Analytical method for aminopyralid in soil (GRM 02.34) by liquid chromatography with tandem mass spectrometry detection

Reports:	MRID 46235602: Lindsey, A. (2004) Method Validation Report for the Determination of Residues of Aminopyralid in Soil by Liquid Chromatography with Tandem Mass Spectrometry Detection Using Dow AgroSciences Method GRM 02.34. Project Number: 021295. Unpublished study prepared by Dow Agrosciences LLC. 56 p. MRID 46235715: Reed, R. (2004) Independent Laboratory Validation of Dow AgroSciences Method GRM 02.34 - Determination of Residues of Aminopyralid in Soil by Liquid Chromatography with Tandem Mass Spectrometry. Project Number: 020158, ML03/1102/DOW. Unpublished study prepared by Morse Laboratories. 125 p.
Document No.:	MRIDs 46235602 & 46235715
Guideline:	850.6100
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 for soil is classified as **acceptable** provided the excursions per minute specified in 9.3.5 and 9.3.10 be increased from 180 to ≥200 excursions per minute. This method may be used to measure aminopyralid in soil with an LOQ of 0.0015 µg/g (1.5 µg/kg) and an LOD of 0.000246 µg/g (0.246 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 46235602, is designed for the quantitative determination of aminopyralid in soil (GRM 02.34) using high performance liquid chromatograph (HPLC) with tandem mass spectrometry (MS/MS) detection (see Table 1). The method was validated for a concentration range of 0.0015 to 0.10 µg/g. The limit of quantitation (LOQ) is 0.0015 µg/g (1.5 µg/kg). The LOQ is less than the lowest toxicological level of concern in soil.[1] An ILV was submitted (MRID 46235715) and results were acceptable after the platform shaker speed was increased to from 180 to ≥200 excursions/minute, as specified in the method.

[1] The target LOQ is based on the lowest measured terrestrial plant endpoint of 0.0004 lbs a.i./A (equal 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).

The soils used in the aerobic soil metabolism studies (Charentilly, Orthic, and Cuckney) were used in the method validation study and recoveries were tested 9, 21, 57, and 79 days after dosing when shaking in an acetonitrile/1 N HCL (90:10) versus the acetone/1 N HCl (90:10) extraction solvents. The acetone:1 N HCl was used in some aerobic soil metabolism studies. The results of the two solvent systems yielded similar extraction recoveries for the different time points. The ILV used soils from the terrestrial field dissipation studies.

Table 1. Analytical Method Summary

Matrix	MRID		EPA Review	Analyte	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	ECM	ILV						
Soil	46235602	46235715		Aminopyralid	01/2004	Dow Agrosciences	HPLC/ MS/MS	1.5 µg/kg

ECM=Environmental Chemistry Method; ILV= Independent Laboratory Validation

I. Principle of the Method

Soil samples were shaken (180 excursions/minute) in acetonitrile/1 N hydrochloric acid (90:10) solution for 60 minutes. After centrifugation, the solution was poured off, acetonitrile/1 N hydrochloric acid (90:10) was added, and the mixture was shaken for 30 minutes. The second extraction solution was then combined with the first and brought to a fixed volume of 40 mL. An aliquot of the solutions was then evaporated to dryness and reconstituted in 1 N hydrochloric acid. The sample was then purified using a polymeric 96-well solid phase extraction plate (Phenomenex Strata-S-well SPE plate). After pulling the sample through the SPE plate, the SPE plate was washed with methanol/water (5:95) and the analyte was eluted with acetonitrile. A stable-isotope labelled internal standard ($^{13}C_2{}^{15}N$-aminopyralid) was added to the eluate. The eluate was evaporated to dryness and the residues were reconstituted in an acetonitrile/pyridine/1-butanol (22:2:1) solution, and derivatized with butyl chloroformate to form the 1-butyl esters (1-BE) of the analyte. After derivatization, the mixture was diluted with methanol/water/acetic acid (50:50:0.1). The purified extract was then analyzed by positive-ion electrospray ESI-HPLC/MS/MS. The standard methodology used to verify methods were completed and raw data were submitted.

The soils used to test the method were used in the aerobic soil metabolism studies (Charentilly, Orthic, and Cuckney). Recovery was also examined 9, 21, 57, and 79 days after dosing and compared the recovery results with the method with the extraction method used in the aerobic soil metabolism studies where an acetone:1 N HCl (90:10) solution was used as the extraction solvent. Raw data were not submitted to support this portion of the study.

II. Recovery Findings

Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) (**Table 2**). The method was quantitative for aminopyralid and the LOQ and LOD appropriate. The first attempt at the ILV yielded low recoveries (20-60%). Increasing the speed setting of the platform shaker from 180 to ≥200 excursions/minute resulted in acceptable recoveries (**Table 3**).

Table 2. Initial Validation Method Recoveries for Analytes in Aminopyralid

Matrix	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil	0	8	<LOQ	--	--	--
	0.003	4	<LOQ	--	--	--
	0.0015	16	80-99	87	5.5	6.3
	0.015	8	81-94	90	3.9	4.4
	0.05	8	81-91	88	3.1	3.5
	0.10	8	82-102	90	6.1	6.8
	0.0015-0.10	88	80-102	88	4.9	5.5

Table 3. Independent Validation Method Recoveries for Analytes in Soil

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Aminopyralid	0.0015	5	70-89	81	6.9	8.6
	0.015	5	93-103	96	4.1	4.2

Table 4 shows results reported by the study author when comparing the extraction solvents for the tested method, and the extraction solvent used in aerobic soil metabolism studies.

Table 4. Comparison of recoveries from aged samples for extraction solvents

Soil Sample	% Extracted and Counted by LSC				%Extracted and Counted by LSC			
	90:10 acetone:1N HCl				90:10 acetonitrile:1N HCl			
	Day 9	Day 21	Day 57	Day 79	Day 9	Day 21	Day 57	Day 79
Sand	96	91	71	66	99	92	73	68
Clay	79	79	32	14	80	79	32	14
Loam	83	76	60	50	91	82	64	53

III. Method Characteristics

The LOQ was calculated using the standard deviation from the 0.0015 µg/g 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. 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 LOQ for soil was 0.000818 µg/g and is lower than the reported LOQ of 0.0015 µg/g. The calculated LOD was 0.000246 µg/g. The percent recovery results and calculated LOD and LOQ reported are consistent with the overall method LOD and LOQ.

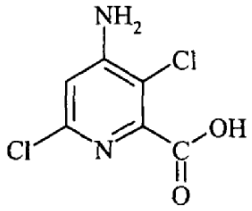
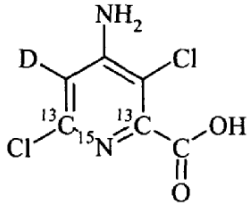
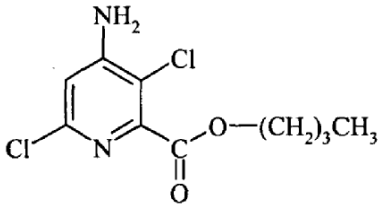
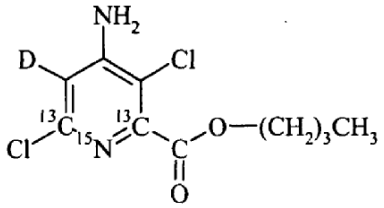
Table 5. Method Characteristics for Aminopyralid Detections using HPLC/MS/MS for water

	Soil
Limit of Quantitation (LOQ)	0.0015 µg/g
Limit of Detection (LOD)	0.000246 µg/g
Linearity (calibration curve r^2 and concentration range)	r^2=0.9997 0.0001 – 0.05 µg/mL
Repeatable	Yes
Reproducible	Yes
Specific	Yes

IV. Method Deficiencies and Reviewer's Comments

The only deficiency is that in order for the ILV to be successful, the speed of the shaker table had to be increased from 180 to ≥200 excursions/minute.

Structure and Chemical Information for Aminopyarlid

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

References Cited

USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. United States Environmental Protection Agency.