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

Reports:	ECM: EPA MRID No.: 51074466. Baum, E. 2020. Method Validation of XDE-659 and its Metabolite in Water. Report prepared by JRF America, Inc., Audubon, Pennsylvania, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 271 pages. JRF America Study No.: AU-2019-11. Dow AgroSciences Study ID: 180503. Final report issued January 15, 2020. ILV: EPA MRID No.: 51074467. Skaggs, C., and P. Afedi. 2020. Independent Laboratory Validation of XDE-659 and Metabolite in Water. Report prepared by SGS North America, Inc., Brookings, South Dakota, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 380 pages. Performing Laboratory Study No.: SGS-19-01-14. Dow AgroSciences Study ID: 190826. Final report issued February 12, 2020.	
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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 51074466). 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 of MRID 51074467). 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 representative chromatograms. The ILV study report did not provide a detailed account of its method.	
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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
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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-2019-11 and Dow AgroSciences Study ID 180503, is designed for the quantitative determination of florylpicoxamid (XDE-659) and X12485649 in water at the stated LOQ of 0.10 µg/L using LC/MS/MS. The LOQ is **less than** the lowest toxicological level of concern in water for florylpicoxamid (0.382 µg/L; LOAEC; MRID 51074440) and X12485649 (0.72 µg/L; NOAEC; MRID 51074438). 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 water matrices (0.10 µg/L).

The ECM and ILV validated the method using different characterized surface, drinking (tap), and ground water matrices. The ILV validated the method for florylpicoxamid and X12485649 in water in the first trial with minor modifications to the analytical parameters. The ILV modifications did not warrant an updated ECM. All ILV and ECM data regarding repeatability, accuracy, precision, and linearity were satisfactory for florylpicoxamid and X12485649 in test water matrices. All ILV and ECM data regarding specificity was satisfactory for florylpicoxamid in test water matrices. The specificity of the method was not acceptable for X12485649 based on ILV representative chromatograms due to a significant nearby contaminant (florylpicoxamid; peak height *ca*. 60-120% of analyte peak height) which was present in all samples. This contamination of X12485649 with florylpicoxamid was also noted in the ECM representative chromatograms; however, the contamination was only observed in minor amounts (*ca*. 30% of the analyte peak height). 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 or experimental error.

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)	51074466[1]	51074467[2]		Water	15/01/2020	Dow AgroSciences LLC	LC/MS/MS	0.10 µg/L
X12485649								

[1] In the ECM, surface water (#204220; pH 7.0, hardness 44 mg equiv. $CaCO_3$/L, conductivity 0.13 mmhos/cm, dissolved organic carbon 3.9 ppm), drinking (tap) water (#204960; pH 8.0, hardness 494 mg equiv. $CaCO_3$/L, conductivity 1.07 mmhos/cm, dissolved organic carbon 0.8 ppm) and groundwater (#204962; pH 7.8, hardness 138 mg equiv. $CaCO_3$/L, conductivity 0.44 mmhos/cm, dissolved organic carbon 2.3 ppm) were used in the study (Appendix IV, pp. 135-137 of MRID 51074466). The surface water was obtained from Site D- Emperor Lake, Chatsworth, Derbyshire, United Kingdom (p. 19). The ground water was obtained from Bennett Residence, West Chester, Pennsylvania. The drinking water was obtained from JRF America, Audubon, Pennsylvania. The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

[2] In the ILV, surface (pond/lake) water (pH 8.0, hardness 265 mg equiv. $CaCO_3$/L, conductivity 0.53 mmhos/cm), drinking (tap) water (pH 8.1, hardness 134 mg equiv. $CaCO_3$/L, conductivity 0.32 mmhos/cm) and ground (well) water (pH 8.1, hardness 504 mg equiv. $CaCO_3$/L, conductivity 0.90 mmhos/cm) were used in the study (Appendix E, pp. 378-380 of MRID 51074467). The control samples were collected by SGS (p. 11). The tap water came from the SGS GLP laboratory (44.3220743, -96.7554363) the well water from SGS Brookings field site (44.304195, -96.667977), and the surface water from a lake in Brookings, SD (44.266182, -96.761461). The water matrices were

characterized by Agvise Laboratories, Northwood, North Dakota.

I. Principle of the Method

Water samples (10 mL) were fortified (0.010 or 0.10 µL of 100 ng/mL fortification solution) and shaken by hand for *ca*. 10 seconds (20:80, v:v; pp. 17-19; Appendix VI, pp. 155, 158-161 of MRID 51074466). An aliquot was taken for LC/MS/MS analysis. The method noted that samples should be analyzed within seven days after extraction. The ECM identified the use of matrix-matched standards as a critical step of the method (pp. 24-25).

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. 161-163 of MRID 51074466). 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 acetonitrile:methanol (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 or 10 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 2.90 minutes for florylpicoxamid and X12485649, respectively.

The ILV performed the ECM methods as written, except for minor modifications to the analytical parameters (p. 12; Table 5, p. 26 of MRID 51074467). 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 µ; 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.

The Limit of Quantification (LOQ) for florylpicoxamid and X12485649 in water was 0.10 µg/L in the ECM and the ILV (p. 17; Appendix VI, p. 154 of MRID 51074466; p. 8 of MRID 51074467). In the ECM, the Limit of Detection (LOD) for florylpicoxamid and X12485649 was set to 0.03 µg/L 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 51074466): 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 0.10 µg/L (LOQ) and 1.0 µg/L (10×LOQ) in three water matrices (Tables 28-55, pp. 36-47). 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 112-129% and 103-132% for florylpicoxamid and X12485649, respectively. The surface water (#204220; pH 7.0, hardness 44 mg equiv. $CaCO_3$/L, conductivity 0.13 mmhos/cm, dissolved organic carbon 3.9 ppm), drinking (tap) water (#204960; pH 8.0, hardness 494 mg equiv. $CaCO_3$/L, conductivity 1.07 mmhos/cm, dissolved organic carbon 0.8 ppm) and groundwater (#204962; pH 7.8, hardness 138 mg equiv. $CaCO_3$/L, conductivity 0.44 mmhos/cm, dissolved organic carbon 2.3 ppm) were used in the study (Appendix IV, pp. 135-137). The surface water was obtained from Site D- Emperor Lake, Chatsworth, Derbyshire, United Kingdom (p. 19). The ground water was obtained from Bennett Residence, West Chester, Pennsylvania. The drinking water was obtained from JRF America, Audubon, Pennsylvania. The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 51074467): Mean recoveries and RSDs were within guidelines for analysis of florylpicoxamid and X12485649 at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ) in three water matrices (pp. 13-14). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The surface (pond/lake) water (pH 8.0, hardness 265 mg equiv. $CaCO_3$/L, conductivity 0.53 mmhos/cm), drinking (tap) water (pH 8.1, hardness 134 mg equiv. $CaCO_3$/L, conductivity 0.32 mmhos/cm) and ground (well) water (pH 8.1, hardness 504 mg equiv. $CaCO_3$/L, conductivity 0.90 mmhos/cm) were used in the study (Appendix E, pp. 378-380). The control samples were collected by SGS (p. 11). The tap water came from the SGS GLP laboratory (44.3220743, -96.7554363) the well water from SGS Brookings field site (44.304195, -96.667977), and the surface water from a lake in Brookings, SD (44.266182, -96.761461). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota. The method for florylpicoxamid and X12485649 in water was validated in the first trial with minor modifications to the analytical parameters (pp. 12, 15; Table 5, p. 26). The ILV modifications did not warrant an updated ECM.

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

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water					
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.03 (LOD)	1	120	--[3]	--	--
	0.10 (LOQ)	5	102-107	105	2	2
	1.0	5	91-95	92	2	2
X12485649	0.03 (LOD)	1	115	--	--	--
	0.10 (LOQ)	5	100-107	103	3	2
	1.0	5	85-90	87	2	2
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.03 (LOD)	1	129	--	--	--
	0.10 (LOQ)	5	98-108	103	4	4
	1.0	5	85-93	91	3	3
X12485649	0.03 (LOD)	1	132	--	--	--
	0.10 (LOQ)	5	102-106	104	1	1
	1.0	5	84-86	85	1	1
	Drinking (Tap) Water					
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.03 (LOD)	1	114	--	--	--
	0.10 (LOQ)	5	78-104	91	10	11
	1.0	5	92-98	95	2	2
X12485649	0.03 (LOD)	1	105	--	--	--
	0.10 (LOQ)	5	80-107	94	10	10
	1.0	5	92-99	96	2	2
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.03 (LOD)	1	113	--	--	--
	0.10 (LOQ)	5	78-106	94	12	13
	1.0	5	92-101	97	4	4
X12485649	0.03 (LOD)	1	112	--	--	--
	0.10 (LOQ)	5	81-103	93	8	9
	1.0	5	94-99	97	2	2
	Groundwater					
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.03 (LOD)	1	112	--	--	--
	0.10 (LOQ)	5	106-110	107	2	1
	1.0	5	99-102	100	2	2
X12485649	0.03 (LOD)	1	103	--	--	--
	0.10 (LOQ)	5	107-111	109	2	2
	1.0	5	90-92	91	1	1
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.03 (LOD)	1	112	--	--	--
	0.10 (LOQ)	5	105-113	110	4	4
	1.0	5	95-99	97	2	2
X12485649	0.03 (LOD)	1	103	--	--	--
	0.10 (LOQ)	5	106-111	108	2	2
	1.0	5	89-92	90.4	1	1

Data (uncorrected recovery results; pp. 21-23; Appendix VI, pp. 163-164) were obtained from Tables 28-55, pp. 36-47 of MRID 51074466.

1 The surface water (#204220; pH 7.0, hardness 44 mg equiv. $CaCO_3$/L, conductivity 0.13 mmhos/cm, dissolved organic carbon 3.9 ppm), drinking (tap) water (#204960; pH 8.0, hardness 494 mg equiv. $CaCO_3$/L, conductivity 1.07 mmhos/cm, dissolved organic carbon 0.8 ppm) and groundwater (#204962; pH 7.8, hardness 138 mg equiv. $CaCO_3$/L, conductivity 0.44 mmhos/cm, dissolved organic carbon 2.3 ppm) were used in the study (Appendix IV, pp. 135-137). The surface water was obtained from Site D- Emperor Lake, Chatsworth, Derbyshire, United Kingdom (p. 19). The ground water was obtained from Bennett Residence, West Chester, Pennsylvania. The drinking water was obtained from JRF America, Audubon, Pennsylvania. The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

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 Water[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Surface (Pond/Lake) Water					
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.10 (LOQ)	5	70-80	73	3.8	5.2
	1.0	5	75-92	84	6.8	8.1
X12485649	0.10 (LOQ)	5	72-78	76	2.7	3.5
	1.0	5	75-80	78	2.2	2.8
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.10 (LOQ)	5	72-75	73	1.5	2.1
	1.0	5	78-88	83	4.6	5.5
X12485649	0.10 (LOQ)	5	73-79	76	2.5	3.4
	1.0	5	76-80	78	1.8	2.3
	Drinking (Tap) Water					
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.10 (LOQ)	5	78-92	85	6.5	7.7
	1.0	5	86-91	88	2.2	2.5
X12485649	0.10 (LOQ)	5	78-86	82	3.6	4.3
	1.0	5	79-87	84	3.1	3.7
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.10 (LOQ)	5	77-92	85	7.0	8.3
	1.0	5	84-94	88	3.6	4.1
X12485649	0.10 (LOQ)	5	76-85	80	3.3	4.1
	1.0	5	80-85	83	1.9	2.3
	Ground (Well) Water					
	Quantitation ion transition					
Florylpicoxamid (XDE-659)	0.10 (LOQ)	5	71-91	85	8.1	9.6
	1.0	5	94-101	96	2.9	3.0
X12485649	0.10 (LOQ)	5	75-88	81	5.0	6.2
	1.0	5	82-92	87	3.7	4.3
	Confirmation ion transition					
Florylpicoxamid (XDE-659)	0.10 (LOQ)	5	72-88	83	6.8	8.2
	1.0	5	87-103	95	5.7	6.0
X12485649	0.10 (LOQ)	5	78-91	86	4.8	5.6
	1.0	5	87-100	94	5.0	5.3

Data (uncorrected recovery results; p. 13) were obtained from pp. 13-14 of MRID 51074467.

1 The surface (pond/lake) water (pH 8.0, hardness 265 mg equiv. $CaCO_3$/L, conductivity 0.53 mmhos/cm), drinking (tap) water (pH 8.1, hardness 134 mg equiv. $CaCO_3$/L, conductivity 0.32 mmhos/cm) and ground (well) water (pH 8.1, hardness 504 mg equiv. $CaCO_3$/L, conductivity 0.90 mmhos/cm) were used in the study (Appendix E, pp. 378-380). The control samples were collected by SGS (p. 11). The tap water came from the SGS GLP laboratory (44.3220743, -96.7554363) the well water from SGS Brookings field site (44.304195, -96.667977), and the surface water from a lake in Brookings, SD (44.266182, -96.761461). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

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 water was 0.10 µg/L in the ECM and the ILV (p. 17; Appendix VI, p. 154 of MRID 51074466; p. 8 of MRID 51074467). In the ECM and ILV, the LOQ was not justified or further defined. The LOD for florylpicoxamid and X12485649 was set to 0.03 µg/L 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 Water

		Florylpicoxamid	**X12485649**
Limit of Quantitation (LOQ)*	ECM	0.10 µg/L	
	ILV		
Limit of Detection (LOD)	ECM	0.03 µg/L	
	ILV		
Linearity (calibration curve r and concentration range) [1]	ECM	r = 0.99805543963 (Q, SW) r = 0.99736212018 (C, SW) r = 0.99937610298 (Q, DW) r = 0.99925660996 (C, DW) r = 0.9993296959 (Q, GW) r = 0.99894319134 (C, GW)	r = 0.99790074513 (Q, SW) r = 0.99742644223 (C, SW) r = 0.9993710298 (Q, DW) r = 0.99939497528 (C, DW) r = 0.99914086769 (Q, GW) r = 0.99908194066 (C, GW)
	ILV	r = 0.99971 (Q, SW) r = 0.99985 (C, SW) r = 0.99890 (Q, DW) r = 0.99876 (C, DW) r = 0.99895 (Q, GW) r = 0.99865 (C, GW)	r = 0.99901 (Q, SW) r = 0.99896 (C, SW) r = 0.99892 (Q, DW) r = 0.99895 (C, DW) r = 0.99969 (Q, GW) r = 0.99531 (C, GW)
	Range	0.01-2.0 ng/mL	
Repeatable	ECM[2]	Yes at LOQ (0.10 µg/L) and 10×LOQ (1.0 µg/L) (three characterized water matrices – surface water, drinking water, and groundwater)	
	ILV[3,4]		
Reproducible		Yes for 0.10 µg/L (LLMV)* and 1.0 µg/L in water matrices	
Specific	ECM	Yes, matrix interferences were <2% of the LOQ (based on peak area). Minor peak tailing was observed.	Yes, matrix interferences were <1% of the LOQ (based on peak area). A minor nearby contaminant (peak height *ca*. 30% of analyte peak height) was present at RT *ca*. 3.23-3.25 min. (florylpicoxamid) in all samples.[5]
	ILV	Yes, no matrix interferences were observed. 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 significant nearby contaminant (peak height *ca*. **60-120%** of analyte peak height) was present at RT *ca*. 4.1 min. (florylpicoxamid) in all samples.[6]

Data were obtained from p. 17; Appendix VI, p. 154 (LOQ/LOD); Tables 28-55, pp. 36-47 (recovery results); Tables 26-47, pp. 36-44 (calibration coefficients); p. 22; Appendix II, Figures 1-3, pp. 69-74; Appendix VI, pp. 159-160 (calibration curves); Appendix II, Figures 4-34, pp. 75-131 (chromatograms) of MRID 51074466; p. 8 (LOQ/LOD); pp. 13-14 (recovery results); p. 8; Appendix C, Figures 1-139, pp. 27-106 (calibration curves & chromatograms) of MRID 51074467. Q = quantitation ion transition; C = confirmation ion transition; SW = Surface Water; DW = Drinking Water; GW = Groundwater.

* 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 ECM and ILV (p. 22 of MRID 51074466; pp. 8, 15 of MRID 51074467).

[2] In the ECM, surface water (#204220; pH 7.0, hardness 44 mg equiv. CaCO$_3$/L, conductivity 0.13 mmhos/cm, dissolved organic carbon 3.9 ppm), drinking (tap) water (#204960; pH 8.0, hardness 494 mg equiv. CaCO$_3$/L, conductivity 1.07 mmhos/cm, dissolved organic carbon 0.8 ppm) and groundwater (#204962; pH 7.8, hardness 138 mg equiv. CaCO$_3$/L, conductivity 0.44 mmhos/cm, dissolved organic carbon 2.3 ppm) were used in the study (Appendix IV, pp. 135-137 of MRID 51074466). The surface water was obtained from Site D- Emperor Lake, Chatsworth, Derbyshire, United Kingdom (p. 19). The ground water was obtained from Bennett Residence, West Chester, Pennsylvania. The drinking water was obtained from JRF America, Audubon, Pennsylvania. The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

[3] In the ILV, surface (pond/lake) water (pH 8.0, hardness 265 mg equiv. CaCO$_3$/L, conductivity 0.53 mmhos/cm),

drinking (tap) water (pH 8.1, hardness 134 mg equiv. $CaCO_3$/L, conductivity 0.32 mmhos/cm) and ground (well) water (pH 8.1, hardness 504 mg equiv. $CaCO_3$/L, conductivity 0.90 mmhos/cm) were used in the study (Appendix E, pp. 378-380 of MRID 51074467). The control samples were collected by SGS (p. 11). The tap water came from the SGS GLP laboratory (44.3220743, -96.7554363) the well water from SGS Brookings field site (44.304195, -96.667977), and the surface water from a lake in Brookings, SD (44.266182, -96.761461). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota.

4 The ILV validated the method for florylpicoxamid and X12485649 in water in the first trial with minor modifications to the analytical parameters (pp. 12, 15; Table 5, p. 26 of MRID 51074467). The ILV modifications did not warrant an updated ECM.

5 See the following examples: Appendix II, Figure 22, p. 109, Figure 29, p. 121, Figure 32, p. 127 of MRID 51074466.

6 See the following examples: Appendix C, Figures 33-34, pp. 45-46, Figures 81-82, pp. 73-74, Figures 125-126, pp. 98-99 of MRID 51074467.

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. 17; Appendix VI, p. 154 of MRID 51074466; p. 8 of MRID 51074467). 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 water matrices (0.10 µg/L).

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*. 60-120% of analyte peak height) which was present at RT *ca*. 4.1 min. in all samples (Appendix C, Figures 33-34, pp. 45-46, Figures 45-46, pp. 52-53, Figures 81-82, pp. 73-74, Figures 93-94, pp. 80-81, Figures 125-126, pp. 98-99, Figures 136-137, p. 105 of MRID 51074467). The reviewer considered this contaminant to be florylpicoxamid based on its RT (*ca*. 4.0 min; p. 12). The ILV study report did not address this contamination.

   This contamination of X12485649 with florylpicoxamid (RT *ca*. 3.24) was also noted in the ECM representative chromatograms; however, the contamination was only observed in minor amounts (*ca*. 30% of the analyte peak height; RT *ca*. 3.23-3.25 min; Appendix II, Figure 22, p. 109, Figure 29, p. 121, Figure 32, p. 127; Appendix VI, p. 161 of MRID 51074466). The ECM study report did not address this contamination.

   In the ECM, the florylpicoxamid and X12485649 stock solutions were prepared separately (Appendix VI, pp. 158-159 of MRID 51074466). So, the presence of florylpicoxamid in ECM and ILV chromatograms of X12485649 was due to cross-contamination or experimental error.

3. The ILV performed the ECM methods as written, except for minor modifications to the analytical parameters (p. 12; Table 5, p. 26 of MRID 51074467). 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). No comment about this mobile phase adjustment was reported in the ILV. 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.

4. Characterized surface, drinking (tap), and ground water matrices were used in the ECM and ILV (of MRID 51074466). The ECM and ILV used different matrices.

5. 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, 16 of MRID 51074467). Communications included the communication of the completion 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 51074466).

6. The reviewer noted that the integrated peak in the ECM XDE-659 confirmatory transition chromatogram of UTC 2 for ground water (RT 3.55-3.80 min.) did not correspond to the RT of XDE-659 (RT *ca*. 3.24 min.; Appendix II, Figure 31, p. 124 of MRID 51074466). This matrix interference was insignificant.

7. The reviewer noted the following typographical error in the ILV Table of Contents: the page number for the “Appendices” was reported as “17” instead of “19” (p. 7 of MRID 51074467). The reviewer also 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.

8. The LOD samples were prepared in the ECM to show that the analytes were distinguishable from the untreated control samples at the LOD (p. 23 of MRID 51074466).

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. 17; Appendix VI, p. 154 of MRID 51074466; p. 8 of MRID 51074467). 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 86-88, pp. 60-62 of MRID 51074466). Matrix-matched calibration standards were used in the ECM and ILV (p. 22 of MRID 51074466; pp. 8, 15; Appendix C, Figures 1-139, pp. 27-106 of MRID 51074467).

11. Stability of XDE-659 and X12485649 stock standard solution was reported as six months (Appendix VI, p. 161 of MRID 51074466). Stability of 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. 161 of MRID 51074466). 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.

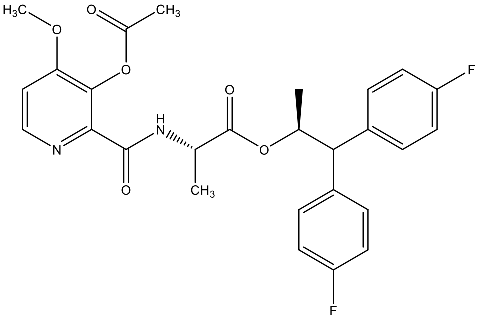
USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency.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

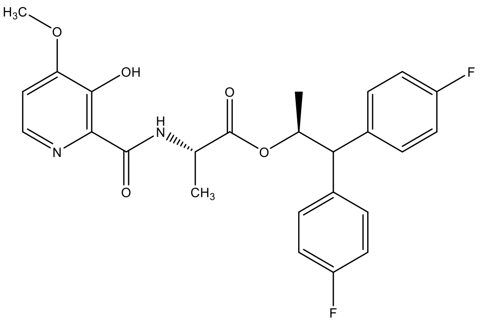
IUPAC Name:	(1*S*)-2,2-Bis(4-fluorophenyl)-1-methylethyl *N*-[(3-acetoxy-4-methoxy-2-pyridyl)carbonyl]-L-alaninate
CAS Name:	(1*S*)-2,2-Bis(4-fluorophenyl)-1-methylethyl *N*-[[3-(acetyloxy)-4-methoxy-2-pyridinyl]carbonyl]-L-alaninate
CAS Number:	1961312-55-9
SMILES String:	COC1=C(OC(C)=O)C(C(N[C@@H](C)C(O[C@@H](C)C(C2=CC=C(F)C=C2)C3=CC=C(F)C=C3)=O)=O)=NC=C1



Structure shown above is equivalent to that shown on Appendix VI, p. 154 of MRID 51074466.

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



Structure shown above is equivalent to that shown on Appendix VI, p. 155 of MRID 51074466.