Analytical method for aminopyralid in compost

Reports:	ECM: EPA MRID No.: 51062701. Beato, B.D. 2019. Method Validation Study for the Determination of Residues of Aminopyralid in Compost by Liquid Chromatography with Tandem Mass Spectrometry. Dow AgroSciences Study ID: 191576. Report prepared, sponsored, and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 61 pages. Final report issued December 19, 2019. ILV: EPA MRID No. 51241201. Skaggs, C. 2020. Independent Laboratory Validation of Aminopyralid in Compost. Sponsor Study ID: 191362. Performing Laboratory Study No.: SGS-19-01-10. Report prepared by SGS North America, Inc., Brookings, South Dakota, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 104 pages. Final report issued June 13, 2020.
Document No.:	MRIDs 51062701 & 51241201
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160), except that the electronic signature device used for solvent preparation sheets was not validated according to internal SOPs (p. 3 of MRID 51062701). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4). ILV: The study was conducted in accordance with USEPA FIFRA GLP (40 CFR Part 160), which are compatible with OECD Principles of GLP standards (as revised 1997), ENV/MC/CHEM(98)17, and OECD, Paris (1998; p. 3 of MRID 51241201). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).
Classification:	This analytical method is classified as **supplemental**. The LOQ is greater than the most sensitive toxicological endpoint. Reported instrument optimization, these ILV modifications in the ILV were necessary for the successful validation of the method. Multiple significant peaks were present very close to the analyte peak. Communication details were not provided.
PC Code:	005100
EFED Final Reviewer:	Joshua Antoline, Ph.D., Chemist Signature: Antoline, Joshua Digitally signed by Antoline, Joshua Date: 2021.03.29 15:31:51 -04'00' Katrina White, Ph.D., Senior Scientist Signature: White, Katrina Digitally signed by White, Katrina Date: 2021.03.29 17:03:58 -04'00'
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist Signature: Lisa Muto Date: 11/13/2020

Mary Samuel, M.S.,
Environmental Scientist

Signature: Marysamuel

Date: 11/13/2020

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Dow AgroSciences Study No. 191576, is designed for the quantitative determination of aminopyralid in compost at an LOQ of 0.5 ng/g using LC/MS/MS. The LOQ is greater than the lowest toxicological level of concern in compost for aminopyralid based on a 6-inch soil depth, a soil density of 1.5 g/cm^3, and a No Observable Adverse Effects Concentration for tomato vegetative vigor of 0.0044 ng/g (MRID 50342206).[1]

Only manure compost was used in the ILV; pasture grass compost and manure compost were used in the ECM. The ILV validated the method for aminopyralid in the third trial with minor modifications to the analytical instrumentation and parameters, including the extension of the gradient and the use of a different mass transition for the internal standard. The first two ILV validations failed due to unacceptable recoveries (<70%). Although the reported ILV modifications only involved instrument optimization, these ILV modifications were necessary for the successful validation of the method. Communication details and failed ILV trial details were not provided.

All ECM and ILV precision, accuracy, and linearity data were acceptable, but the specificity of the method for aminopyralid was difficult to determine in manure compost based on ILV representative chromatograms and in pasture compost based on ECM representative chromatograms. Multiple peaks (peak height *ca*. 50-500% of LOQ peak height) which had RT +0.1 to +0.5 min. of the analyte peak were observed in the quantitation ion transition chromatograms, however the study authors were able to quantify the aminopyralid residues. ECM representative 10×LOQ chromatograms were not provided.

[1] 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. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Aminopyralid	51062701	51241201		Compost	12/19/2019	Dow AgroSciences LLC	LC/MS/MS	0.5 ng/g

1 In the ECM, pasture and manure compost were obtained from Dow Agrosciences LLC Samples Management Group (p. 12 of MRID 51062701). No further information regarding test matrices was provided; it was reported that complete source documentation was included in the study file.

2 In the ILV, the manure compost used in this study was provided by Dow Agrosciences; the compost source was not further specified (p. 10 of MRID 51241201). No further information regarding test matrix was provided.

I. Principle of the Method

Samples (1.00 ± 0.05 g) of compost were weighed into 50-mL centrifuged tubes and fortified, as necessary, at the LOD, LOQ, 10×LOQ, and 80×LOQ (p. 11; Appendix I, p. 57-59 of MRID 51062701). The samples were extracted with 20 mL of 0.1N sodium hydroxide via shaking for 60 minutes on a flatbed shaker (*ca*. 280 excursions/minute). The samples were then centrifuged (5 minutes at 3000 rpm) and decanted into a separate vial (acceptable stopping point if samples are refrigerated). An aliquot (7.00 mL) of the supernatant was pipetted into 16-mm screw cap glass tubes then 660 µL of concentrated (12.1N) HCl was added. The sample was capped and placed in a water bath set at 90°C for 90 minutes. After cooling without removing the caps, 340 µL water was added. The samples were centrifuged (3000 rpm for 10 minutes) then an aliquot of at least 6.5 mL of the supernatant (without precipitate) was removed for clean-up. The sample was purified via Oasis MAX SPE cartridge (150 mg, 6 mL) pre-conditioned with 4 mL each of methanol then water (elution rate 1-2 seconds between drops for clean-up). The sample (6.00 mL) was applied with two vial rinsings of 3 mL of water. The cartridge was washed with 2 x 4 mL of methanol:water:acetic acid (50:49:1, v:v:v). After the cartridge was dried for at least 30 seconds, the analyte was eluted with 2 x 3.50 mL aliquots of ethyl acetate:trifluoroacetic acid (98:2, v:v) into culture tubes (16 x 100 mm) containing 20 µL of the 1-butanol:glycerol (90:10, v:w) solution. The sample was evaporated (*ca*. 1 hour) to dryness using a Turbovap set at 40°C and a gentle stream of nitrogen. The samples were mixed with 50.0 µL of the 100 ng/mL internal standard solution in acetonitrile then evaporated to dryness using a Turbovap set at 40°C and a gentle stream of nitrogen (the method noted that it was critical that all methanol and water was removed from the sample via evaporation prior to derivatization). The residue was reconstituted in 200 µL of acetonitrile:pyridine:1-butanol (22:2:1, v:v:v) then derivatized by pipetting 100 µL of acetonitrile:butyl chloroformate (90:10, v:v). After vortexing for a few seconds, the mixture was allowed to react at room temperature for *ca*. 15 minutes. The reaction was quenched with sonication for *ca*. 30 seconds with 250 µL of 0.1% formic acid in water. The samples were filtered (13 mm, 0.2 µm PTFE), transferred to low volume autosampler vials or autosampler vials with low volume glass inserts, then analyzed by LC-MS/MS. Calibration standards which were fortified with the internal standard were also derivatized.

Samples were analyzed for analytes by Agilent 1290 Infinity HPLC (Waters HSS T3 column, 2.1 mm x 100 mm, 1.8 µm, and KrudKatcher Ultra pre-column filter, 0.5 µm x 0.004 in.; column

temperature 40°C) using a mobile phase of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid [percent A:B at 0.0-0.5 min. 45:55, 2.0 min. 40:60, 2.5-3.5 min. 5:95, 4.0-5.0 min. 45:55] with AB SCIEX QTrap 5500 MS using MS/MS-ESI (electrospray ionization; temperature 600°C) detection in positive polarity and multiple reaction monitoring (MRM; pp. 11, 13; Appendix I, pp. 52, 60-61 of MRID 51062701). Injection volume was 20 µL. Aminopyralid was identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 262.9→133.9 and *m/z* 264.9→135.9 (*m/z* 269.0→195.0 for aminopyralid IS). The confirmation ion transition *m/z* 263→161 was not used due to too much interference with some matrices at low concentrations. Expected retention time was *ca*. 1.6 minutes for aminopyralid (Figure 8, p. 38).

In the ILV, the ECM was performed as written, except for modifications to the analytical instrumentation and parameters, including the extension of the gradient from 5.0 to 6.0 minutes and the mass transition used for the internal standard (*m/z* 269→111 for aminopyralid IS; pp. 8, 11, 13; Appendix B, Table 4, p. 21 of MRID 51241201). A Shimadzu Nexera XR HPLC (Acquity UPLC HSS T3 column, 2.1 mm x 100 mm, 1.8 µm; column temperature 40°C) coupled with AB Biosystems/MDS Sciex API 6500+ MS using MS/MS-ESI in positive mode was used. No pre-column filter was reported. Significant parameters were the same as the ECM. Aminopyralid was identified using the same two ion transitions as those used in the ECM. Expected retention time was *ca*. 1.9 minutes for aminopyralid. No other modifications to the ECM were reported.

The Limit of Quantification (LOQ) for aminopyralid was reported as 0.5 ng/g in the ECM and ILV (pp. 11, 15-16; Table 13, p. 26; Appendix I, p. 57 of MRID 51062701; p. 8 of MRID 51241201). The Limit of Detection (LOD) for aminopyralid was reported as 0.150 ng/g in the ECM and ILV. In the ECM, the LOQ and LOD were calculated as 0.119-0.211 ng/g and 0.0357-0.0633 ng/g, respectively, for pasture compost and 0.135-0.165 ng/g and 0.0405-0.0495 ng/g, respectively, for manure compost. The LOQ was calculated using the standard deviation of the average recovery based on the procedure describe in Keith *et al.*

II. Recovery Findings

ECM (MRID 51062701): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of aminopyralid in two compost matrices at the fortification levels of 0.5 ng/g (LOQ), 5.0 ng/g (10×LOQ), and 40 ng/g (80×LOQ; n = 6 for all analyses; Tables 3-12, pp. 21-26). Recoveries for pasture compost were reported as uncorrected and corrected for residues quantified in the matrix blanks (0.132-0.146 ng/g). Corrected values are achieved by subtracting out the contribution from the untreated pasture grass control sample(s) analyzed within the same analytical batch (p. 14). Recoveries for manure compost were only reported as uncorrected. Aminopyralid was identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Two samples were prepared at LOD for both analytes for both matrices (n = 2); LOD recoveries ranged 165-181% for pasture compost (uncorrected), 99-116% for manure compost (uncorrected), and 68-86% for pasture manure (corrected; DER Excel Attachment 2). Pasture and manure compost were obtained from Dow Agrosciences LLC Samples Management Group (p. 12). No further information regarding test matrices was provided; it was reported that complete source documentation was included in the study file.

ILV (MRID 51241201): Mean recoveries and RSDs were within guideline requirements for analysis of aminopyralid in one compost matrix at the fortification levels of 0.5 ng/g (LOQ), 5.0 ng/g (10×LOQ), and 40 ng/g (80×LOQ; n = 5 for all analyses pp. 9, 12; Appendix B, Table 1, p. 19). Aminopyralid was identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were not comparable for the LOQ analyses but were comparable for 10×LOQ and 80×LOQ. The manure compost used in this study was provided by Dow Agrosciences; the compost source was not further specified (p. 10). No further information regarding test matrix was provided. The method for aminopyralid in compost was validated in the third trial with minor modifications to the analytical instrumentation and parameters, including the extension of the gradient from 5.0 to 6.0 minutes and the mass transition used for the internal standard (*m/z* 269→111 for aminopyralid IS; pp. 8, 11, 13; Appendix B, Table 4, p. 21). The first two ILV validations failed due to unacceptable recoveries (<70%). Although the reported ILV modifications only involved instrument optimization, these ILV modifications were necessary for the successful validation of the method.

Table 2. Initial Validation Method Recoveries for Aminopyralid in Compost[1,2]

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Pasture Compost – Uncorrected Recoveries					
	Quantitation Ion Transition					
Aminopyralid	0.15 (LOD)	**2**[3]	**165**, **179**	--[4]	--	--
	0.50 (LOQ)	6	111-118	114.8	2.6	2.2
	5.0	6	91-93	92.0	0.9	1.0
	40.0	6	88-93	90.6	1.7	1.9
	Confirmatory Ion Transition					
Aminopyralid	0.15 (LOD)	**2**[3]	**167**, **181**	--	--	--
	0.50 (LOQ)	6	106-118	110	4.3	3.9
	5.0	6	92-99	95.9	2.6	2.7
	40.0	6	91-96	93.6	1.8	1.9
	Manure Compost – Uncorrected Recoveries					
	Quantitation Ion Transition					
Aminopyralid	0.15 (LOD)	**2**[3]	99, 116	--	--	--
	0.50 (LOQ)	6	92-101	96.6	3.3	3.4
	5.0	6	93-98	96.3	2.2	2.3
	40.0	6	93-98	95.4	1.8	1.9
	Confirmatory Ion Transition					
Aminopyralid	0.15 (LOD)	**2**[3]	105, 111	--	--	--
	0.50 (LOQ)	6	91-97	93.8	2.7	2.9
	5.0	6	89-100	96.0	5.2	5.5
	40.0	6	94-99	96.3	1.7	1.8
	Pasture Compost – Corrected Recoveries[5]					
	Quantitation Ion Transition					
Aminopyralid	0.15 (LOD)	**2**[3]	**68**, 82	--	--	--
	0.50 (LOQ)	6	82-88	85.8	2.4	2.8
	5.0	6	88-91	89.0	0.9	1.0
	40.0	6	88-93	90.2	1.7	1.9
	Confirmatory Ion Transition					
Aminopyralid	0.15 (LOD)	**2**[3]	79, 86	--	--	--
	0.50 (LOQ)	6	77-89	82.0	4.0	4.8
	5.0	6	90-97	93.2	2.6	2.8
	40.0	6	91-96	93.3	1.9	2.0

Data (uncorrected recovery results unless noted otherwise; Figure 5, p. 35) were obtained from Tables 3-12, pp. 21-26 of MRID 51062701.

1 Aminopyralid was identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 262.9→133.9 and *m/z* 264.9→135.9 (Appendix I, p. 61). The confirmation ion transition m/z 263→161 was not used due to too much interference with some matrices at low concentrations.

2 Pasture and manure compost were obtained from Dow Agrosciences LLC Samples Management Group and homogenized prior to use (p. 12). No further information regarding test matrices was provided; it was reported that complete source documentation was included in the study file.

3 Reviewer-calculated recoveries are reported since the recoveries were not calculated in the study report (see DER Excel Attachment 2).

4 Could not be calculated, n = 2.

5 Calculated in the study report for LOQ, 10×LOQ and 40×LOQ fortification levels. Corrected values are achieved by subtracting out the contribution from the untreated pasture grass control sample(s) analyzed within the same analytical batch (p. 14).

Table 3. Independent Validation Method Recoveries for Aminopyralid in Compost[1,2]

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Manure Compost					
	Quantitation Ion Transition					
Aminopyralid	0.5 (LOQ)	5	71-80	75	3.2	4.3
	5.0	5	78-82	80	1.6	2.1
	40	5	87-89	88	0.8	1.0
	Confirmatory Ion Transition					
Aminopyralid	0.5 (LOQ)	5	86-108	100	9.1	9.0
	5.0	5	82-85	84	1.5	1.8
	40	5	91-93	92	1.0	1.1

Data (uncorrected recovery results, Appendix D, Figure 5, p. 78) were obtained from pp. 9, 12; Appendix B, Table 1, p. 19 of MRID 51241201.

1 Aminopyralid was identified using two ion transitions (quantitative and confirmatory, respectively *m/z* 262.9→133.9 and *m/z* 264.9→135.9 (p. 12). These were the same as those of the ECM.

2 The manure compost used in this study was provided by Dow Agrosciences; the compost source was not further specified (p. 10). No further information regarding test matrix was provided.

III. Method Characteristics

The LOQ for aminopyralid was reported as 0.5 ng/g in the ECM and ILV (pp. 11, 15-16, 19; Table 13, p. 26; Appendix I, p. 57 of MRID 51062701; p. 8 of MRID 51241201). The LOD for aminopyralid was reported as 0.150 ng/g in the ECM and ILV, which was equivalent to 30% of the LOQ. Following the method of Keith *et al*., the LOD and LOQ for determination of aminopyralid in compost were calculated in the ECM using the standard deviation from the LOQ recovery results, 0.0119-0.0211 ng/g (Q/C). The LOD was calculated as three times the standard deviation (3*s*), and the LOQ was calculated as ten times the standard deviation (10*s*) of the recovery results. In the ECM, the LOQ and LOD were calculated as 0.119-0.211 ng/g and 0.0357-0.0633 ng/g, respectively, for pasture compost and 0.135-0.165 ng/g and 0.0405-0.0495 ng/g, respectively, for manure compost. The calculated LOQ and LOD values supported the Method LOQ and LOD values.

Table 4. Method Characteristics

Analyte		Aminopyralid	
Limit of Quantitation (LOQ)*	ECM (method)	0.5 ng/g	
	ECM (calc)	0.119 ng/g (Q, pasture)[1] 0.199-0.211 ng/g (C, pasture)[1]	0.165 ng/g (Q, manure) 0.135 ng/g (C, manure)
	ILV (method)	0.5 ng/g	
Limit of Detection (LOD)	ECM (method)	0.150 ng/g (30% of the LOQ)	
	ECM (calc)	0.0357 ng/g (Q, pasture)[1] 0.0596-0.0633 ng/g (C, pasture)[1]	0.0495 ng/g (Q, manure) 0.0405 ng/g (C, manure)
	ILV (method)	0.150 ng/g (30% of the LOQ)	
Linearity (calibration curve r and concentration range)	ECM	r = 1.0000 (Q) r = 0.9999 (C)	
	ILV	r = 0.99915 (Q) r = 0.99924 (C)	
	Range	0.150-50.0 ng/g	
Repeatable	ECM[1,2]	Yes at the LOQ, 10×LOQ, and 80×LOQ in pasture and manure compost matrices.[1]	
	ILV[3,4]	Yes at the LOQ, 10×LOQ, and 80×LOQ in manure compost matrix.	
Reproducible		Yes at the LOQ, 10×LOQ, and 80×LOQ.	
Specific	ECM	Yes. For manure compost, matrix interferences at analyte RT (*ca*. 1.6 min.) were *ca*. 0-12% (Q) and *ca*. 8-16% (C)[5] of the LOQ (based on peak area).[6] For pasture compost, two significant peaks (peak height *ca*. 50-150% of LOQ peak height; RTs *ca*. 1.7 and 1.8 min.) near analyte peak in all Q matrix chromatograms. Q analyte peak only identifiable by RT (*ca*. 1.6 min.). Matrix interferences at analyte RT were *ca*. 12-24% (Q) and *ca*. 17-27% (C)[5] of the LOQ (based on peak area).[6,7] Minor baseline noise in all representative chromatograms, except 80×LOQ representative chromatograms in which baseline noise was insignificant. Representative 10×LOQ chromatograms were **not** presented for either matrix.	
	ILV	Yes. For manure compost, four significant peaks (peak height *ca*. 50-500% of LOQ peak height; RTs *ca*. 1.4, 2.1 and 2.3 min.) near analyte peak in all Q matrix chromatograms. Q analyte peak only identifiable by RT (*ca*. 1.84 min.). Matrix interferences at analyte RT were *ca*. 0-5% (Q) and *ca*. 0-35% (C)[5] of the LOQ (based on peak area).[8]	

Data were obtained from pp. 11, 15-16, 19; Table 13, p. 26; Appendix I, p. 57 (LOD/LOQ); Tables 3-12, pp. 21-26 (recovery data); Figures 3-4, pp. 33-34 (calibration data and figures); Figures 8-19, pp. 38-49 (chromatograms) of MRID 51062701; pp. 9, 12; Appendix B, Table 1, p. 19 (LOD/LOQ); Appendix D, Tables 3-12, pp. 64-69 (recovery data); p. 8; Appendix C, pp. 22, 33 (calibration data and figures); Appendix C, pp. 23-43 (chromatograms) of MRID 51241201. Q = Quantitation ion transition; C = Confirmation ion transition.

* Since the method LOQ was validated based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the LOQ.

1 Data from the corrected and uncorrected recoveries of pasture manure compost was reported.

2 In the ECM, pasture and manure compost were obtained from Dow Agrosciences LLC Samples Management Group (p. 12 of MRID 51062701). No further information regarding test matrices was provided; it was reported that complete source documentation was included in the study file.

3 In the ILV, the manure compost used in this study was provided by Dow Agrosciences; the compost source was not further specified (p. 10 of MRID 51241201). No further information regarding test matrix was provided.

4 The ILV validated the method for aminopyralid in compost in the third trial with minor modifications to the analytical instrumentation and parameters, including the extension of the gradient from 5.0 to 6.0 minutes and the mass transition used for the internal standard (*m/z* 269→111 for aminopyralid IS; pp. 8, 11, 13; Appendix B, Table 4, p. 21 of MRID 51241201). The first two ILV validations failed due to unacceptable recoveries (<70%).

Although the reported ILV modifications only involved instrument optimization, these ILV modifications were necessary for the successful validation of the method; therefore, an updated ECM should be submitted which includes an instrument optimization step prior to sample analysis.

5 Deviations in the confirmation ion analyses do not affect the specificity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

6 Based on Figures 11-13, pp. 41-43, and Figures 16-17, pp. 46-47 of MRID 51062701 (reagent blank interferences included). The significant peak at RT 1.7 min. was also observed in the aminopyralid internal standard chromatograms for pasture and manure compost (peak height *ca*. 50-150% of LOQ peak height; not observed in reagent blank; Figures 12-19, pp. 42-49).

7 Matrix interferences in the pasture compost matrix were reviewer-calculated as *ca*. 29% (Q) and *ca*. 26-29% (C)[5] of the LOQ (based on quantified residues; Tables 3-4, pp. 21-22 of MRID 51062701).

8 Based on Appendix C, Figures 8-10, pp. 29-31, Figures 18-21, pp. 39-42, of MRID 51241201.

IV. Method Deficiencies and Reviewer's Comments

1. The LOQ is greater than the lowest toxicological level of concern in compost for aminopyralid based on a 6-inch soil depth, a soil density of 1.5 g/cm^3, and a No Observable Adverse Effects Concentration for tomato vegetative vigor of 0.0044 ng/g (MRID 50342206).

2. There were multiple peaks (peak height *ca*. 50-500% of LOQ peak height) which had retention time of +0.1 to +0.5 min. of the analyte peak in all Q matrix chromatograms (Figures 11-13, pp. 41-43; Figures 16-17, pp. 46-47 of MRID 51062701; Appendix C, pp. Figures 8-10, pp. 29-31, Figures 18-21, pp. 39-42, of MRID 51241201). The aminopyralid Q analyte peak was identifiable by retention time. Two possible reasons for these significant peaks were as follows: extraction or clean-up methods may have needed to be adjusted to isolate aminopyralid, or there are problems with the derivatization portion of the method. However, the study authors were able to quantify the aminopyralid residues in both studies

   A peak at RT +0.1 min. of the analyte peak was also observed in the ECM aminopyralid internal standard chromatograms for pasture and manure compost (peak height *ca*. 50-150% of LOQ peak height; not observed in reagent blank; Figures 12-19, pp. 42-49 of MRID 51062701). This provides some support to an issue with the derivatization portion of the method.

3. The following ILV minor modifications to the analytical instrumentation and parameters were reported: the extension of the gradient from 5.0 to 6.0 minutes and the mass transition used for the internal standard (*m/z* 269→111 for aminopyralid IS; pp. 8, 11, 13; Appendix B, Table 4, p. 21 of MRID 51241201). Although the reported ILV modifications only involved instrument optimization, these ILV modifications were necessary for the successful validation of the method.

   The first two ILV validations failed due to unacceptable recoveries (<70%); however, no further data was provided about the failed trials (p. 13; Appendix B, Table 4, p. 21 of MRID 51241201). It could not be determined how low the recoveries were, what ILV modifications were made for the second trial, and how the ILV modifications increased

the recoveries. The reviewer noted that the extension of the gradient should not have affected the LC/MS outcome since the extension only affected the gradient after 5.0 minutes and aminopyralid eluted at *ca*. 2 min. The use of a different mass transition for the internal standard may have increased detection and affected the quantification of aminopyralid analyte.

4. Only manure compost was used in the ILV; pasture and manure compost were used in the ECM (p. 12 of MRID 51062701; p. 10 of MRID 51241201).

5. In the ECM, representative 10×LOQ chromatograms were not provided. Representative chromatograms from all matrices and fortifications should be provided for review.

6. Communication details were not provided. The ILV reported that communications occurred between the ILV Study Director (C. Skaggs) and Dow AgroSciences Study Representative (Leandro Ap. G. Deziderio) were documented but not provided (pp. 1, 6, 13-14 of MRID 51241201). The only communication which was reported was the communication of the successful completion of the third ILV trial on April 21, 2020. No one from Dow AgroSciences was allowed to visit the ILV testing facility. Leandro Ap. G. Deziderio was not listed in the ECM personnel (p. 6 of MRID 51062701).

7. ECM results for pasture compost were presented as corrected and uncorrected due to the inability to obtain control pasture compost samples without residues of aminopyralid (p. 14; Tables 3-4, pp. 21-22 of MRID 51062701). The ECM noted that residues of aminopyralid in control pasture were reviewer-calculated as *ca*. 29% (Q) and *ca*. 26-29% (C) of the LOQ (based on quantified residues). These aminopyralid residues were also noted in the chromatograms of pasture compost (Figure 12, p. 42). OCSPP guidelines state that recoveries should be uncorrected.

   The deviations in the confirmation ion analyses do not affect the specificity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

8. Matrix effects were found to be insignificant (≤20%) for aminopyralid in the test matrices in the ECM (quantitation and confirmatory transitions) with the use of an internal standard for both matrices and corrected recoveries for pasture compost matrix only (pp. 17-18; Tables 17-19, pp. 28-30 of MRID 51062701). Solvent-based standards were used in the ECM.

   In the ILV, matrix effects were found to be significant (>20%) in the test matrix with only aminopyralid (-56%; quantitation transition; p. 13; Table 2, p. 19 of MRID 51241201). Following the ECM, the ILV used the stable isotope internal standard to normalize the matrix effects (no data provided). Solvent-based standards were used in the ILV.

9. In the ECM, it was reported that the method was the same as the analytical methods used to support ^{14}C-metabolism studies (p. 15 of MRID 51062701).

10. Certificates of Analysis for aminopyralid and the internal standard were provided in the ECM and ILV (98.6-100% purity; Figures 1-2, pp. 31-32 of MRID 51062701; Appendix A, pp. 17-18 of MRID 51241201).

11. Carryover was assessed in the ECM (p. 16 of MRID 51062701). No carryover was observed.

12. Since stable-isotope labeled internal standards were used, isotopic cross-over was evaluated in the ECM (pp. 12-13; Table 2, p. 21 of MRID 51062701). The concentration range of calibration curve and concentration of internal standard were chosen to minimize cross-over. No significant mass spectral isotopic crossover was observed.

13. In the ECM, the calibration solutions and stock solutions were found to be stable in acetonitrile up to 198 days of refrigerated storage in a separate study (pp. 16, 19 of MRID 51062701). The final sample extracts were found to be stable up to 4 days at *ca*. 10°C (p. 17; Tables 14-16, pp. 26-27).

14. In the ECM, it was reported that the extraction efficiency of the method was not studied in the study, but residue studies in wheat and grass were referenced (pp. 14-15, 19 of MRID 51062701).

15. It was reported for the ILV that one validation sample set required *ca*. 8 hours (p. 12 of MRID 51241201). In the ECM, it was reported that one validation sample set required *ca*. 1.5 days, including analysis time (p. 14 of MRID 51062701).

V. References

Keith, L.H.; Crummett, W.; Deegan, J., Jr.; Libby, R.A.; Taylor, J.K.; Wentler, G. *Anal. Chem.* 1983, *55*, 2210-2218 (p. 19 of MRID 51062701).

USEPA. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, *Environmental Chemistry Methods and Associated Independent Laboratory Validation*. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

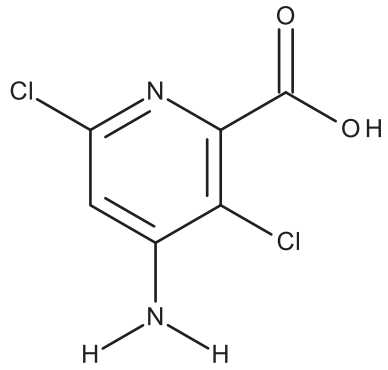
USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319, and Revision 2; 1994 and 2016.

Attachment 1: Chemical Names and Structures

Aminopyralid

IUPAC Name: 4-Amino-3,6-dichloropyridine-2-carboxylic acid
CAS Name: 4-Amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS Number: 150114-71-9
SMILES String: [H]N([H])c1cc(nc(c1Cl)C(=O)O)Cl



Attachment 2: Calculations Spreadsheet

005100_51062701+_850.6100_Calculations.x