Test Material:	XDE-848 Benzyl Ester
MRID:	49677801
Title:	Method Validation Study for the Determination of Residues of XDE-848 and Five Metabolites (X11438848, X12300837, X11966341, X12131932 and X12393505) in Ground, Surface, and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry
MRID:	49677802
Title:	Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of XDE-848 Benzyl Ester and Five Metabolites (X11438848, X12300837, X11966341, X12131932 and X12393505) in Water
EPA PC Code:	030093
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 2/26/16

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 2/26/16

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 2/26/16

Analytical method for XDE-848 benzyl ester and its transformation products, X11438848, X12300837, X11966341, X12131932 and X12393505, in surface, ground and drinking water

Reports: ECM: EPA MRID No.: 49677801. Huang, T-Y, M.J. Walter. 2015. Method Validation Study for the Determination of Residues of XDE-848 and Five Metabolites (X11438848, X12300837, X11966341, X12131932 and X12393505) in Ground, Surface, and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry. Laboratory Study ID: 140962. Report prepared, sponsored and submitted by Regulatory Sciences and Government Affairs, Dow AgroSciences LLC, Indianapolis, Indiana; 267 pages. Final report issued August 6, 2015.

ILV: EPA MRID No. 49677802. Austin, R. 2015. Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of XDE-848 Benzyl Ester and Five Metabolites (X11438848, X12300837, X11966341, X12131932 and X12393505) in Water. Dow AgroSciences Protocol No.: 140962. Battelle Study No.: YR/14/027. Report prepared by Battelle UK Ltd., Essex, United Kingdom, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 176 pages. Final report issued July 31, 2015.

Document No.: MRIDs 49677801 & 49677802

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP; 1998; p. 3 of MRID 49677801). 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 and OECD GLP standards (1998), as well as the UK Department of Health (Directive 2004/9/EC; p. 3; Appendix 3, p. 176 of MRID 49677802). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-4; Appendix 3, p. 176). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).

Classification: This analytical method is considered supplemental. In the ILV, no samples were prepared at 10×LOQ. In the ILV, chromatograms for three of the six analytes showed matrix interferences which affected the peak attenuation of the analyte in all matrices. The sets of representative chromatograms were not complete in the ECM and ILV. A new ILV, including testing at LOQ and 10×LOQ is required. Further, representative chromatograms are needed on the ECM.

PC Code: 030093

Reviewer: José Meléndez **Date:** November 10, 2016

Signature: JOSE MELENDEZ Digitally signed by JOSE MELENDEZ DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=JOSE MELENDEZ, dnQualifier=0000008590 Date: 2016.11.10 06:56:13 -05'00'

Executive Summary

The analytical method, DowAgroSciences Protocol No. 140962, is designed for the quantitative determination of florpyrauxifen-benzyl (XDE-848; XDE-848 BE; XDE-848 benzyl ester) in drinking, ground and surface water matrices at the LOQ of 0.02 µg/L using LC/MS/MS and the five metabolites X11966341, X12131932, X12393505, X12300837, and X11438848 in drinking, ground and surface water matrices at the LOQ of 0.05 µg/L using LC/MS/MS. The LOQ is equal to the lowest toxicological level of concern in water for XDE-848 benzyl ester[1]. The LOQs are less than the lowest toxicological level of concern in water for XDE-848 benzyl ester's five metabolites[2]. The ECM was validated by the ILV in the first trial for all six analytes in all three matrices with insignificant modifications to the analytical parameters; however, no samples were prepared at 10×LOQ in the ILV. The water matrices were adequately characterized in the ECM and ILV. In chromatograms for all matrices of the ILV, peak attenuation of XDE-848 and X12131932 was disrupted at the peak base by a nearby significant contaminant, and minor baseline noise disrupted peak attenuation of X11966341. Sample recoveries were corrected in the ECM.

Table 1. Analytical Method Summary[1,2,3]

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						
Florpyrauxifen-benzyl (X11959130)	49677801	49677802		Water	06/08/2015	Dow AgroSciences LLC	LC/MS/MS	0.02 µg/L (0.02 ng/mL)
XDE-848 hydroxy acid (X11966341)								0.05 µg/L (0.05 ng/mL)
Des-chloro XDE-848 benzyl ester (X12131932)								
Des-chloro XDE-848 acid (X12393505)								
XDE-848 benzyl hydroxy (X12300837)								
XDE-848 acid (X11438848)								

1 Florpyrauxifen-benzyl = [XDE-848; XDE-848 BE; XDE-848 benzyl ester; TSN301734; X11959130; SX-1552; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; X11966341 = [XDE-848 hydroxy acid; TSN305649; 1552-OHA; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylic acid]; X12131932 = [Dechlorinated XDE-848 benzyl ester; TSN305649; 1552-DBE;

1 The lowest toxicological level of concern is IC_{50} = 0.0162 µg a.i./L ~ 0.02 µg/L, for XDE-848 benzyl ester, for Eurasian Watermilfoil (MRID 49677805).

2 The lowest toxicological level of concern for the degradates appears to be an IC_{50} = 0.497 µg a.i./L ~ 0.5 µg/L, for XDE-848 acid, for Eurasian Watermilfoil (MRID 49677806).

benzyl 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; X12393505 = [Dechlorinated XDE-848 acid; TSN304479; 1552-DA; 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid]; X12300837 = [XDE-848 hydroxy benzyl ester; TSN305650; 1552-OHBE; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylate]; and X11438848 = [XDE-848 acid; TSN301691; 1552-Acid; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid].

2 For the ECM, drinking (tap) water (001-0001; pH 8.2, dissolved organic carbon 2.7 ppm), surface (pond) water (002-0001; pH 7.9, dissolved organic carbon 3.3 ppm), and ground (monitoring well) water (003-0001; pH 8.2, dissolved organic carbon 1.8 ppm) were used (p. 17 of MRID 49677801).

3 For the ILV, surface water (15/003 Surface H_2O Res; pH 8.0, dissolved organic carbon 3.1 ppm), ground water (12/044 Highland Spring; pH 8.2, dissolved organic carbon 0.1 ppm), and drinking water (15/002 Drinking H_2O Res; pH 8.1, dissolved organic carbon 3.7 ppm) were used **(**p. 17; Appendix 2, pp. 171-175 of MRID 49677802).

I. Principle of the Method

During the entire procedure, no plastic or Nalgene should be used (critical step; Appendix I, p. 262 of MRID 49677801). Samples (10 mL) of water in 45-mL glass vials were fortified, as necessary, then acidified with 100 µL of formic acid via vortex (5 seconds; p. 16; Appendix I, pp. 253-254, 263-266). The sample was mixed with 500 µL of acetonitrile:methanol (50:50, v:v) via vortex (5 seconds). The sample was purified using an Oasis HLB solid phase extraction (SPE) cartridge (60-mg, 3-mL). The SPE column was pre-conditioned with acetonitrile: methanol (50:50, v:v) and water containing 0.1% formic acid (3 mL) with full vacuum for 5 seconds after each elution. The sample was applied to the column (*ca*. 0.5 mL/min rate) with full vacuum for 5 seconds after elution; the eluate was discarded. The vial was rinsed with 1.0 mL of HPLC grade water, and the water was applied to the column (*ca*. 1 mL/min rate), with full vacuum for 5 seconds after elution. After drying the column with full vacuum for 5 minutes, the analytes were eluted with 4 x 1.5-mL aliquots as follows: 2 x 0.5-mL aliquots of acetonitrile: methanol (50:50, v:v) were added to the sample vial for rinsing then applied to the SPE column; 1 x 0.5-mL aliquots of acetonitrile were added to the sample vial for rinsing then applied to the SPE column; and 1 x 0.5-mL aliquots of acetonitrile:methanol (50:50, v:v) were applied directly to the SPE column. The analytes were eluted via gravity with full vacuum for 5 seconds after each elution. The keeper [50 µL of glycerol:methanol (10:90, v:v)] and mixed internal standard (100 µL of 0.01 µg/mL mixed XDE-848 internal standard) was added to the eluate. After vortexing gently, the sample was evaporated to dryness on a Turbo-Vap set at 40°C with 7 psi nitrogen (approximately 30-35 minutes). The residue was reconstituted with 1000 µL of acetonitrile:methanol:formic acid (50:50:0.1, v:v:v). After vortexing, 1000 µL of water containing 0.1% formic acid was added to the sample. After vortexing for 5 seconds, an aliquot of the sample was transferred to a LC/MS/MS vial for analysis.

The method noted that XDE-848 benzyl ester has carryover so a blank injection is required after each sample with concentrations above 5 ng/mL (Appendix I, pp. 265-266 of MRID 49677801). Additionally, the method noted that 1) the water volume could be adjusted as long as all proportions remain the same; 2) samples which are not clear may require filtration prior to SPE (example filter, Filter Aid 400, 3M Empore); and 3) all stock, fortification and calibration solutions should be stored in the refrigerator.

Samples were analyzed for XDE-848 and its metabolites using an Agilent 1290 Infinity LC system coupled to an AB Sciex API5500 LC/MS/MS (Appendix I, pp. 254, 260-261 of MRID 49677801). The instrumental conditions consisted of a Phenomenex Kinetex 1.7µ PFP 100A column (100 x 2.1 mm, 1.7-µm; column temperature ambient, *ca*. 20°C), Phenomenex SecurityGuard ULTRA UHPLC PFP for 2.1 ID columns, a gradient mobile phase of (A) water containing 0.1% formic acid and (B) methanol containing 0.1% formic acid [percent A:B (v:v) at -3.0-0.0 min. 50.0:50.0, 7.0-9.0 min. 0.0:100.0], MS/MS detection in positive electrospray mode MS (MRM; temperature, 500°C), and injection volume 15 µL. Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* 441 → 91 and *m/z* 439 → 91 for XDE-848 BE; *m/z* 335 → 254 and *m/z* 337 → 256 for X11966341; *m/z* 405 → 65 and *m/z* 407 → 91 for X12131932; *m/z* 315 → 234 and *m/z* 315 → 124 for X12393505; *m/z* 425 → 91 and *m/z* 427 → 91 for X12300837; and *m/z* 349 → 268 and *m/z* 351 → 270 for X11438848 (Tables 20-31, pp. 68-70; Appendix I, p. 261). Retention times were

observed at *ca*. 5.5, 1.9, 5.3, 2.2, 4.75, and 3.0 min. for XDE-848 BE, X11966341, X12131932, X12393505, X12300837, and X11438848, respectively (Figure 45, pp. 188-191).

In the ILV, the sample processing of the ECM was performed exactly as written (pp. 17-18; Appendix 1, pp. 164-168 of MRID 49677802). Samples were analyzed for XDE-848 and its metabolites using an Agilent 1290 Infinity LC system coupled to an AB Sciex QTRAP 6500 LC/MS/MS. All instrumental parameters were the same, except for the following: gradient of mobile phase [percent A:B (v:v) at 0.00 min. 50:50, 7.00-9.00 min. 0:100, 9.10-12.00 min. 50:50], MS/MS detection in positive Turbo Spray IonDrive mode MS (MRM; temperature, 500°C), and injection volume 30 µL. Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* **439 → 91** and *m/z* **441 → 91** for XDE-848 BE; *m/z* 335 → 254 and *m/z* 337 → 256 for X11966341; *m/z* 405 → 65 and *m/z* 407 → 91 for X12131932; *m/z* 315 → 234 and *m/z* 315 → 124 for X12393505; *m/z* **427 → 91** and *m/z* **425 → 91** for X12300837; and *m/z* **351 → 270** and *m/z* **349 → 268** for X11438848 (**bolded** transitions were those for which the quantification and confirmation ions transitions were reversed from the ECM). Retention times were observed at *ca*. 6.0, 2.0, 5.8, 2.5, 5.3, and 3.4 min. for XDE-848 BE, X11966341, X12131932, X12393505, X12300837, and X11438848, respectively (Figures 34-39, pp. 121-126; Appendix 1, p. 169). The ILV study author noted that the increase in the injection volume was due to poor sensitivity at the lower injection volume (p. 22). None of the minor ILV modifications to the instrumental parameters had an effect on the outcome of the study.

LOQ/LOD

The LOQ and LOD in the ECM and ILV were 0.02 µg/L and 0.006 µg/L, respectively, for XDE-848 and 0.05 µg/L and 0.015 µg/L, respectively, for the five metabolites of XDE-848 (pp. 16, 22; Tables 80-81, pp. 113-114 of MRID 49677801; pp. 16, 22 of MRID 49677802).

II. Recovery Findings

ECM (MRID 49677801): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of XDE-848 (XDE-848 BE; XDE-848 benzyl ester) in drinking, ground and surface water matrices at fortification levels of 0.02 µg/L (LOQ), 0.2 µg/L (10×LOQ) and 2 µg/L (100×LOQ) and the five metabolites, X11966341, X12131932, X12393505, X12300837, and X11438848, in drinking, ground and surface water matrices at fortification levels of 0.05 µg/L (LOQ), 0.5 µg/L (10×LOQ) and 5 µg/L (100×LOQ; Tables 68-79, pp. 107-112). For all analytes, two ion transitions were monitored using LC/MS/MS; performance data (recovery results) of the quantitative and confirmatory results were comparable. The ECM calculations allowed for recovery data to be corrected for residues found in the control samples; residues were quantified in several of the control samples (*ca*. 30% of all analyte/matrix control samples; Tables 32-67, pp. 71-106; Figures 23-34, pp. 166-177). Recoveries from samples fortified at 0.006/0.015 µg/L (LOD) ranged (ions/matrices combined) from 83-122% for XDE-848 BE, 80-107% for X11966341, 87-107% for X12131932, 73-113% for X12393505, 87-113% for X12300837, and 67-140% for X11438848 (n = 2 for each matrix/analyte; Tables 32-67, pp. 71-106; DER Attachment 2). The water matrices were well characterized at and obtained from the Sample Management Group of Dow AgroSciences LLC (sources not further specified; p. 17). Drinking (tap) water (001-0001; pH 8.2, dissolved organic carbon 2.7 ppm), surface (pond) water (002-0001; pH 7.9, dissolved organic carbon 3.3 ppm), and ground (monitoring well) water (003-0001; pH 8.2, dissolved organic carbon 1.8 ppm) were used in the study.

ILV (MRID 49677802): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of XDE-848 (XDE-848 BE; XDE-848 benzyl ester) in drinking, ground and surface water matrices at fortification levels of 0.02 µg/L (LOQ) and 2.0 µg/L (100×LOQ) and the five metabolites, X11966341, X12131932, X12393505, X12300837, and X11438848, in drinking, ground and surface water matrices at fortification levels of 0.05 µg/L (LOQ) and 5.0 µg/L (100×LOQ; uncorrected recovery results; Tables 50-61, pp. 76-81; Figure 23, p. 110). No samples were prepared at 10×LOQ. For all analytes, two ion transitions were monitored using LC/MS/MS; performance data (recovery results) of the quantitative and confirmatory results were comparable. Recoveries from samples fortified at 0.006/0.015 µg/L (LOD) ranged (ions/matrices combined) from 77-96% for XDE-848 BE, 81-147% for X11966341, 53-95% for X12131932, 90-106% for X12393505, 95-99% for X12300837, and 81-104% for X11438848 (n = 1 for each matrix/analyte; Tables 14-49, pp. 40-75; DER Attachment 2). The water matrices were well characterized by Agvise Laboratories, Northwood, North Dakota (sources not further specified; p. 17; Appendix 2, pp. 171-175). Surface water (15/003 Surface H2O Res; pH 8.0, dissolved organic carbon 3.1 ppm), ground water (12/044 Highland Spring; pH 8.2, dissolved organic carbon 0.1 ppm), and drinking water (15/002 Drinking H2O Res; pH 8.1, dissolved organic carbon 3.7 ppm) were used in the study. The method was validated in the first trial for all analytes in drinking, surface and ground water matrices with insignificant modifications to the analytical parameters (p. 22).

Table 2. Initial Validation Method Recoveries for XDE-848 (XDE-848 BE; XDE-848 Benzyl Ester) and Its Five Metabolites, X11966341, X12131932, X12393505, X12300837, and X11438848, in Drinking, Ground and Surface Water[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground (Well) Water						
	Quantitation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**2**	92, 103	--	--	--
	0.02 (LOQ)	10	95-106	98	3.19	3.24
	0.2	6	96-104	99	2.99	3.03
	2	10	92-100	96	2.05	2.13
X11966341	0.015 (LOD)	**2**	93, 100	--	--	--
	0.05 (LOQ)	10	88-102	95	4.26	4.49
	0.5	6	84-101	94	6.41	6.84
	5	10	90-106	98	4.90	5.01
X12131932	0.015 (LOD)	**2**	93, 100	--	--	--
	0.05 (LOQ)	10	94-107	98	3.86	3.94
	0.5	6	94-103	98	3.42	3.48
	5	10	96-106	100	3.13	3.13
X12393505	0.015 (LOD)	**2**	113	--	--	--
	0.05 (LOQ)	10	88-98	93	3.84	4.15
	0.5	6	90-94	92	1.61	1.76
	5	10	83-98	91	4.20	4.63
X12300837	0.015 (LOD)	**2**	93, 107	--	--	--
	0.05 (LOQ)	10	90-100	96	3.27	3.42
	0.5	6	85-98	90	4.52	5.11
	5	10	88-98	92	2.99	3.26
X11438848	0.015 (LOD)	**2**	100	--	--	--
	0.05 (LOQ)	10	90-114	100	7.36	7.38
	0.5	6	92-98	95	1.91	2.00
	5	10	90-105	98	4.17	4.27
	Confirmation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**2**	93, 98	--	--	--
	0.02 (LOQ)	10	93-105	100	3.35	3.36
	0.2	6	96-103	99	2.81	2.84
	2	10	88-99	95	3.11	3.28
X11966341	0.015 (LOD)	**2**	80, 100	--	--	--
	0.05 (LOQ)	10	80-117	94	9.80	10.4
	0.5	6	83-104	95	8.24	8.63
	5	10	93-104	98	4.41	4.51
X12131932	0.015 (LOD)	**2**	93, 100	--	--	--
	0.05 (LOQ)	10	94-109	98	4.22	4.30
	0.5	6	93-104	98	4.08	4.15
	5	10	96-103	100	2.65	2.64
X12393505	0.015 (LOD)	**2**	80, 87	--	--	--
	0.05 (LOQ)	10	78-96	87	4.81	5.55

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.5	6	89-97	92	2.94	3.21
	5	10	85-100	92	4.77	5.18
X12300837	0.015 (LOD)	**2**	100, 113	--	--	--
	0.05 (LOQ)	10	94-105	98	3.88	3.94
	0.5	6	88-98	94	3.64	3.89
	5	10	91-103	97	4.04	4.18
X11438848	0.015 (LOD)	**2**	93, 113	--	--	--
	0.05 (LOQ)	10	94-106	99	3.52	3.54
	0.5	6	94-100	96	1.85	1.93
	5	10	91-107	97	4.79	4.93
Surface (Pond) Water						
	Quantitation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**2**	98, 122	--	--	--
	0.02 (LOQ)	10	89-107	98	4.97	5.08
	0.2	6	97-107	101	4.54	4.51
	2	10	97-106	101	2.69	2.67
X11966341	0.015 (LOD)	**2**	80, 100	--	--	--
	0.05 (LOQ)	10	88-104	93	4.86	5.21
	0.5	6	90-102	95	4.26	4.46
	5	10	94-104	99	3.96	3.99
X12131932	0.015 (LOD)	**2**	87, 93	--	--	--
	0.05 (LOQ)	10	80-100	89	5.97	6.69
	0.5	6	84-104	94	8.57	9.13
	5	10	92-113	100	6.93	6.92
X12393505	0.015 (LOD)	**2**	80, 113	--	--	--
	0.05 (LOQ)	10	80-94	89	4.76	5.37
	0.5	6	86-98	91	4.82	5.30
	5	10	84-94	90	4.06	4.53
X12300837	0.015 (LOD)	**2**	93	--	--	--
	0.05 (LOQ)	10	85-98	93	4.20	4.50
	0.5	6	92-97	94	1.68	1.78
	5	10	93-100	96	2.55	2.65
X11438848	0.015 (LOD)	**2**	113, 127	--	--	--
	0.05 (LOQ)	10	95-105	101	3.81	3.77
	0.5	6	93-107	97	5.04	5.20
	5	10	93-104	99	3.45	3.48
	Confirmation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**2**	83, 88	--	--	--
	0.02 (LOQ)	10	88-108	95	5.88	6.21
	0.2	6	94-111	100	6.19	6.16
	2	10	96-108	101	3.75	3.72
X11966341	0.015 (LOD)	**2**	93, 107	--	--	--
	0.05 (LOQ)	10	77-110	100	9.76	9.73
	0.5	6	88-102	94	4.99	5.28

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	5	10	94-103	99	3.16	3.19
X12131932	0.015 (LOD)	**2**	87	--	--	--
	0.05 (LOQ)	10	79-102	89	7.02	7.91
	0.5	6	84-102	92	6.95	7.52
	5	10	93-112	100	6.41	6.41
X12393505	0.015 (LOD)	**2**	73, 100	--	--	--
	0.05 (LOQ)	10	79-98	89	6.32	7.08
	0.5	6	88-94	90	2.04	2.26
	5	10	82-93	90	3.58	4.00
X12300837	0.015 (LOD)	**2**	87, 100	--	--	--
	0.05 (LOQ)	10	88-97	94	3.03	3.22
	0.5	6	91-100	94	3.34	3.57
	5	10	92-100	96	3.10	3.22
X11438848	0.015 (LOD)	**2**	87, **140**	--	--	--
	0.05 (LOQ)	10	85-118	101	10.0	9.96
	0.5	6	94-104	98	3.95	4.04
	5	10	94-104	98	3.21	3.26
Drinking (Tap) Water						
	Quantitation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**2**	98, 113	--	--	--
	0.02 (LOQ)	10	95-107	102	3.24	3.17
	0.2	6	96-104	100	2.73	2.74
	2	10	96-103	100	2.39	2.39
X11966341	0.015 (LOD)	**2**	87, 100	--	--	--
	0.05 (LOQ)	10	87-115	95	7.77	8.15
	0.5	6	88-97	93	2.99	3.20
	5	10	95-101	97	1.84	1.90
X12131932	0.015 (LOD)	**2**	100, 107	--	--	--
	0.05 (LOQ)	10	92-102	98	3.04	3.11
	0.5	6	94-98	95	2.12	2.23
	5	10	95-104	101	2.65	2.62
X12393505	0.015 (LOD)	**2**	80, 100	--	--	--
	0.05 (LOQ)	10	80-94	86	4.74	5.53
	0.5	6	89-92	90	1.15	1.29
	5	10	83-90	87	1.95	2.24
X12300837	0.015 (LOD)	**2**	87, 100	--	--	--
	0.05 (LOQ)	10	87-99	96	3.45	3.61
	0.5	6	92-101	95	3.18	3.36
	5	10	94-100	97	2.26	2.33
X11438848	0.015 (LOD)	**2**	80, 87	--	--	--
	0.05 (LOQ)	10	92-110	99	5.85	5.93
	0.5	6	97-106	100	4.03	4.01
	5	10	96-105	100	3.23	3.21

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Confirmation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**2**	93, 100	--	--	--
	0.02 (LOQ)	10	94-100	97	2.21	2.28
	0.2	6	94-100	97	2.36	2.43
	2	10	94-104	98	2.90	2.96
X11966341	0.015 (LOD)	**2**	100, 107	--	--	--
	0.05 (LOQ)	10	82-113	95	9.67	10.1
	0.5	6	86-98	93	4.12	4.41
	5	10	94-103	98	3.51	3.57
X12131932	0.015 (LOD)	**2**	100, 107	--	--	--
	0.05 (LOQ)	10	93-101	97	2.35	2.41
	0.5	6	93-96	95	1.15	1.22
	5	10	96-104	101	2.55	2.53
X12393505	0.015 (LOD)	**2**	80, 107	--	--	--
	0.05 (LOQ)	10	79-97	87	6.35	7.28
	0.5	6	89-94	91	1.76	1.94
	5	10	83-90	87	2.37	2.71
X12300837	0.015 (LOD)	**2**	93	--	--	--
	0.05 (LOQ)	10	88-100	93	3.93	4.21
	0.5	6	91-102	95	3.75	3.95
	5	10	92-99	96	2.25	2.34
X11438848	0.015 (LOD)	**2**	**67**, 73	--	--	--
	0.05 (LOQ)	10	87-106	97	6.56	6.78
	0.5	6	94-103	99	2.90	2.93
	5	10	96-106	101	3.14	3.12

Data (recovery results corrected for residues found in the controls; Figures 23-34, pp. 166-177) were obtained from Tables 32-67, pp. 71-106 (LOD results) and Tables 68-79, pp. 107-112 of MRID 49677801 and DER Attachment 2 (LOD calculations).

1 Florpyrauxifen-benzyl = [XDE-848; XDE-848 BE; XDE-848 benzyl ester; TSN301734; X11959130; SX-1552; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; X11966341 = [XDE-848 hydroxy acid; TSN305649; 1552-OHA; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylic acid]; X12131932 = [Dechlorinated XDE-848 benzyl ester; TSN305649; 1552-DBE; benzyl 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; X12393505 = [Dechlorinated XDE-848 acid; TSN304479; 1552-DA; 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid]; X12300837 = [XDE-848 hydroxy benzyl ester; TSN305650; 1552-OHBE; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylate]; and X11438848 = [XDE-848 acid; TSN301691; 1552-Acid; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid].

2 The water matrices were well characterized (p. 17). Drinking (tap) water (001-0001; pH 8.2, dissolved organic carbon 2.7 ppm), surface (pond) water (002-0001; pH 7.9, dissolved organic carbon 3.3 ppm), and ground (monitoring well) water (003-0001; pH 8.2, dissolved organic carbon 1.8 ppm) were used in the study.

3 Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* 441 → 91 and *m/z* 439 → 91 for XDE-848 BE; *m/z* 335 → 254 and *m/z* 337 → 256 for X11966341; *m/z* 405 → 65 and *m/z* 407 → 91 for X12131932; *m/z* 315 → 234 and *m/z* 315 → 124 for X12393505; *m/z* 425 → 91 and *m/z* 427 → 91 for X12300837; and *m/z* 349 → 268 and *m/z* 351 → 270 for X11438848.

Table 3. Independent Validation Method Recoveries for XDE-848 (XDE-848 BE; XDE-848 Benzyl Ester) and Its Five Metabolites, X11966341, X12131932, X12393505, X12300837, and X11438848, in Drinking, Ground and Surface 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					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**1**	96	--	--	--
	0.02 (LOQ)	5	92-100	96	2.9	3.0
	2.0	5	90-98	94	2.9	3.1
X11966341	0.015 (LOD)	**1**	91	--	--	--
	0.05 (LOQ)	5	86-103	97	6.3	6.6
	5.0	5	95-100	98	2.1	2.2
X12131932	0.015 (LOD)	**1**	95	--	--	--
	0.05 (LOQ)	5	95-100	98	1.9	2.0
	5.0	5	95-99	97	1.5	1.6
X12393505	0.015 (LOD)	**1**	90	--	--	--
	0.05 (LOQ)	5	93-110	100	6.9	6.9
	5.0	5	94-98	97	1.7	1.7
X12300837	0.015 (LOD)	**1**	96	--	--	--
	0.05 (LOQ)	5	90-100	95	4.3	4.6
	5.0	5	90-96	93	2.6	2.8
X11438848	0.015 (LOD)	**1**	104	--	--	--
	0.05 (LOQ)	5	90-102	97	5.3	5.5
	5.0	5	95-102	100	2.9	2.9
	Confirmation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**1**	91	--	--	--
	0.02 (LOQ)	5	92-96	95	1.7	1.8
	2.0	5	91-97	93	2.2	2.3
X11966341	0.015 (LOD)	**1**	89	--	--	--
	0.05 (LOQ)	5	84-95	91	4.3	4.7
	5.0	5	93-99	96	2.5	2.7
X12131932	0.015 (LOD)	**1**	85	--	--	--
	0.05 (LOQ)	5	92-97	93	2.2	2.4
	5.0	5	94-99	97	1.9	1.9
X12393505	0.015 (LOD)	**1**	95	--	--	--
	0.05 (LOQ)	5	93-101	98	3.8	3.9
	5.0	5	94-97	96	1.1	1.2
X12300837	0.015 (LOD)	**1**	95	--	--	--
	0.05 (LOQ)	5	91-99	96	3.3	3.4
	5.0	5	91-96	94	2.2	2.3
X11438848	0.015 (LOD)	**1**	97	--	--	--
	0.05 (LOQ)	5	91-103	97	4.8	4.9
	5.0	5	97-101	99	1.6	1.7

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
	Quantitation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**1**	87	--	--	--
	0.02 (LOQ)	5	89-95	93	2.6	2.8
	2.0	5	95-99	97	1.5	1.5
X11966341	0.015 (LOD)	**1**	107	--	--	--
	0.05 (LOQ)	5	92-102	97	4.0	4.1
	5.0	5	93-102	97	3.6	3.7
X12131932	0.015 (LOD)	**1**	81	--	--	--
	0.05 (LOQ)	5	85-91	88	2.2	2.5
	5.0	5	96-99	98	1.1	1.2
X12393505	0.015 (LOD)	**1**	97	--	--	--
	0.05 (LOQ)	5	82-88	86	2.7	3.1
	5.0	5	88-92	90	1.6	1.8
X12300837	0.015 (LOD)	**1**	99	--	--	--
	0.05 (LOQ)	5	96-102	100	2.6	2.6
	5.0	5	98-100	99	0.8	0.8
X11438848	0.015 (LOD)	**1**	97	--	--	--
	0.05 (LOQ)	5	97-104	100	2.6	2.6
	5.0	5	92-98	95	2.4	2.5
	Confirmation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**1**	77	--	--	--
	0.02 (LOQ)	5	91-97	93	2.8	3.0
	2.0	5	96-101	98	2.1	2.1
X11966341	0.015 (LOD)	**1**	81	--	--	--
	0.05 (LOQ)	5	84-103	95	7.3	7.6
	5.0	5	90-99	95	3.3	3.5
X12131932	0.015 (LOD)	**1**	**69**	--	--	--
	0.05 (LOQ)	5	79-88	83	3.8	4.6
	5.0	5	98-99	98	0.5	0.6
X12393505	0.015 (LOD)	**1**	93	--	--	--
	0.05 (LOQ)	5	81-88	83	2.9	3.5
	5.0	5	88-91	90	1.1	1.3
X12300837	0.015 (LOD)	**1**	97	--	--	--
	0.05 (LOQ)	5	97-107	100	4.0	4.0
	5.0	5	95-99	97	1.5	1.5
X11438848	0.015 (LOD)	**1**	91	--	--	--
	0.05 (LOQ)	5	92-97	94	2.2	2.3
	5.0	5	92-98	95	2.6	2.7
Drinking Water						
	Quantitation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**1**	77	--	--	--
	0.02 (LOQ)	5	93-100	96	2.9	3.0
	2.0	5	94-98	96	1.9	1.9

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
X11966341	0.015 (LOD)	**1**	89	--	--	--
	0.05 (LOQ)	5	91-100	95	4.6	4.8
	5.0	5	94-95	94	0.5	0.6
X12131932	0.015 (LOD)	**1**	**58**	--	--	--
	0.05 (LOQ)	5	80-92	86	4.3	4.9
	5.0	5	92-97	96	2.2	2.3
X12393505	0.015 (LOD)	**1**	98	--	--	--
	0.05 (LOQ)	5	93-101	96	3.1	3.3
	5.0	5	92-97	95	1.8	1.9
X12300837	0.015 (LOD)	**1**	96	--	--	--
	0.05 (LOQ)	5	98-107	102	3.4	3.3
	5.0	5	92-101	96	3.6	3.8
X11438848	0.015 (LOD)	**1**	81	--	--	--
	0.05 (LOQ)	5	93-109	100	6.4	6.4
	5.0	5	93-100	98	2.9	3.0
	Confirmation ion transition					
Florpyrauxifen-benzyl (XDE-848 BE; XDE-848 Benzyl Ester)	0.006 (LOD)	**1**	87	--	--	--
	0.02 (LOQ)	5	95-104	99	3.5	3.5
	2.0	5	94-102	99	3.0	3.0
X11966341	0.015 (LOD)	**1**	147	--	--	--
	0.05 (LOQ)	5	87-110	95	9.4	9.8
	5.0	5	94-98	96	1.5	1.6
X12131932	0.015 (LOD)	**1**	**53**	--	--	--
	0.05 (LOQ)	5	77-88	81	4.4	5.4
	5.0	5	88-96	93	2.9	3.2
X12393505	0.015 (LOD)	**1**	106	--	--	--
	0.05 (LOQ)	5	95-103	99	2.9	2.9
	5.0	5	93-99	95	2.3	2.4
X12300837	0.015 (LOD)	**1**	95	--	--	--
	0.05 (LOQ)	5	97-105	101	3.5	3.5
	5.0	5	92-98	94	2.5	2.6
X11438848	0.015 (LOD)	**1**	86	--	--	--
	0.05 (LOQ)	5	91-104	97	4.8	5.0
	5.0	5	96-101	99	1.9	2.0

Data (uncorrected recovery results; Figure 23, p. 110) were obtained from Tables 14-49, pp. 40-75 (LOD results) and Tables 50-61, pp. 76-81 of MRID 49677802 and DER Attachment 2 (LOD calculations).

1 Florpyrauxifen-benzyl = [XDE-848; XDE-848 BE; XDE-848 benzyl ester; TSN301734; X11959130; SX-1552; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; X11966341 = [XDE-848 hydroxy acid; TSN305649; 1552-OHA; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylic acid]; X12131932 = [Dechlorinated XDE-848 benzyl ester; TSN305649; 1552-DBE; benzyl 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; X12393505 = [Dechlorinated XDE-848 acid; TSN304479; 1552-DA; 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid]; X12300837 = [XDE-848 hydroxy benzyl ester; TSN305650; 1552-OHBE; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylate]; and X11438848 = [XDE-848 acid; TSN301691; 1552-Acid; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid].

2 The water matrices were well characterized (p. 17; Appendix 2, pp. 171-175). Surface water (15/003 Surface H2O Res; pH 8.0, dissolved organic carbon 3.1 ppm), ground water (12/044 Highland Spring; pH 8.2, dissolved organic carbon 0.1 ppm), and drinking water (15/002 Drinking H2O Res; pH 8.1, dissolved organic carbon 3.7 ppm) were used in the study.

3 Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* **439 → 91** and *m/z* **441 → 91** for XDE-848 BE; *m/z* 335 → 254 and *m/z* 337 → 256 for X11966341; *m/z* 405 → 65 and *m/z* 407 → 91 for X12131932; *m/z* 315 → 234 and *m/z* 315 → 124 for X12393505; *m/z* **427 → 91** and *m/z* **425 → 91** for X12300837; and *m/z* **351 → 270** and *m/z* **349 → 268** for X11438848 (**bolded** transitions were those for which the quantification and confirmation ions transitions were reversed from the ECM).

III. Method Characteristics

In the ECM and ILV, the established LOQ and LOD were 0.02 µg/L and 0.006 µg/L, respectively, for XDE-848 and 0.05 µg/L and 0.015 µg/L, respectively, for the five metabolites of XDE-848 (pp. 16, 22; Tables 80-81, pp. 113-114 of MRID 49677801; pp. 16, 22 of MRID 49677802). Following the method of Keith, L. H., *et al*. (see section **V. References** below), the LOD and LOQ for determination of XDE-848 and its metabolites in water were calculated in the ECM using the standard deviation from the 0.006 µg/L or 0.015 µg/L recovery results. 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. The calculated values of the ECM support the LOQ and LOD established for the study and are presented in **Table 4** below. In the ILV, the LOQ and LOD were reported from the ECM without justification or calculation.

Table 4. Method Characteristics

		XDE-848 (XDE-848 BE)	X11966341	X12131932	X12393505	X12300837	X11438848
Limit of Quantitation (LOQ)	Established	0.02 µg/L	0.05 µg/L				
	Calculated (ECM)	0.00516-0.01247 µg/L	0.0213-0.0490 µg/L	0.0117-0.0351 µg/L	0.0192-0.0317 µg/L	0.0151-0.0210 µg/L	0.0176-0.0501 µg/L
Limit of Detection (LOD)	Established	0.006 µg/L	0.015 µg/L				
	Calculated (ECM)	0.00155-0.00188 µg/L	0.00639-0.0147 µg/L	0.00352-0.0105 µg/L	0.00576-0.00952 µg/L	0.00454-0.00630 µg/L	0.00528-0.0150 µg/L
Linearity (Least squares calibration curve r and concentration range)	ECM[1]	r^2 = 0.9996-1.0000 (Q) r^2 = 0.9998-1.0000 (C)	r^2 = 0.9992-0.9998 (Q) r^2 = 0.9986-0.9998 (C)	r^2 = 0.9996-1.0000 (Q) r^2 = 0.9994-1.0000 (C)	r^2 = 0.9968-0.9998 (Q) r^2 = 0.9954-1.0000 (C)	r^2 = 0.9988-1.0000 (Q) r^2 = 0.9994-1.0000 (C)	r^2 = **0.9936**-0.9998 (Q) r^2 = **0.9944**-0.9998 (C)
		0.03-4.0 ng/mL	0.075-10 ng/mL				
	ILV[1]	r^2 = 0.9988-0.9996 (Q) r^2 = 0.9986-0.9996 (C)	r^2 = 0.9990-0.9996 (Q) r^2 = 0.9996-0.9998 (C)	r^2 = 0.9990-0.9996 (Q) r^2 = 0.9992-0.9996 (C)	r^2 = 0.9988-0.9998 (Q) r^2 = 0.9988-1.0000 (C)	r^2 = 0.9984-0.9998 (Q) r^2 = 0.9992-0.9998 (C)	r^2 = 0.9994-0.9998 (Q) r^2 = 0.9994-0.9996 (C)
		0.03-4.0 ng/mL	0.075-10 ng/mL				
Repeatable	ECM[2]	Yes at LOQ (n = 10), 10×LOQ (n = 6) and 100×LOQ (n = 10).					
	ILV[3]	Yes at LOQ and 100×LOQ (n = 5). **No** at 10×LOQ, no samples were prepared.					
Reproducible		Yes at LOQ and 100×LOQ (n = 5). **No** at 10×LOQ, no samples were prepared.					
Specific	ECM	Yes, only minor interferences (<50% of the LOD) at the retention time of the analytes were observed in the matrix controls. Residues in the matrix controls were quantified as <LOQ. No representative chromatograms were provided for the reagent blank and fortifications at the LOD or 10×LOQ, only calibrants, controls, LOQ and 100×LOQ. Peaks were well defined and distinct from the baseline. Insignificant baseline interference was observed in LOQ chromatograms for X11966341.					
	ILV	Yes, only minor interferences (<10% of the LOQ) at the retention time of the analytes were observed in the matrix controls. Residues in the matrix controls were quantified as <LOD. No representative chromatograms were provided for the reagent blank and fortifications at the LOD or 100×LOQ, only calibrants, controls and LOQ.					

		XDE-848 (XDE-848 BE)	X11966341	X12131932	X12393505	X12300837	X11438848
		Peak attenuation was disrupted at base by a nearby significant contaminant (*ca*. 50% height of LOQ).	Minor baseline noise was observed which disrupted peak attenuation.	Peak attenuation was disrupted at base by a nearby significant contaminant (height ≥LOQ).	Baseline was free of interferences.		

Data were obtained from pp. 16, 22; Tables 20-31, pp. 68-70 (Correlation Coefficients); Tables 32-67, pp. 71-106 (Control residues and LOD results); Tables 68-79, pp. 107-112 (Summary Recovery Results); Tables 80-81, pp. 113-114 (Calculated LOQ/LOD); Figures 11-22, pp. 154-165 (Linear Regressions); Figures 45-60, pp. 188-251 (Chromatograms) of MRID 49677801 ; pp. 16, 22; Tables 2-13, pp. 28-39 (Correlation Coefficients); Tables 14-49, pp. 40-75 (Control residues and LOD results); Tables 50-61, pp. 76-81 (Summary Recovery Results); Figures 11-22, pp. 98-109 (Linear regressions); Figures 34-75, pp. 121-162 (Chromatograms) of MRID 49677802 and DER Attachment 2. Q = Quantitative HPLC analysis; C = Confirmatory HPLC analysis.

* Florpyrauxifen-benzyl = [XDE-848; XDE-848 BE; XDE-848 benzyl ester; TSN301734; X11959130; SX-1552; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; X11966341 = [XDE-848 hydroxy acid; TSN305649; 1552-OHA; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylic acid]; X12131932 = [Dechlorinated XDE-848 benzyl ester; TSN305649; 1552-DBE; benzyl 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; X12393505 = [Dechlorinated XDE-848 acid; TSN304479; 1552-DA; 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid]; X12300837 = [XDE-848 hydroxy benzyl ester; TSN305650; 1552-OHBE; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylate]; and X11438848 = [XDE-848 acid; TSN301691; 1552-Acid; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid].

1 ECM and ILV standard curves were weighted 1/x for XDE-848, X11966341, X12131932, X12393505, X12300837, and X11438848. ECM r^2 values are reviewer-generated for the analytes from reported r values of 0.9968-1.0000 (Q) and 0.9972-1.0000 (C; analytes combined; calculated from data in Tables 20-31, pp. 68-70 and Figures 11-22, pp. 154-165 of MRID 49677801; see DER Attachment 2). ILV r^2 values are reviewer-generated for the analytes from reported r values of 0.9992-0.9999 (Q) and 0.9993-1.0000 (C; analytes/ions combined; calculated from data in Tables 2-13, pp. 28-39 and Figures 11-22, pp. 98-109 of MRID 49677802; see DER Attachment 2).

2 For the ECM, drinking (tap) water (001-0001; pH 8.2, dissolved organic carbon 2.7 ppm), surface (pond) water (002-0001; pH 7.9, dissolved organic carbon 3.3 ppm), and ground (monitoring well) water (003-0001; pH 8.2, dissolved organic carbon 1.8 ppm) were used (p. 17 of MRID 49677801).

3 For the ILV, surface water (15/003 Surface H2O Res; pH 8.0, dissolved organic carbon 3.1 ppm), ground water (12/044 Highland Spring; pH 8.2, dissolved organic carbon 0.1 ppm), and drinking water (15/002 Drinking H2O Res; pH 8.1, dissolved organic carbon 3.7 ppm) were used (p. 17; Appendix 2, pp. 171-175 of MRID 49677802).

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, no samples were prepared at 10×LOQ (Tables 14-61, pp. 40-81 of MRID 49677802). OCSPP guidelines require that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10×LOQ) for each analyte.

2. In the ILV, chromatograms for three of the six analytes showed matrix interferences which affected the peak attenuation of the analyte in all matrices (Figures 34-75, pp. 121-162 of MRID 49677802). For XDE-848, peak attenuation was disrupted at base by a nearby significant contaminant (*ca*. 50% height of LOQ). For X12131932, peak attenuation was disrupted at base by a nearby significant contaminant (height ≥LOQ). For X11966341, minor baseline noise was observed which disrupted peak attenuation.

   In the ECM and ILV, representative chromatograms were not complete. In the ECM, representative chromatograms were not provided for the reagent blank and fortifications at the LOD or 10×LOQ, only calibrants, controls, LOQ and 100×LOQ (Figures 45-60, pp. 188-251 of MRID 49677801). A reagent blank was included in the validation (p. 18). In the ILV, representative chromatograms were not provided for the reagent blank and fortifications at the LOD or 100×LOQ, only calibrants, controls and LOQ (Figures 34-75, pp. 121-162 of MRID 49677802). A reagent blank was included in the validation (p. 19).

3. In the ECM, sample recoveries were corrected. The ECM calculations allowed for recovery data to be corrected for residues found in the control samples; residues were quantified in several of the control samples (*ca*. 30% of all analyte/matrix control samples; Tables 32-67, pp. 71-106; Figures 23-34, pp. 166-177 of MRID 49677801).

4. Although the water matrices were well characterized in the ECM and ILV, the specific water source of each of the matrices was not reported (p. 17 of MRID 49677801; p. 17; Appendix 2, pp. 171-175 of MRID 49677802).

5. The toxicological level of concern was not reported for the analytes in water. A LOQ above toxicological levels of concern results in an unacceptable method classification.

6. The reviewer noted the following typographical error in the ECM: the calculated LOD from the standard deviation of the LOQ sample was reported as "0.01888 µg/L", instead of "0.001888 µg/L", for the confirmation ion of XDE-848 Benzyl Ester in ground water (Table 81, p. 114 of MRID 49677801).

7. Radiolabeled internal standards or dechlorinated standards were used to determine isotopic crossover, as well as to facilitate analyses in the occurrence of matrix effects (pp. 16-17, 24; Appendix 1, p. 261 of MRID 49677801).

8. The ILV reported that no communications occurred between the ILV laboratory and the study director (p. 22 of MRID 49677802).

9. In the ECM, the stability of the calibration standards, stock solutions and sample extracts were studied. The stock solutions of all analytes, except X12331932, were stable for up to 211 days under refrigeration storage and protected from light; stock solutions of X12331932 were stable for up to 53 days under refrigeration storage and protected from light (pp. 22-23; Tables 82-117, pp. 115-132 of MRID 49677801). The calibration and fortification solutions of all analytes were stable for up to 53 days under refrigeration/ freezer storage and protected from light. The final extracts of all analytes were stable for up to 8 days under refrigeration storage (p. 23; Tables 118-123, pp. 133-135).

   In the ECM and ILV, matrix effects were also studied (p. 24; Tables 124-129, pp. 136-141 of MRID 49677801; p. 21; Tables 62-67, pp. 82-87 of MRID 49677802). Matrix effects were determined to be insignificant (±20%) for all matrices in the ECM; however, the use of internal standards was recommended. In the ILV, matrix effects were determined to be significant in some matrices (up to -30%), and the use of internal standards were considered necessary to reduce matrix effects.

10. It was reported for the ILV that the analytical procedure for one set of 19 samples (five calibration standards, two controls, one LOD sample, five LOQ samples, five 100×LOQ samples and one reagent blank) required approximately 5 hours for laboratory preparation (p. 19 of MRID 49677802). The LC/MS/MS was conducted unattended (8 hours or overnight). The interpretation of data required approximately 4 hours. The overall time to complete a set of samples (14 samples, not including calibration standards) was 1.5 calendar days.

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. 26 of MRID 49677801).

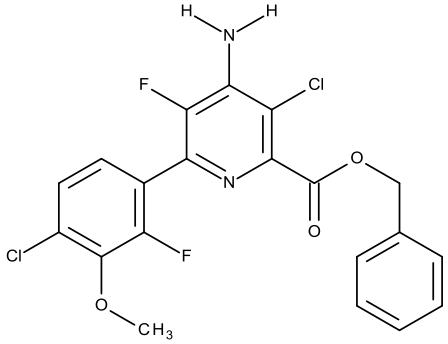
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Attachment 1: Chemical Names and Structures

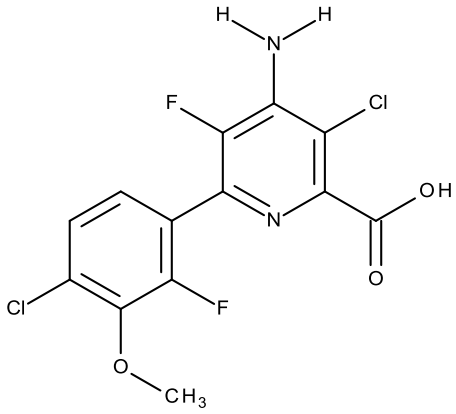
XDE-848 Benzyl Ester (Rinskor, XR-848-BE, XR-848 Benzyl, X11959130, TSN301734)

IUPAC Name:	Benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate
CAS Name:	Phenylmethyl ester 3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-2-pyridinecarboxylic acid
CAS Number:	1390661-72-9
SMILES String:	[H]N([H])c1c(c(nc(c1Cl)C(=O)OCc2ccccc2)c3ccc(c(c3F)OC)Cl)F



XDE-848 acid (X11433848, TSN304667)

IUPAC Name:	4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid
CAS Name:	--
CAS Number:	--
SMILES String:	[H]N([H])c1c(c(nc(c1Cl)C(=O)O)c2ccc(c(c2F)OC)Cl)F



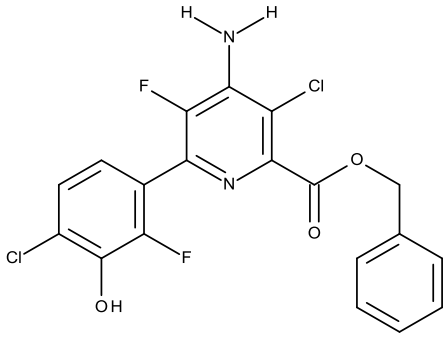
XDE-848 Hydroxy Benzyl Ester (X12300837; TSN305650; XDE-848 BH; Benzyl hydroxyl; 1552-OHBE; OHBE)

IUPAC Name: Benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylate

CAS Name: --

CAS Number: --

SMILES String: [H]N([H])c1c(c(nc(c1Cl)C(=O)OCc2ccccc2)c3ccc(c(c3F)O)Cl)F



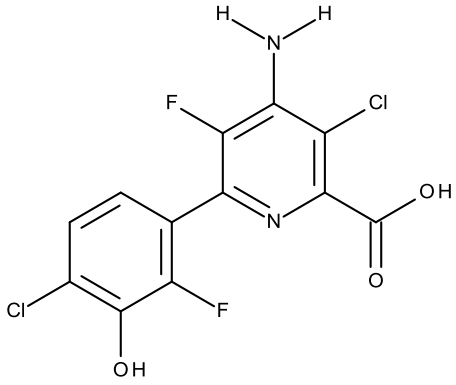
XDE-848 Hydroxy Acid (X11966341; TSN305649; XDE-848 HA; Hydroxy acid; 1552-OHA; OHA)

IUPAC Name: 4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-hydoxyphenyl)-5-fluoropyridine-2-carboxylic acid

CAS Name: --

CAS Number: --

SMILES String: [H]N([H])c1c(c(nc(c1Cl)C(=O)O)c2ccc(c(c2F)O)Cl)F



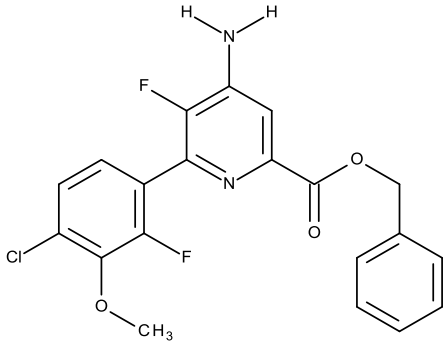
Dechlorinated XDE-848 Benzyl Ester (X12131932; TSN304497; De-chloro BE; Dechlorinated 848 BE; 1552-DBE; DBE)

IUPAC Name: Benzyl 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate

CAS Name: --

CAS Number: --

SMILES String: [H]N([H])c1cc(nc(c1F)c2ccc(c(c2F)OC)Cl)C(=O)OCc3ccccc3



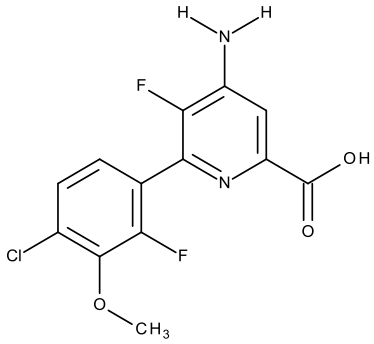
Dechlorinated XDE-848 Acid (X12393505; TSN304479; De-chloro acid; Dechlorinated 848 BE; 1552-DA; DA)

IUPAC Name: 4-Amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid

CAS Name: --

CAS Number: --

SMILES String: [H]N([H])c1cc(nc(c1F)c2ccc(c(c2F)OC)Cl)C(=O)O



Attachment 2: Calculations

Chemical: XDE-848
PC: 030093
MRIDs: 49677801/49677802
Guideline: 850.6100

ECM Recoveries at LOD of XDE-848 and its Products

	XDE-848 BE		
Fortified (µg a.i./L)	Found (µg/L)	Recovery (%)	
0.0060	Quantiation ion		
	0.0068	113	Tap Water
	0.0059	98	
	0.0073	122	Pond Water
	0.0059	98	
	0.0062	103	Well Water
	0.0055	92	
	Confirmation ion		
	0.0056	93	Tap Water
	0.0060	100	
	0.0050	83	Pond Water
	0.0053	88	
	0.0059	98	Well Water
	0.0056	93	

Results from Tables 32-67, pp. 71-106 of MRID 49677801.

	X11966341		X12131932		X12393505	
Fortified (µg a.i./L)	Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)
0.0150					Quantiation ion	
	0.0150	100	0.0160	107	0.0150	100
	0.0130	87	0.0150	100	0.0120	80
	0.0120	80	0.0130	87	0.0120	80
	0.0150	100	0.0140	93	0.0170	113
	0.0150	100	0.0150	100	0.0170	113
	0.0140	93	0.0140	93	0.0170	113
					Confirmation ion	
	0.0160	107	0.0150	100	0.0160	107
	0.0150	100	0.0160	107	0.0120	80
	0.0140	93	0.0130	87	0.0150	100
	0.0160	107	0.0130	87	0.0110	73
	0.0120	80	0.0150	100	0.0120	80
	0.0150	100	0.0140	93	0.0130	87

Results from Tables 32-67, pp. 71-106 of MRID 49677801.

X12300837		X11438848		
Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)	
0.0150	100	0.0130	87	Tap Water
0.0130	87	0.0120	80	
0.0140	93	0.0190	127	Pond Water
0.0140	93	0.0170	113	
0.0160	107	0.0150	100	Well Water
0.0140	93	0.0150	100	
0.0140	93	0.0100	67	Tap Water
0.0140	93	0.0110	73	
0.0130	87	0.0130	87	Pond Water
0.0150	100	0.0210	140	
0.0170	113	0.0170	113	Well Water
0.0150	100	0.0140	93	

Chemical: XDE-848
PC: 030093
MRIDs: 49677801/49677802
Guideline: 850.6100

ECM Recoveries at LOD of XDE-848 and its Products

	XDE-848 BE		
Fortified (µg a.i./L)	Found (µg/L)	Recovery (%)	
0.0060	Quantiation ion		
	0.0057	96	Pond Water
	0.0052	87	Well Water
	0.0046	77	Tap Water
	Confirmation ion		
	0.0054	91	Pond Water
	0.0046	77	Well Water
	0.0052	87	Tap Water

Results from Tables 14-49, pp. 40-75 of MRID 49677802.

	X11966341		X12131932		X12393505	
Fortified (µg a.i./L)	Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)
0.0150					Quantiation ion	
	0.0137	91	0.0143	95	0.0135	90
	0.0161	107	0.0121	81	0.0146	97
	0.0134	89	0.0086	58	0.0147	98
					Confirmation ion	
	0.0133	89	0.0128	85	0.0143	95
	0.0121	81	0.0103	69	0.0140	93
	0.0220	147	0.0079	53	0.0159	106

Results from Tables 14-49, pp. 40-75 of MRID 49677802.

X12300837		X11438848		
Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)	
0.0144	96	0.0156	104	Pond Water
0.0149	99	0.0145	97	Well Water
0.0144	96	0.0121	81	Tap Water
0.0142	95	0.0145	97	Pond Water
0.0145	97	0.0136	91	Well Water
0.0143	95	0.0129	86	Tap Water

Chemical: XDE-848
PC: 030093
MRIDs: 49677801/49677802
Guideline: 850.6100

ECM Calibration Curve Correlation Coefficients r (1/x weighting) converted to r2

	Water				
	First Ion Transition (Q)		Second Ion Transition (C)		
Analyte	Reported r	Calculated r2	Reported r	Calculated r2	
XDE-848 BE	1.0000	1.0000	1.0000	1.0000	High
	0.9998	0.9996	0.9999	0.9998	Low
X11966341	0.9999	0.9998	0.9999	0.9998	High
	0.9996	0.9992	0.9993	0.9986	Low
X12131932	1.0000	1.0000	1.0000	1.0000	High
	0.9998	0.9996	0.9997	0.9994	Low
X12393505	0.9999	0.9998	1.0000	1.0000	High
	0.9984	0.9968	0.9977	0.9954	Low
X12300837	1.0000	1.0000	1.0000	1.0000	High
	0.9994	0.9988	0.9997	0.9994	Low
X11438848	0.9999	0.9998	0.9999	0.9998	High
	0.9968	0.9936	0.9972	0.9944	Low

Results (r values) from Tables 20-31, pp. 68-70 and Figures 11-22, pp. 154-165 of MRID 49677801.

ILV Calibration Curve Correlation Coefficients r (1/x weighting) converted to r2

	Water				
	First Ion Transition (Q)		Second Ion Transition (C)		
Analyte	Reported r	Calculated r2	Reported r	Calculated r2	
XDE-848 BE	0.9998	0.9996	0.9998	0.9996	High
	0.9994	0.9988	0.9993	0.9986	Low
X11966341	0.9998	0.9996	0.9999	0.9998	High
	0.9995	0.9990	0.9998	0.9996	Low
X12131932	0.9998	0.9996	0.9998	0.9996	High
	0.9995	0.9990	0.9996	0.9992	Low
X12393505	0.9999	0.9998	1.0000	1.0000	High
	0.9994	0.9988	0.9994	0.9988	Low
X12300837	0.9999	0.9998	0.9999	0.9998	High
	0.9992	0.9984	0.9996	0.9992	Low
X11438848	0.9999	0.9998	0.9998	0.9996	High
	0.9997	0.9994	0.9997	0.9994	Low

Results (r values) from Tables 2-13, pp. 28-39 and Figures 11-22, pp. 98-109 of MRID 49677802.