Analytical method for florylpicoxamid (XDE-659) and its metabolite X12485649 in soil and sediment

Reports:	ECM: EPA MRID No.: 51074468. Tarsi, M. 2020. Method Validation for Determination of Residues of XDE-659 and X12485649 in Soil by LC-MS/MS. Report prepared by JRF America, Inc., Audubon, Pennsylvania, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 311 pages. JRF America Study No.: AU-2019-09. Dow AgroSciences Study ID: 190830. Final report issued January 29, 2020. ILV: EPA MRID No.: 51074469. Skaggs, C., and P. Afedi. 2020. Independent Laboratory Validation of XDE-659 and Metabolite in Soil. Report prepared by SGS North America, Inc., Brookings, South Dakota, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 358 pages. Performing Laboratory Study No.: SGS-19-01-13. Dow AgroSciences Study ID: 190829. Final report issued February 12, 2020.	
Document No.:	MRIDs 51074468 & 51074469	
Guideline:	850.6100	
Statements:	ECM: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160), which are compatible with OECD GLP (as revised 1997) ENV/MC/CHEM(98)17 (1998; p. 3 of MRID 51074468). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity statement was included with the Quality Assurance statement. ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160), which are compatible with OECD GLP (as revised 1997) ENV/MC/CHEM(98)17 (1998; p. 3). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity statement was included with the Quality Assurance statement.	
Classification:	This analytical method is classified as **acceptable** for XDE-659 and **supplemental** for X12485649. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. The specificity of the method was not acceptable for X12485649 based on ILV and ECM representative chromatograms. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the one ILV soil matrix covered the range of soils used in the submitted florylpicoxamid terrestrial field dissipation study. No sediment matrix was included in the ILV. The ILV study report did not provide a detailed account of its method.	
PC Code:	119032	
EFED Final Reviewer:	Zoe Ruge Physical Scientist	Signature: Zoe Ruge Date: 07/28/2021
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Lisa Muto Date: 10/30/2020

Mary Samuel, M.S., Signature: Mary Samuel
Environmental Scientist
Date: 10/30/2020

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, JRF America Study No. AU-2019-09 and Dow AgroSciences Study ID 190830, is designed for the quantitative determination of florylpicoxamid (XDE-659) and X12485649 in soil and sediment at the stated LOQ of 0.05 µg/g using LC/MS/MS. The LOQ is **less than** the lowest toxicological level of concern in soil and sediment for florylpicoxamid (1.80 µg/g; NOAEC; MRID 51074444) and X12485649 (7.0 µg/g; NOAEC; MRID 51074444). Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for florylpicoxamid and X12485649 in the tested soil matrices (0.05 µg/g).

The ECM validated the method using three characterized soil matrices (clay loam, sandy loam, and silt loam) and one characterized sediment matrix (silt loam). One uncharacterized, undescribed soil matrix was used in the ILV; no sediment matrix was included. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the one ILV soil matrix covered the four soils used in the submitted florylpicoxamid terrestrial field dissipation study. The ILV validated the method for florylpicoxamid and X12485649 in soil in the first trial with minor modifications to the analytical parameters including the modification of one of the LC mobile phases. The ILV modifications did not warrant an updated ECM, but more information should have been provided to discuss the reason that the ILV LC mobile phase was modified in both formic acid concentration and acetonitrile:methanol ratio.

All ILV and ECM data regarding repeatability, accuracy, precision, and linearity were satisfactory for florylpicoxamid and X12485649 in test soil/sediment matrices. All ILV and ECM data regarding specificity was satisfactory for florylpicoxamid in test soil/sediment matrices. The specificity of the method was not acceptable for X12485649 based on ILV and ECM representative chromatograms due to a significant contaminant (florylpicoxamid; peak height >100% (ILV) and *ca*. 30-45% (ECM) of analyte peak height) which was present in all samples. The florylpicoxamid and X12485649 stock solutions were prepared separately, so the presence of florylpicoxamid in ECM and ILV chromatograms of X12485649 was determined by the reviewer to be due to cross-contamination, experimental error, or degradation.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Florylpicoxamid (XDE-659)	51074468[1]	51074469[2]		Soil/ Sediment	29/01/2020	Dow AgroSciences LLC	LC/MS/MS	0.05 µg/g
X12485649								

1 In the ECM, silt loam sediment (Sediment 204219; 20% sand, 68% silt, 12% clay; pH 7.8 in 1:1 soil:water ratio; 8.9% organic matter (Walkley-Black); 5.2% organic carbon (Walkley-Black); cation exchange capacity 11.0 meq/100 g), clay loam soil (Soil 204956; 37% sand, 26% silt, 37% clay; pH 7.7 in 1:1 soil:water ratio; 3.7% organic matter (loss on ignition); 2.1% organic carbon (Walkley-Black); cation exchange capacity 10.3 meq/100 g), sandy loam soil (Soil 204957; 67% sand, 24% silt, 9% clay; pH 7.7 in 1:1 soil:water ratio; 3.2% organic matter (loss on ignition); 1.7% organic carbon (Walkley-Black); cation exchange capacity 7.5 meq/100 g), and silt loam soil (Soil 204958; 15% sand, 62% silt, 23% clay; pH 5.8 in 1:1 soil:water ratio; 3.4% organic matter (loss on ignition); 1.7% organic carbon (Walkley-Black); cation exchange capacity 12.6 meq/100 g) were used in the study (p. 19; Appendix V, pp. 172-175 of MRID 51074468). The matrices were obtained from DowAgroSciences and characterized by Agvise Laboratories, Northwood, North Dakota. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

2 In the ILV, soil (M 1132) was used in the study and provided by the Sponsor (p. 10 of MRID 51074469). The soil characterization data and soil texture were not reported in the study report; however, the reviewer noted that ECM Soil 204958 had the Sample ID AM Fischteich 07/19-M1132. It was possible that the ILV soil matrix was the same soil matrix as ECM Soil 204958. The soil textures could not be verified by the reviewer using USDA-NRCS technical support tools.

I. Principle of the Method

Soil/sediment samples (5.0 ± 0.05 g) were fortified (250 µL of 1.00 µg/L or 10.0 µg/L fortification solutions) and extracted twice with 20 mL of 0.25% H_2PO_4 in acetonitrile:water (80:20, v:v) via shaking for 30 minutes (pp. 17-19; Appendix VI, pp. 185, 188-190 of MRID 51074468). After centrifugation (5 minutes at 2000 rpm), the combined extracts were combined, filtered (0.45 µm PTFE filter) and analyzed by LC/MS/MS. The method noted that samples should be analyzed within seven days after extraction.

Samples were analyzed for florylpicoxamid and X12485649 using an Agilent 1290 Infinity HPLC coupled with an AB Sciex 6500 QTrap TripQuad MS with an ESI ionization mode operated in the positive polarity with multiple reaction monitoring (MRM; pp. 18-21; Appendix VI, pp. 191-193 of MRID 51074468). The following LC conditions were used: Zorbax Eclipse Plus Phenyl Hexyl column (3 mm x 50 mm, 1.8 µm; column temperature 30°C), mobile phase of (A) 0.1% formic acid in LC/MS water and (B) 0.1% formic acid in LC/MS grade methanol:acetonitrile (80:20, v:v) [mobile gradient phase of percent A:B (v:v) at 0.00 min. 50:50, 1.30-2.00 min. 40:60, 3.00 min. 10:90, 3.10-4.50 min. 0:100, 4.60-5.00 min. 50:50] and injection volume of 5 µL. MS source temperature was 600°C. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 512.960→231.100 and *m/z* 512.960→108.900 for florylpicoxamid and *m/z* 470.813→230.900 and *m/z* 470.813→109.000 for X12485649. Reported retention times were *ca*. 3.24 and 2.89 minutes for florylpicoxamid and X12485649, respectively.

The ILV performed the ECM methods as written, except for minor modifications to the analytical parameters including the modification of one of the LC mobile phases (p. 11; Table 3, p. 23 of MRID 51074469). Samples were analyzed for florylpicoxamid and X12485649 using a Shimadzu Nexera XR HPLC coupled with an AB Biosystems/MDS Sciex API 6500+ LC/MS/MS with a

TurboIonSpray ionization mode operated in the positive polarity with MRM. The LC/MS/MS parameters were similar to those of the ECM. The following LC conditions were used: Zorbax Eclipse Plus Phenyl-Hexyl column (3.0 mm x 50 mm, 1.8 µm; column temperature 30°C), mobile phase of (A) 0.1% formic acid in LC/MS water and (B) **1.0%** formic acid in **acetonitrile:methanol (8:2, v:v)** [mobile gradient phase of percent A:B (v:v) at 0.0 min. 50:50, 1.3-2.0 min. 40:60, 3.0 min. 10:90, 3.1-5.5 min. 0:100, 5.6-8.0 min. 50:50] and injection volume of 10 µL (**bolded** value differed from the ECM). MS source temperature was 600°C. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 513.1→231.1 and *m/z* 513.1→109.0 for florylpicoxamid and *m/z* 471.1→231.1 and *m/z* 471.1→109.0 for X12485649. These were similar to those of the ECM. Reported retention times were *ca*. 4.0 and 3.6 minutes for florylpicoxamid and X12485649, respectively. The ILV modifications did not warrant an updated ECM, but more information should have been provided to discuss the reason that the ILV LC mobile phase was modified.

The Limit of Quantification (LOQ) for florylpicoxamid and X12485649 in soil was 0.05 µg/g in the ECM and the ILV (p. 24; Appendix VI, p. 188 of MRID 51074468; pp. 8-9 of MRID 51074469). In the ECM, the Limit of Detection (LOD) for florylpicoxamid and X12485649 was set to 0.015 µg/g in the ECM and the ILV. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 51074468): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of florylpicoxamid and X12485649 at fortification levels of 0.05 µg/g (LOQ) and 0.5 µg/g (10×LOQ) in one sediment matrix and three soil matrices (Tables 42-61, pp. 48-56). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. A sample was prepared at LOD for both analytes for all matrices (n = 1); LOD recoveries ranged 95-109% and 107-116% for florylpicoxamid and X12485649, respectively. The silt loam sediment (Sediment 204219; 20% sand, 68% silt, 12% clay; pH 7.8 in 1:1 soil:water ratio; 8.9% organic matter (Walkley-Black); 5.2% organic carbon (Walkley-Black); cation exchange capacity 11.0 meq/100 g), clay loam soil (Soil 204956; 37% sand, 26% silt, 37% clay; pH 7.7 in 1:1 soil:water ratio; 3.7% organic matter (loss on ignition); 2.1% organic carbon (Walkley-Black); cation exchange capacity 10.3 meq/100 g), sandy loam soil (Soil 204957; 67% sand, 24% silt, 9% clay; pH 7.7 in 1:1 soil:water ratio; 3.2% organic matter (loss on ignition); 1.7% organic carbon (Walkley-Black); cation exchange capacity 7.5 meq/100 g), and silt loam soil (Soil 204958; 15% sand, 62% silt, 23% clay; pH 5.8 in 1:1 soil:water ratio; 3.4% organic matter (loss on ignition); 1.7% organic carbon (Walkley-Black); cation exchange capacity 12.6 meq/100 g) were used in the study (p. 19; Appendix V, pp. 172-175). The matrices were obtained from DowAgroSciences and characterized by Agvise Laboratories, Northwood, North Dakota. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

ILV (MRID 51074469): Mean recoveries and RSDs were within guidelines for analysis of florylpicoxamid and X12485649 at fortification levels of 0.05 µg/g (LOQ) and 0.5 µg/g (10×LOQ) in one soil matrix (p. 9). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The soil (M 1132) was used in the study and provided by the Sponsor (p. 10). The soil characterization data and soil texture were not reported in the study report; however, the reviewer noted that ECM Soil 204958 had the Sample ID AM Fischteich 07/19-M1132. It was possible that the ILV soil matrix was the same soil matrix as ECM Soil 204958. The soil texture could not be verified by the reviewer using USDA-NRCS technical support tools. Sediment was not included. The method for florylpicoxamid and X12485649 in soil was validated in the first trial with minor modifications to the analytical parameters including the modification of one of the LC mobile phases (pp. 11, 13; Table 3, p. 23). The ILV modifications did not warrant an updated ECM, but more information should have been provided to discuss the reason that the ILV LC mobile phase was modified in both formic acid concentration and acetonitrile:methanol ratio.

Table 2. Initial Validation Method Recoveries for Florylpicoxamid and X12485649 in Soil and Sediment[1,2]

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Silt Loam Sediment 204219					
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.015 (LOD)	1	95	--[3]	--	--
	0.05 (LOQ)	5	99-112	105	5	5
	0.5	5	96-102	99	2	2
X12485649	0.015 (LOD)	1	110	--	--	--
	0.05 (LOQ)	5	104-110	108	2	2
	0.5	5	97-100	99	2	2
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.015 (LOD)	1	97	--	--	--
	0.05 (LOQ)	5	94-112	103	8	7
	0.5	5	89-105	98	6	6
X12485649	0.015 (LOD)	1	107	--	--	--
	0.05 (LOQ)	5	103-112	108	4	3
	0.5	5	97-101	99	2	2
	Clay Loam Soil 204956					
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.015 (LOD)	1	103	--	--	--
	0.05 (LOQ)	5	101-109	105	3	3
	0.5	5	96-101	99	2	2
X12485649	0.015 (LOD)	1	109	--	--	--
	0.05 (LOQ)	5	106-109	108	1	1
	0.5	5	100-103	102	1	1
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.015 (LOD)	1	100	--	--	--
	0.05 (LOQ)	5	106-115	109	4	4
	0.5	5	91-99	96	3	3
X12485649	0.015 (LOD)	1	110	--	--	--
	0.05 (LOQ)	5	106-109	107	1	1
	0.5	5	100-102	101	1	1
	Sandy Loam Soil 204957					
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.015 (LOD)	1	102	--	--	--
	0.05 (LOQ)	5	98-105	103	3	3
	0.5	5	95-107	102	5	5
X12485649	0.015 (LOD)	1	112	--	--	--
	0.05 (LOQ)	5	109-114	110	2	1
	0.5	5	99-101	100	1	1
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.015 (LOD)	1	99	--	--	--
	0.05 (LOQ)	5	100-105	103	2	2
	0.5	5	99-105	101	2	2
X12485649	0.015 (LOD)	1	110	--	--	--
	0.05 (LOQ)	5	111-114	112	1	1
	0.5	5	99-103	101	2	2
	Silt Loam Soil 204958					

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.015 (LOD)	1	103	--	--	--
	0.05 (LOQ)	5	99-111	107	5	5
	0.5	5	96-107	101	4	4
X12485649	0.015 (LOD)	1	115	--	--	--
	0.05 (LOQ)	5	102-115	111	6	5
	0.5	5	101-105	104	2	2
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.015 (LOD)	1	109	--	--	--
	0.05 (LOQ)	5	105-111	109	2	2
	0.5	5	97.5-105	102	3	3
X12485649	0.015 (LOD)	1	116	--	--	--
	0.05 (LOQ)	5	104-115	112	5	4
	0.5	5	103-108	106	2	2

Data (uncorrected recovery results; pp. 22-23; Appendix VI, pp. 192-193) were obtained from Tables 42-61, pp. 48-56 of MRID 51074468.

1 The silt loam sediment (Sediment 204219; 20% sand, 68% silt, 12% clay; pH 7.8 in 1:1 soil:water ratio; 8.9% organic matter (Walkley-Black); 5.2% organic carbon (Walkley-Black); cation exchange capacity 11.0 meq/100 g), clay loam soil (Soil 204956; 37% sand, 26% silt, 37% clay; pH 7.7 in 1:1 soil:water ratio; 3.7% organic matter (loss on ignition); 2.1% organic carbon (Walkley-Black); cation exchange capacity 10.3 meq/100 g), sandy loam soil (Soil 204957; 67% sand, 24% silt, 9% clay; pH 7.7 in 1:1 soil:water ratio; 3.2% organic matter (loss on ignition); 1.7% organic carbon (Walkley-Black); cation exchange capacity 7.5 meq/100 g), and silt loam soil (Soil 204958; 15% sand, 62% silt, 23% clay; pH 5.8 in 1:1 soil:water ratio; 3.4% organic matter (loss on ignition); 1.7% organic carbon (Walkley-Black); cation exchange capacity 12.6 meq/100 g) were used in the study (p. 19; Appendix V, pp. 172-175). The matrices were obtained from DowAgroSciences and characterized by Agvise Laboratories, Northwood, North Dakota. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 512.960→231.100 and *m/z* 512.960→108.900 for florylpicoxamid and *m/z* 470.813→230.900 and *m/z* 470.813→109.000 for X12485649.

3 Could not be calculated, n = 1.

Table 3. Independent Validation Method Recoveries for Florylpicoxamid and X12485649 in Soil[1,2]

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Soil					
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.05 (LOQ)	5	105-106	106	0.5	0.5
	0.5	5	91-109	101	7.6	7.6
X12485649	0.05 (LOQ)	5	109-112	111	1.3	1.2
	0.5	5	100-104	102	1.6	1.6
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.05 (LOQ)	5	107-110	109	1.3	1.2
	0.5	5	97-106	102	3.4	3.3
X12485649	0.05 (LOQ)	5	105-108	106	1.1	1.1
	0.5	5	100-103	102	1.2	1.2

Data (uncorrected recovery results; p. 8) were obtained from p. 9 of MRID 51074469.

1 The soil (M 1132) was used in the study and provided by the Sponsor (p. 10). The soil characterization data and soil texture were not reported in the study report; however, the reviewer noted that ECM Soil 204958 had the Sample ID AM Fischteich 07/19-M1132. It was possible that the ILV soil matrix was the same soil matrix as ECM Soil 204958. The soil texture could not be verified by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 513.1→231.1 and *m/z* 513.1→109.0 for florylpicoxamid and *m/z* 471.1→231.1 and *m/z* 471.1→109.0 for X12485649. These ion transitions were similar to those of the ECM.

III. Method Characteristics

The LOQ for florylpicoxamid and X12485649 in soil was 0.05 µg/g in the ECM and the ILV (p. 24; Appendix VI, p. 188 of MRID 51074468; p. 8 of MRID 51074469). In the ECM and ILV, the LOQ was not justified or further defined. The LOD for florylpicoxamid and X12485649 was set to 0.015 µg/g in the ECM and the ILV. In the ILV, the LOD value was defined as 30% of the LOQ. No justification for LOD was reported in the ECM. No calculations or comparisons to background noise were reported for LOQ or LOD in the ECM or ILV.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics in Soil

		Florylpicoxamid	X12485649
Limit of Quantitation (LOQ)*	ECM	0.05 µg/g	
	ILV		
Limit of Detection (LOD)	ECM	0.015 µg/g	
	ILV		
Linearity (calibration curve r and concentration range) [1]	ECM	r = 0.9994425372 (Q, SD) r = 0.99961891019 (C, SD) r = 0.99959392256 (Q, CL) r = 0.99952196903 (C, CL) r = 0.99969453715 (Q, SL) r = 0.99960507494 (C, SL) r = 0.99954704 (Q, SIL) r = 0.99934630005 (C, SIL)	r = 0.99921670160 (Q, SD) r = 0.99916154008 (C, SD) r = 0.99921261200 (Q, CL) r = 0.99931788289 (C, CL) r = 0.99916157343 (Q, SL) r = 0.999175123 (C, SL) r = 0.99936423480 (Q, SIL) r = 0.99932073884 (C, SIL)
		0.100-80.0 ng/mL	
	ILV	r = 0.99813 (Q) r = 0.99836 (C)	r = 0.99960 (Q) r = 0.99994 (C)
		1.00-800 ng/mL	
Repeatable	ECM[2]	Yes at LOQ (0.05 µg/g) and 10×LOQ (0.5 µg/g) [three characterized soil matrices (clay loam, sandy loam, and silt loam) and one characterized sediment matrix (silt loam)]	
	ILV[3,4]	Yes at LOQ (0.05 µg/g) and 10×LOQ (0.5 µg/g) [one uncharacterized soil matrix (no soil texture reported)]	
Reproducible		Yes for 0.05 µg/g (LLMV)* and 0.5 µg/g in soil matrices	
Specific	ECM	Yes, matrix interferences were <1% of the LOQ (based on peak area). Minor peak tailing was observed.	**No**, matrix interferences were <1% of the LOQ (based on peak area); however, a significant nearby contaminant (peak height *ca.* **30-45%** of analyte peak height) was present at RT *ca.* 3.24 min. (florylpicoxamid) in all samples.[5]
	ILV	Yes, matrix interferences were <1% of the LOQ (based on peak area). Nearby minor baseline noise interfered with C peak integration and attenuation.	**No**, matrix interferences were <1% of the LOQ (based on peak area); however, a very significant nearby contaminant (peak height **>100%** of analyte peak height) was present at RT *ca.* 4.1 min. (florylpicoxamid).[6]

Data were obtained from p. 24; Appendix VI, p. 188 (LOQ/LOD); Tables 42-61, pp. 48-56 (recovery results); p. 21; Tables 2-17, pp. 28-35 (calibration coefficients); p. 22; Appendix II, Figures 1-4, pp. 81-88 (calibration curves); Appendix II, Figures 20-40, pp. 119-160 (chromatograms) of MRID 51074468; p. 8 (LOQ/LOD); p. 9 (recovery results); pp. 8, 22; Appendix C, Figures 1-44, pp. 24-47 (calibration curves & chromatograms) of MRID 51074469. Q = quantitation ion transition; C = confirmation ion transition; SD = Silt Loam Sediment; CL = Clay Loam Soil; SL = Sandy Loam Soil; SIL = Silt Loam Soil.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 Matrix-matched calibration standards were used in the ILV (p. 22 of MRID 51074469). Solvent-based calibration standards were reportedly used in the ECM, but the reviewer assumed that this was a typographical error and matrix-matched calibration standards were actually used in the ECM based on significant matrix effects (pp. 22, 24-25; Tables 96-97, pp. 75-76 of MRID 51074468).

2 In the ECM, silt loam sediment (Sediment 204219; 20% sand, 68% silt, 12% clay; pH 7.8 in 1:1 soil:water ratio; 8.9% organic matter (Walkley-Black); 5.2% organic carbon (Walkley-Black); cation exchange capacity 11.0 meq/100 g), clay loam soil (Soil 204956; 37% sand, 26% silt, 37% clay; pH 7.7 in 1:1 soil:water ratio; 3.7% organic matter (loss on ignition); 2.1% organic carbon (Walkley-Black); cation exchange capacity 10.3 meq/100 g), sandy loam soil (Soil 204957; 67% sand, 24% silt, 9% clay; pH 7.7 in 1:1 soil:water ratio; 3.2% organic matter (loss on ignition); 1.7%

organic carbon (Walkley-Black); cation exchange capacity 7.5 meq/100 g), and silt loam soil (Soil 204958; 15% sand, 62% silt, 23% clay; pH 5.8 in 1:1 soil:water ratio; 3.4% organic matter (loss on ignition); 1.7% organic carbon (Walkley-Black); cation exchange capacity 12.6 meq/100 g) were used in the study (p. 19; Appendix V, pp. 172-175 of MRID 51074468). The matrices were obtained from DowAgroSciences and characterized by Agvise Laboratories, Northwood, North Dakota.

3 In the ILV, soil (M 1132) was used in the study and provided by the Sponsor (p. 10 of MRID 51074469). The soil characterization data and soil texture were not reported in the study report; however, the reviewer noted that ECM Soil 204958 had the Sample ID AM Fischteich 07/19-M1132. It was possible that the ILV soil matrix was the same soil matrix as ECM Soil 204958.

4 The ILV validated the method for florylpicoxamid and X12485649 in soil in the first trial with minor modifications to the analytical parameters including the modification of one of the LC mobile phases (pp. 11, 13; Table 3, p. 23). The ILV modifications did not warrant an updated ECM, but more information should have been provided to discuss the reason that the ILV LC mobile phase was modified in both formic acid concentration and acetonitrile:methanol ratio.

5 See the following examples: Appendix II, Figure 23, p. 126, Figure 29, p. 138, Figure 33, p. 146, Figure 39, p. 158 of MRID 51074468.

6 See Appendix C, Figures 30-31, p. 40, and Figures 41-42, p. 46 of MRID 51074469.

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ (p. 24; Appendix VI, p. 188 of MRID 51074468; p. 8 of MRID 51074469). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for florylpicoxamid and X12485649 in the tested soil matrices (0.05 µg/g).

2. The specificity of the method was not acceptable for X12485649 based on ILV representative chromatograms due to a significant nearby contaminant (peak height *ca.* >100% of analyte peak height) which was present at RT *ca.* 4.1 min. in all samples (Appendix C, Figures 30-31, p. 40, and Figures 41-42, p. 46 of MRID 51074469). The reviewer considered this contaminant to be florylpicoxamid based on its RT (*ca*. 4.0 min; p. 11). The ILV study report did not address this contamination.

   The specificity of the method was not acceptable for X12485649 based on ECM representative chromatograms, as well. This contamination of X12485649 with florylpicoxamid (RT *ca*. 3.24) was also noted in the ECM representative chromatograms in significant amounts (*ca*. 30-45% of the analyte peak height; RT *ca.* 3.24 min; Appendix II, Figures 23-25, pp. 126-130, Figure 28-30, pp. 136-140, Figure 33-35, pp. 146-150, Figure 38-40, pp. 156-160; Appendix VI, p. 191 of MRID 51074468). The ECM study report did not address this contamination.

   In the ECM, the florylpicoxamid and X12485649 stock solutions were prepared separately (Appendix VI, pp. 188-189 of MRID 51074468). So, the presence of florylpicoxamid in ECM and ILV chromatograms of X12485649 was due to cross-contamination, experimental error, or degradation.

3. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method since only one uncharacterized soil matrix was tested. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low

organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil/soil matrix would need to be included in an ILV in order to cover the range of soils used in the submitted florylpicoxamid terrestrial field dissipation study MRID 51074418. The ILV soil (M 1132) was provided by the Sponsor, but the soil characterization data and soil texture were not reported in the study report (p. 10 of MRID 51074469). The reviewer noted that ECM Soil 204958 had the Sample ID AM Fischteich 07/19-M1132. It was possible that the ILV soil matrix was the same soil matrix as ECM Soil 204958.

In the submitted florylpicoxamid terrestrial field dissipation study MRID 51074418, the following four soils were used (disturbed soil data; Tables 24-27, pp. 76-79 of MRID 51074418): California loamy sand soil (0-6" depth; 2% clay, 0.34% organic carbon); Florida sand soil (BARE 0-6" depth; 7% clay, 0.38% organic carbon); North Dakota clay soil (0-6" depth; 55% clay, 2.00% organic carbon); and Ontario loam soil (0-6" depth; 16% clay, 1.30% organic carbon).

No sediment matrix was included in the ILV.

4. The ILV performed the ECM methods as written, except for minor modifications to the analytical parameters including the modification of one of the LC mobile phases (p. 11; Table 3, p. 23 of MRID 51074469). The ILV reported that mobile phase B was (B) **1.0**% formic acid in **acetonitrile:methanol (8:2, v:v)** and not **0.1**% formic acid in LC/MS grade **methanol:acetonitrile: (80:20, v:v)**. No comment about this mobile phase adjustment was reported in the ILV. The ILV modifications did not warrant an updated ECM, but more information should have been provided to discuss the reason that the ILV LC mobile phase was modified in both formic acid concentration and acetonitrile:methanol ratio. The reviewer did not know if this percentage difference was a typographical error. The reviewer also noted that the ILV study report did not provide a detailed account of its method, such as fortification solution and calibration solution preparation.

   The reviewer noted that, in the florylpicoxamid method validation in water (see accompanying DER), the ILV reported that mobile phase B was **1.0%** formic acid in **acetonitrile:methanol (8:2, v:v)** which was not the same as the ECM mobile phase B which was **0.1**% formic acid in LC/MS grade **acetonitrile:methanol (80:20, v:v**; p. 20; Appendix VI, pp. 161-162 of MRID 51074466; Table 5, p. 26 of MRID 51074467). The reviewer noted that, while the formic acid concentration changed, the acetonitrile:methanol ratio remained the same.

5. According to the ILV communication summary, the first trial samples were re-extracted since the incorrect quantitation mass transition was used in the first extraction (p. 14 of MRID 51074469). This re-extraction is still considered the first ILV trial.

6. The communications between the ILV study director (Christopher Skaggs, SGS North America, Inc.) and Dow AgroSciences sponsor representative (Leandro Ap. G. Deziderio) were reportedly documented but not summarized or included in the ILV study report (pp. 1, 6, 14 of MRID 51074469). Communications included communication of the successful ILV trial. Communications should be reported to ensure the independence of the ILV from the ECM. Leandro Deziderio was the Dow AgroSciences sponsor representative for the ECM (JRF America, Inc.), as well (p. 6 of MRID 51074468).

7. The reviewer noted that it would have been preferable to list the individual Appendices (Appendices A-E) in the ILV Table of Contents for ease of study material location (p. 7 of MRID 51074469).

8. The LOD samples were prepared in the ECM to show that the analytes were distinguishable from the untreated control samples at the LOD (pp. 23-24 of MRID 51074468).

9. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 24; Appendix VI, p. 188 of MRID 51074468; p. 8 of MRID 51074469). In the ECM and ILV, the LOQ was not justified or further defined. In the ILV, the LOD value was defined as 30% of the LOQ. No justification for LOD was reported in the ECM. No calculations or comparisons to background noise were reported for LOQ or LOD in the ECM or ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

   Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

10. Significant (>20%) matrix effects were reported in the ECM (pp. 24-25; Tables 96-97, pp. 75-76 of MRID 51074468). Matrix-matched calibration standards were used in the ILV (p. 22 of MRID 51074469). Solvent-based calibration standards were reportedly used in the ECM, but the reviewer assumed that this was a typographical error and matrix-matched calibration standards were actually used in the ECM based on significant matrix effects (pp. 22, 24-25; Tables 96-97, pp. 75-76 of MRID 51074468).

11. Stability of florylpicoxamid (XDE-659) and X12485649 stock standard solution was reported as six months (Appendix VI, p. 189 of MRID 51074468). Stability of florylpicoxamid (XDE-659) and X12485649 calibration standards and fortification solutions was reported as 1 month.

12. The total time required to complete one set of 14 samples was reported as 8 working hours in the ECM (Appendix VI, p. 190 of MRID 51074468). No time requirement for the method was included in the ILV.

V. References

U.S. Environmental Protection Agency. 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:

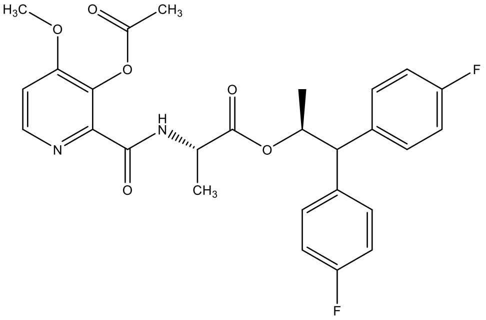
https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

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

Florylpicoxamid (XDE-659)

IUPAC Name:	(1*S*)-2,2-Bis(4-fluorophenyl)-1-methylethyl *N*-[(3-acetoxy-4-methoxy-2-pyridyl)carbonyl]-L-alaninate
CAS Name:	(1*S*)-2,2-Bis(4-fluorophenyl)-1-methylethyl *N*-[[3-(acetyloxy)-4-methoxy-2-pyridinyl]carbonyl]-L-alaninate
CAS Number:	1961312-55-9
SMILES String:	COC1=C(OC(C)=O)C(C(N[C@@H](C)C(O[C@@H](C)C(C2=CC=C(F)C=C2)C3=CC=C(F)C=C3)=O)=O)=NC=C1



X12485649

IUPAC Name:	(2S)-1,1-Bis(4-fluorophenyl)propan-2-yl N-[(3-hydroxy-4-methoxypyridin-2-yl)carbonyl]-L-alaninate
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	OC1=C(OC)C=CN=C1C(N[C@@H](C)C(O[C@@H](C)C(C2=CC=C(F)C=C2)C3=CC=C(F)C=C3)=O)=O