LOQ.

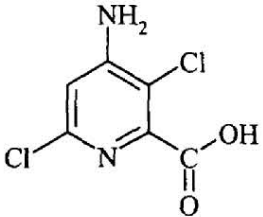
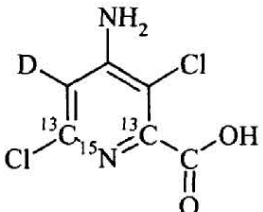
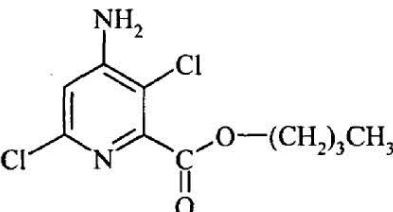
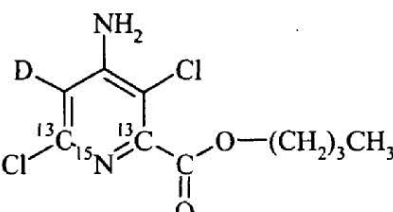
Table 4. Method Characteristics for Aminopyralid Detections using HPLC/MS/MS

| | Tap Water | Groundwater | Surface Water |
|---|---------------------------------|---------------------------------|---------------------------------|
| Limit of Quantitation (LOQ) | 0.0137 µg/L | 0.0087 µg/L | 0.0055 µg/L |
| Limit of Detection (LOD) | 0.0041 µg/L | 0.0026 µg/L | 0.0017 µg/L |
| Linearity (calibration curve r^2 and concentration range) | $r^2 > 0.9997$ 0.05 – 5 µg/L | $r^2 > 0.9997$ 0.05 – 5 µg/L | $r^2 > 0.9997$ 0.05 – 5 µg/L |
| Repeatable | Yes | Yes | Yes |
| Reproducible | Yes | Yes | Yes |
| Specific | Yes | Yes | Yes |

IV. Method Deficiencies and Reviewer's Comments

There were no major deficiencies in the study. The recovery of one sample from the ILV was 121%. This only occurred in one sample and is not considered a major deficiency.

Structure and Chemical Information for Aminopyralid

| Common Name of Compound | Structure and CAS Name |
|---|--|
| <p>Aminopyralid</p> <p>Molecular Formula: $C_6H_4Cl_2N_2O_2$</p> <p>Formula Weight 207.02</p> <p>Nominal Mass: 206</p> <p>CAS Number: 150114-71-9</p> |  <p>4-amino-3,6-dichloro-2-pyridinecarboxylic acid</p> |
| <p>$^{13}C_2^2H^{15}N$-Aminopyralid</p> <p>Molecular Formula: $^{13}C_2C_4^2HH_3Cl_2^{15}NNO_2$</p> <p>Formula Weight 211.00</p> <p>Nominal Mass: 210</p> <p>CAS Number: not available</p> |  <p>4-amino-3,6-dichloro-2-pyridinecarboxylic acid-1-^{15}N-2,6-^{13}C-5-<i>d</i></p> |
| <p>Aminopyralid 1-Butyl Ester</p> <p>Molecular Formula: $C_{10}H_{12}Cl_2N_2O_2$</p> <p>Formula Weight 263.12</p> <p>Nominal Mass: 262</p> <p>CAS Number: not available</p> |  <p>4-amino-3,6-dichloro-2-pyridinecarboxylic acid, 1-butyl ester</p> |
| <p>$^{13}C_2^2H^{15}N$-Aminopyralid 1-Butyl Ester</p> <p>Molecular Formula: $^{13}C_2C_8^2HH_{11}Cl_2^{15}NNO_2$</p> <p>Formula Weight 267.11</p> <p>Nominal Mass: 266</p> <p>CAS Number: not available</p> |  <p>4-amino-3,6-dichloro-2-pyridinecarboxylic acid-1-^{15}N-2,6-^{13}C-5-<i>d</i>, 1-butyl ester</p> |