

Analytical method for aminopyralid in water (Method GRM 07.10) and soil (GRM 07.09) by liquid chromatography with tandem mass spectrometry detection

Reports: ECM: MRID 47572602. Wendelburg, B. (2008) Validation Report for Methods GRM 07.07 - Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometric Detection, GRM 07.08 - Determination of Residues of Aminopyralid in Bovine and Poultry Tissues, Milk, and Eggs by Liquid Chromatography with Tandem Mass Spectrometric Detection, **GRM 07.09 - Determination of Residues of Aminopyralid in Soil by Liquid Chromatography with Tandem Mass Spectrometric Detection**, and **GRM 07.10 - Determination of Residues of Aminopyralid in Drinking Water, Ground Water, and Surface Water by Liquid Chromatography with Tandem Mass Spectrometric Detection. Project Number: 071121. Unpublished study prepared by Dow AgroSciences, LLC. 207 p.**

ILV for soil and water: MRID 48939201. D.K. Moore, A.C. Newcombe, and P.L. Havens. Dissipation of Aminopyralid in Flowing Water Systems Under Operational Conditions. Study completion: October 12, 2012; study identification number 110423. Performing laboratories: ARCADIS US, Inc., Dow AgroSciences LLC, AGVISE Laboratories, and Covance Laboratories Inc.

ILV: MRID 48939202. A.C. Newcombe, T. Negley and P.L. Havens. Dissipation and Aquatic Organism Residue of Aminopyralid in Small Ponds. Study completion: June 21, 2012; study identification number 101046. Performing laboratories: ARCADIS US, Inc., Dow AgroSciences LLC, and AGVISE Laboratories.

ECM similar to ECM in water: 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 Unpublished study prepared by Dow AgroSciences LLC. 48 p.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 for MRID 46235601: 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 Unpublished study prepared by Morse Laboratories. 113 p.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 47572602, 48939201, 48939202, 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.

Classification: The analytical method for water is classified as **acceptable**. This method may be used to measure aminopyralid in water with a limit of quantitation (LOQ) of 0.05 µg/L and a Limit of Detection (LOD) of 0.015 µg/L.

The analytical method for soil is classified **acceptable**. This method may be used to measure aminopyralid in soil with an LOQ of 0.001 µg/g (1 µg/kg) and an LOD of 0.0001 µg/g (0.4 µg/kg):

PC Code: 005100

Reviewer: Katrina White, Ph.D.
Biologist

Katrina White 1/2/14

Secondary Reviewer: Jim Carleton, Ph.D.
Senior Scientist

Jim Carleton 1/2/14

Executive Summary

This analytical method, MRID 47572602, is designed for the quantitative determination of aminopyralid in tap, surface, and ground water (GRM 07.10) and soil (GRM 07.09) using liquid chromatograph (LC) with tandem mass spectrometry (MS) detection (see Table 1). The MRID also provides methods for measuring aminopyralid in Agricultural Commodities and Bovine and Poultry Tissues, Milk, and Eggs; however, these methods are not reviewed in this document. Formal independent laboratory validations were not provided; however, they were conducted as part of the aquatic field dissipation studies (MRID 48939201). Information on method validation is also available in MRID 48939202; however, the validation was performed in the same lab where the method was developed. Those results are not summarized here.

Water

The method for water 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 This method did not have an ILV submitted. However, an ECM (MRID 46235601) and ILV (MRID 46235713) were submitted for a method that is essentially the same except for the final step. In MRID 46235601, in the final step of the procedure the sample is diluted with methanol:water:acetic acid (50:50:0.1) before analyzing with high-performance liquid chromatography with mass spectrometry (HPLC/MS/MS). The corresponding step in MRID 47572602 dilutes with 4790 µL methanol/water (40:60) solution containing 0.05% formic acid and 5 mM ammonium formate to the sample vials. This step is not expected to significantly change the method because it occurs after the extraction from water is completed. Additionally, validation of the method was conducted in support of the aquatic field dissipation study (MRID

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).

48939201). Therefore, an additional ILV is not needed. For the validation in the field dissipation study, an individual recovery was low (68%) and an individual recovery was high (119%) resulting in a high RSD of 27%. Guideline recommendations recommend that %RSDs should be less than 20%. Overall the method was acceptable.

Soil

The method for soil was validated for a concentration range of 0.001 to 0.20 µg/g. The limit of quantitation (LOQ) is 0.001 µg/g (1 µg/kg). The LOQ is less than the lowest toxicological level of concern in soil.² A formal ILV was not submitted in support of the method; however, a validation was performed in support of the aquatic field dissipation study for soil and sediment. Mean recoveries were all within guideline recommendations of 70-120% above the LOQ. There were some individual recoveries that were below 70% (65 and 69%). The %RSD was also high at 0.1 µg/g (%RSD=23%) where it is recommended that the %RSD be below 20%. All mean recoveries were within range for the method report (MRID 47572602). Overall the method is acceptable.

Table 1. Analytical Method Summary

Matrix	MRID		EPA Review	Analyte	Method	Registrant	Analys	Limit of Quantitation (LOQ)
	ECM	ILV						
Water	47572602	See discussion above		Aminopyralid	02/28/2008	Dow Agrosiences	HPLC/MS/MS	0.05 µg/L
Soil	47572602	None submitted						1 µg/kg

ECM=Environmental Chemistry Method; ILV= Independent Laboratory Validation

I. Principle of the Method

Water

Fifty µL of sulfuric acid was added to a 10-mL water sample. Next a Phenomenex Strata X SPE cartridge was prepared by adding 3-mL methanol, followed by 3-mL water/concentrated sulfuric acid (99.5:0.5). Then the cartridge was dried under full vacuum. Finally, the water sample was pulled through the cartridge at approximately 2 mL/min. The vial was rinsed with 2-mL of water/methanol/formic acid (90:10:1) and pulled through the cartridge. The cartridge was dried with vacuum. Finally, aminopyralid was eluted from the SPE cartridge at approximately 1mL/min with three 2.0-mL aliquots of methyl *tert*-butyl ether/methanol (90:10). An aliquot of an aminopyralid stable isotope (¹³C₂²H¹⁵N-aminopyralid) 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. The

² The target LOQ is based on the lowest measured terrestrial plant endpoint of 0.0004 lbs a.i./A (equall to 2 µg/kg-soil) assuming a 1.5 g/mL soil density and six-inch soil depth (MRID 46235825). See calculations recommended in the Environmental Chemistry Method Guidance (USEPA, 2012).

sample is then vortexed for five minutes. The sample is then diluted with 4790 μL methanol/water (40:60) solution containing 0.05% formic acid and 5 mM ammonium formate to the sample vials. Finally, the samples were analyzed by high performance liquid chromatography with positive-ion electrospray (ESI) tandem mass spectrometry (HPLC/MS/MS).

Soil

Soil samples were shaken in methanol/2 N sodium hydroxide (90:10) solution. After centrifugation, the solution was poured off and the extraction was performed again. The second extraction solution was then combined with the first and brought to a fixed volume. An aliquot of the solutions was then evaporated to dryness and reconstituted in water. The sample was then purified using a mixed-mode polymeric anion-exchange solid-phase extraction (Cartridge). After elution from the SPE cartridge with an ethyl/acetate/trifluoroacetic acid (99:1) solution, a stable-isotope labelled internal standard ($^{13}\text{C}_2\text{H}^{15}\text{N}$ -aminopyralid) was added, and the eluate was then evaporated to dryness. The residue was reconstituted in an acetonitrile/pyridine/1-butanol (22:2:1) solution, and derivitized with butyl chloroformate to for the 1-butyl esters (1-BE) of the analyte and internal standard. After derivatization, the mixture was diluted with methanol/water (40:60) with 0.5% formic acid and 5 mM ammonium formate solution and then analyzed by HPLC/MS/MS.

II. Recovery Findings

Water

For the initial validation of the method, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD $\leq 20\%$). For the ILV, mean percent recoveries were not within the recommended 70 to 120% at the LOD. Reliable quantitative results are not expected below the LOQ. At the LOQ, one recovery was low (68%) and one recovery was high (119%) resulting in a high RSD of 27%. While there were a couple of samples out of range the rest of the samples had acceptable recoveries and the samples that were out of range were only slightly out of range (68% and 119% with %RSD of 27%). The method was quantitative for aminopyralid and the LOQ and LOD appropriate.

Table 2. Initial Validation Method Recoveries for Aminopyralid in Water

Matrix	Fortification Level ($\mu\text{g/L}$)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Tap Water	0.05	6	91-101	95	4.3	4.5
	0.50	5	96-99	97	1.3	1.3
	0.05 – 5.0	11	96	95	3.4	3.5
Groundwater	0.05	6	89-99	93	4.0	4.3
	0.50	5	93-101	97	3.6	3.8
	0.05 - 5.0	11	89-101	94	4.1	4.4
Surface Water	0.05	7	83-105	94	8.7	9.2
	5.0	6	88-101	97	4.5	4.7
	0.05 – 5.0	13	83-105	96	7.0	7.3

Table 3. Independent Validation Method Recoveries for Analytes in Water (MRID 48939201)

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Aminopyralid	0.015	22	22-161	88	53	60
	0.05	22	68-119	92	27	30
	5.0	22	72-101	89	14	15

Soil

For the initial validation of the method (MRID 47572602), mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%). Some individual recoveries were 65%. For the ILV, mean recoveries were all within guideline recommendations for the fortification levels above the LOQ. There was an individual recovery at 69%. The %RSD was also high at 0.1 µg/g (%RSD=23%). It is recommended that the %RSD be below 20%. The method was quantitative for aminopyralid and the LOQ and LOD appropriate.

Table 2. Initial Validation Method Recoveries for Analytes in Aminopyralid (MRID 4757202)

Matrix	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil	0.001	21	65-82	73	3.6	4.9
	0.2	18	74-85	81	3.2	3.9
	0.001-0.2	39	65-85	77	5.4	7.1

Table 3. Independent Validation Method Recoveries for Aminopyralid in Soil and Sediment (MRID 48939201)

Matrix	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil	0.0003	4	84-177	126	48	38
	0.001	4	75-89	84	6	7
	0.01	1	71	--	--	--
	0.1	3	69-87	80	18	23
Sediment	0.0003	6	74-107	93	12	13
	0.001	6	74-113	91	14	15
	0.1	6	73-84	78	4	5

III. Method Characteristics

The LOQ was calculated using the standard deviation from the 0.05 µg/L recovery results for water and the 0.001 µg/g for soil. 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.

For soil, the calculated LOQ for soil was 0.0004 µg/g and is lower than the reported LOQ of 0.001 µg/L. The calculated LOD was 0.0001 µg/g.

For water, LOQs ranged from 0.0200 to 0.0435 µg/L, which are all lower than the reported LOQ of 0.05 µg/L. Calculated LODs range from 0.0060 to 0.0130 µg/L.

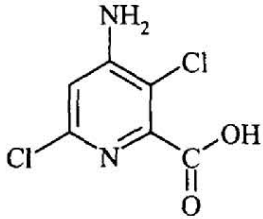
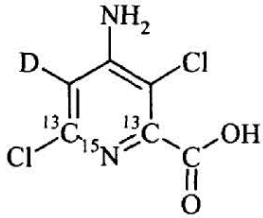
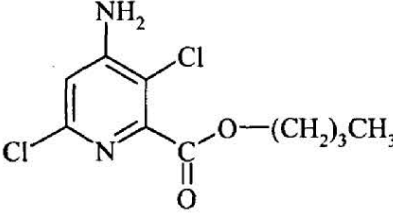
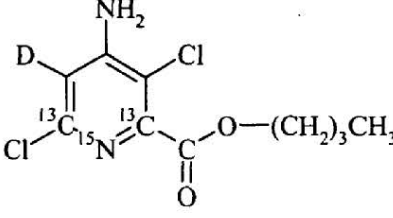
Table 4. Method Characteristics for Aminopyralid Detections using HPLC/MS/MS

	Tap Water (n=6)	Groundwater (n=6)	Surface Water (n=7)	Soil (n=21)
Limit of Quantitation (LOQ)	0.0215 µg/L	0.0200 µg/L	0.0435 µg/L	0.0004 µg/g
Limit of Detection (LOD)	0.0065 µg/L	0.0060 µg/L	0.0130 µg/L	0.0001 µg/g
Linearity (calibration curve r^2 and concentration range)	$r^2 > 0.9997$ 0.03 – 25 µg/L			$R^2 = 0.9996$ 0.03 – 25 µg/L
Repeatable	Yes	Yes	Yes	Yes
Reproducible	Yes	Yes	Yes	Yes
Specific	Yes	Yes	Yes	Yes

IV. Method Deficiencies and Reviewer’s Comments

The ECMs for soil and water did not have an associated ILV; however, there was validation information in an aquatic field dissipation study (MRID 48939201). Additionally, the water method is very similar to an ECM with an ILV (MRID 46235601 and MRID 46235713). The final step (before HPLC analysis) in MRID 47572602 dilutes with 4790 µL methanol/water (40:60) solution containing 0.05% formic acid and 5 mM ammonium formate to the sample vials. In MRID 46235601, in the corresponding step the sample is diluted with methanol:water:acetic acid (50:50:0.1).

Structure and Chemical Information for Aminopyralid

Common Name of Compound	Structure and CAS Name
<p>Aminopyralid</p> <p>Molecular Formula: C₆H₄Cl₂N₂O₂</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>¹³C₂²H¹⁵N-Aminopyralid</p> <p>Molecular Formula: ¹³C₂C₄²HH₃Cl₂¹⁵NNO₂</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-1-¹⁵N-2,6-¹³C-5-<i>d</i></p>
<p>Aminopyralid 1-Butyl Ester</p> <p>Molecular Formula: C₁₀H₁₂Cl₂N₂O₂</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>¹³C₂²H¹⁵N-Aminopyralid 1-Butyl Ester</p> <p>Molecular Formula: ¹³C₂C₈²HH₁₁Cl₂¹⁵NNO₂</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-1-¹⁵N-2,6-¹³C-5-<i>d</i>, 1-butyl ester</p>