


Analytical method for florpyrauxifen-benzyl and its metabolites X11438848 and X11966341 in compost

- Reports:** ECM: EPA MRID No.: 51025001. Smith, K.A. 2018. Method Validation Study for the Determination of Residues of Florpyrauxifen-benzyl, X11438848 and X11966341 in Compost by Liquid Chromatography with Tandem Mass Spectrometry. Dow AgroSciences Study ID: 171407. Report prepared, sponsored, and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 81 pages. Final report issued September 5, 2018.
- ILV: EPA MRID No. 51025002. Kovacevic, E. 2018. Independent Laboratory Validation for the Determination of Residues of Florpyrauxifen Benzyl (XDE 848 BE), X11438848 and X11966341 in Compost by Liquid Chromatography with Tandem Mass Spectrometry. Sponsor Study ID: 180868. Eurofins Agrosience Services EcoChem GmbH Study Code: S18-00377. Report prepared by Eurofins Agrosience Services EcoChem GmbH, Niefern-Öschelbronn, Germany, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 126 pages. Final report issued September 13, 2018.
- Document No.:** MRIDs 51025001 & 51025002
- Guideline:** 850.6100
- Statements:** ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 51025001). 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 OECD GLP standards (as revised 1997) ENV/MC/CHEM(98)17, which are based on OECD GLP, which are accepted by regulation authorities throughout the European Community, the United States of America (EPA and FDA), and Japan (MHW, MAFF and METI; p. 3; Appendix F, p. 126 of MRID 51025002). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix F, p. 126). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).
- Classification:** This analytical method is classified as **supplemental**. The average LOQs for florpyrauxifen-benzyl and its metabolite X11438848 are above the lowest toxicological levels of concern in terrestrial plants. The specificity of the method for florpyrauxifen-benzyl at the LOQ was not supported by ILV representative chromatograms. The ILV used one unspecified compost matrix. The specificity of the method for X11966341 in pasture compost was not supported by ECM representative chromatograms. ECM representative 10×LOQ chromatograms were not provided.
- PC Code:** 030093

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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, Dow AgroSciences Study No. 171407, is designed for the quantitative determination of florpyrauxifen-benzyl and its metabolites X11438848 and X11966341 in compost at respective LOQs of 0.15 ng/g, 0.45 ng/g, and 9.0 ng/g using LC/MS/MS. The respective LOQs in compost are greater than the lowest toxicological levels of concern in terrestrial plants for florpyrauxifen-benzyl (0.026 ng/g) and X11438848 (0.215 ng/g), but lower than the lowest toxicological levels of concern in terrestrial plants for X11966341 (38 ng/g).¹ The ECM performed the method using pasture and manure compost. The ILV validated the method for florpyrauxifen-benzyl, X11438848, and X11966341 using one unspecified compost in the first trial with insignificant modifications to the analytical instrumentation and the substitution of Phenomenex roQ QuEChERS kit with an unspecified QuEChERS Citrat kit. All ECM and ILV precision, accuracy, and linearity data were acceptable; however, ILV recoveries for X11966341 were acceptable but low (*ca.* 70%) for all fortifications/ions. The specificity of the method for florpyrauxifen-benzyl at the LOQ was not supported by ILV representative chromatograms since the LOQ peak was only distinguishable from baseline noise by retention time; ILV specificity data for X11438848, and X11966341 were acceptable. All ECM specificity data for the LOQ fortification were acceptable, except in the case of X11966341 in pasture compost since the LOQ analyte peak was very broad and undefined. The florpyrauxifen-benzyl LOQ peak was small compared to baseline noise in ECM representative chromatograms. ECM representative 10×LOQ chromatograms were not provided. Solvent-based standards were used in

¹ Parent (florpyrauxifen-benzyl) and X11438848 (florpyrauxifen acid) endpoints represent vegetative vigor dicot soybean (dry weight). X11966341 (hydroxy acid) endpoints represent seedling emergence dicot carrot (survival). Conversions from lb a.i./A to ng/g achieved using the following equation (parent example): $[4.69 \times 10^{-5} \text{ lb a.i./acre}] \times [1/6 \text{ inches}] \times [1/1.33 \text{ kg/L}] \times [4.54 (10^5) \text{ mg/lb}] \times [3.94 \text{ inches/dm}] \times [2.47 (10^{-6}) \text{ acres/dm}^2] = 2.6 \times 10^{-5} \text{ mg a.i./kg soil} = 2.6 \times 10^{-5} \text{ } \mu\text{g/g}$. Assumptions: a 6-inch soil depth (default value), a soil bulk density of 1.33 kg/L (from the study MRID 49677762), and lowest endpoint EC₂₅ = 4.69 × 10⁻⁵ lb a.i./acre, for dicots (for soybean, from the ecotoxicity study MRID 49677762).

the ECM and ILV; the reviewer noted that matrix-matched standards should have been used for X11966341 in pasture compost based on significant matrix effects.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Florpyrauxifen-benzyl	51025001 ¹	51025002 ²		Compost	05/09/2018	Dow AgroSciences LLC	LC/MS/MS	0.15 ng/g
X11438848								0.45 ng/g
X11966341								9.0 ng/g

1 In the ECM, pasture and manure compost were obtained from Woods End Laboratories Inc. (p. 11 of MRID 51025001). Fresh cut grass and alfalfa were mixed and composted for 96 days to form the pasture compost. Cow manure and rye straw were mixed and composted for 95 days to create the manure compost. Composting procedures were cited by DAS Study ID 170933 (pp. 11, 19).

2 In the ILV, compost used in this study was untreated material purchased from a local market; the compost source was not further specified (p. 24 of MRID 51025002). The sample material was thoroughly homogenized in a cutter with dry ice and stored at $\leq -18^{\circ}\text{C}$ in the dark until fortification and extraction.

I. Principle of the Method

Samples (1.0 ± 0.05 g) of compost were weighed into 50-mL centrifuged tubes and fortified, as necessary, at the LOD, LOQ, $10 \times \text{LOQ}$, and $50 \times \text{LOQ}$ (p. 10; Appendix I, pp. 73-74, 76, 80-81 of MRID 51025001). The samples were extracted with 20 mL of acetonitrile:0.1N hydrochloric acid (90:10, v:v) via shaking for at 1 hour on a flat-bed shaker (*ca.* 180 excursions/minute). The samples were then centrifuged (5 minutes at 2000 rpm) and decanted into a separate 50 mL tube containing a pouch of Phenomenex roQ QuEChERS (EN Method; 4.0 g MgSO_4 , 1.0 g NaCl, 1.0 g SCD, 0.5 g SCDS, Phenomenex catalog number KS0-8909). After vortexing briefly, 10 mL of 1N HCl was added to each tube followed by centrifugation (5 minutes at 2000 rpm). An aliquot (4 mL) of the upper layer was transferred to a 45-mL vial containing 100 μL of keeper solution and 25 μL of 0.1 $\mu\text{g}/\text{mL}$ or 0.01 $\mu\text{g}/\text{mL}$ internal standard. High-concentration samples should be diluted 10-fold by reducing the aliquot to 0.4 mL. The sample was evaporated to near dryness using a Turbovap set at 40°C and a gentle stream of nitrogen for *ca.* 1.3 L/minute. The samples were treated with 1 mL of 1N HCl, mixed, and incubated at 80°C for 1 hour. After cooling to room temperature, 1 mL of ethyl acetate was added with vortexing. Samples were applied to a 15-mL Supel QuE Z-Sep tube with a vial rinsing of 1 mL of ethyl acetate. The 15-mL Supel QuE Z-Sep tube was mixed via vortex then centrifuged for 5 minutes at 2500 rpm. Acetonitrile (*ca.* 3-4 mL) was added to the 15-mL Supel QuE Z-Sep tube then the aqueous layer was flash-frozen in a dry ice bath. The ethyl acetate layer was carefully removed without transfer of any of the aqueous layer. The 15-mL Supel QuE Z-Sep tube was returned to the dry ice bath (20-30 seconds at a 45° angle) and the organic layer removal was repeated. The tubes were dried using a 96-well nitrogen dry-down apparatus set at 45°C with nitrogen flow of *ca.* 40 L/minute. The residue was reconstituted with 125 μL of methanol with vortex mixing then 175 μL of 0.1% formic acid in water was added. Samples were analyzed by LC/MS/MS.

Samples were analyzed for analytes by Agilent 1290 Infinity HPLC (Phenomenex Kinetex 1.7 μm column, 2.1 mm x 100 mm, 1.7 μm ; column temperature 30°C) using a mobile phase of (A) water with 0.1% formic acid and (B) acetonitrile:methanol (80:20, v:v) with 0.1% formic acid [percent A:B at 0.0-0.5 min. 70:30, 2 min. 35:65, 6 min. 30:70, 6.01-7 min. 0:100, 7.01 min. 70:30] with AB SCIEX Q Trap 5500 MS using MS/MS-ESI (electrospray ionization; temperature 750°C) detection in positive polarity and multiple reaction monitoring (MRM; Appendix I, pp. 74, 78-79 of MRID 51025001). Injection volume was 20 μL (may be adjusted to 60 μL for sensitivity, if necessary). Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): m/z 439 \rightarrow 91 and m/z 441 \rightarrow 91 for florpyrauxifen-benzyl, m/z 349 \rightarrow 268 and m/z 349 \rightarrow 253 for X11438848, and m/z 335 \rightarrow 254 and m/z 337 \rightarrow 256 for X11966341 (m/z 447 \rightarrow 91 for florpyrauxifen-benzyl IS (X12293407), m/z 357 \rightarrow 276 for X11438848 IS (X12293409), and m/z 341 \rightarrow 260 for X11966341 IS (X12401027)). Expected retention times were *ca.* 4.8, 2.6, and 1.6 minutes for florpyrauxifen-benzyl, X11438848 and X11966341, respectively (Figure 15, pp. 56-57).

In the ILV, the ECM was performed as written, except that the Phenomenex roQ QuEChERS kit was replaced with QuEChERS Citrat kit (no source/item No. reported) and insignificant modifications to the analytical instrumentation (pp. 16, 24; Appendix A, pp. 35-44, 48 of MRID 51025002). An Agilent 1290 Infinity II HPLC (Phenomenex Kinetex 1.7 μm column, 2.1 mm x 100 mm, 1.7 μm , column temperature 30°C) coupled with Applied Biosystems API6500 MS using MS/MS-ESI was used for all analyses. The LC/MS parameters were the same as those of the ECM. Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): m/z 439 \rightarrow 91 and m/z 441 \rightarrow 91 for florpyrauxifen-benzyl, m/z 349 \rightarrow 268 and m/z 349 \rightarrow 253 for X11438848, and m/z 335 \rightarrow 254 and m/z 337 \rightarrow 256 for X11966341 (m/z 447 \rightarrow 91 for florpyrauxifen-benzyl IS (X12293407), m/z 357 \rightarrow 276 for X11438848 IS (X12293409), and m/z 341 \rightarrow 260 for X11966341 IS (X12401027)). Expected retention times were *ca.* 5.4, 3.0, and 1.6 minutes for florpyrauxifen-benzyl, X11438848 and X11966341, respectively. No other modifications to the ECM were reported.

The Limits of Quantitation (LOQs) in the ECM and ILV were reported as 0.15 ng/g, 0.45 ng/g, and 9.0 ng/g for florpyrauxifen-benzyl, X11438848, and X11966341, respectively (pp. 10, 14-15; Table 17, p. 36 of MRID 51025001; pp. 28-29 of MRID 51025002). The Limits of Detection (LODs) in the ECM and ILV were reported as 0.05 ng/g, 0.15 ng/g, and 3.0 ng/g for florpyrauxifen-benzyl, X11438848, and X11966341, respectively. In the ECM, the LOQs and LODs for manure compost were calculated as 0.080-0.144 ng/g and 0.024-0.043 ng/g, respectively, for florpyrauxifen-benzyl, 0.214-0.227 ng/g and 0.064-0.068 ng/g, respectively, for X11438848, and 2.28-2.73 ng/g and 0.685-0.819 ng/g, respectively, for X11966341. In the ECM, the LOQs and LODs for pasture compost were calculated as 0.139-0.164 ng/g and 0.042-0.049 ng/g, respectively, for florpyrauxifen-benzyl, 0.231-0.355 ng/g and 0.069-0.106 ng/g, respectively, for X11438848, and 2.10-4.21 ng/g and 0.629-1.263 ng/g, respectively, for X11966341. In the ILV, the LOQs and LODs for compost were calculated as 0.0268-0.0436 ng/g and 0.00805-0.0131 ng/g, respectively, for florpyrauxifen-benzyl, 0.167-0.186 ng/g and 0.0502-0.0559 ng/g, respectively, for X11438848, and 0.976-1.28 ng/g and 0.293-0.384 ng/g, respectively, for X11966341.

II. Recovery Findings

ECM (MRID 51025001): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis in two compost matrices of florpyrauxifen-benzyl at the fortification levels of 0.15 ng/g (LOQ), 1.5 ng/g (10 \times LOQ), and 7.5 ng/g (50 \times LOQ), X11438848 at the fortification levels of 0.45 ng/g (LOQ), 4.5 ng/g (10 \times LOQ), and 22.5 ng/g (50 \times LOQ), and X11966341 at the fortification levels of 9 ng/g (LOQ), 90 ng/g (10 \times LOQ), and 450 ng/g (50 \times LOQ; n = 9-10 for all analyses; Tables 3-16, pp. 23-35). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable for florpyrauxifen-benzyl and X11438848, but not for X11966341 where confirmation ion recoveries were notably lower. Recovery results were corrected for residues quantified in the controls (Tables 3-14, pp. 23-34; Figure 13, p. 53). Residues in the controls accounted for *ca.* 1.3-6.4% of the LOQ for all three analytes (both ions/matrices), except for the confirmation ion analysis of florpyrauxifen-benzyl in pasture compost (*ca.* 10-17%; Tables 3-14, pp. 23-34). Recoveries for the LOD samples were reviewer-calculated as 82-174% for florpyrauxifen-benzyl, 64-141% for X11438848, and 64-130% for X11966341 (matrices/ions combined); mean recovery, s.d. and RSDs could not be determined since n = 2 (Tables 3-6, pp. 20-23; DER Attachment 2). Pasture and manure compost were obtained from Woods End Laboratories Inc. (p. 11). Fresh cut grass and alfalfa were mixed and composted for 96 days to form the pasture compost. Cow manure and rye straw were mixed and composted for 95 days to create the manure compost. Composting procedures were cited by DAS Study ID 170933 (pp. 11, 19).

ILV (MRID 51025002): Mean recoveries and RSDs were within guideline requirements for analysis in one compost matrix of florpyrauxifen-benzyl at the fortification levels of 0.15 ng/g (LOQ), 1.5 ng/g (10 \times LOQ), and 7.5 ng/g (50 \times LOQ), X11438848 at the fortification levels of 0.45 ng/g (LOQ), 4.5 ng/g (10 \times LOQ), and 22.5 ng/g (50 \times LOQ), and X11966341 at the fortification levels of 9 ng/g (LOQ), 90 ng/g (10 \times LOQ), and 450 ng/g (50 \times LOQ; n = 5; pp. 27-28). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. LOD samples were prepared but not quantified for recovery. Compost used in this study was untreated material purchased from a local market; the compost source was not further specified (p. 24). The sample material was thoroughly homogenized in a cutter with dry ice and stored at \leq -18 $^{\circ}$ C in the dark until fortification and extraction. The method for florpyrauxifen-benzyl in compost was validated in the first trial with insignificant modifications to the analytical instrumentation and the substitution of Phenomenex roQ QuEChERS kit with an unspecified QuEChERS Citrat kit (pp. 16, 24, 31; Appendix A, pp. 35-44, 48).

Table 2. Initial Validation Method Recoveries for Florpyrauxifen-benzyl and its metabolites X11438848 and X11966341 in Compost^{1,2,3}

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Manure Compost						
Quantitation Ion Transition						
Florpyrauxifen-benzyl	0.05 (LOD)	2	100, 126	--	--	--
	0.15 (LOQ)	10	90-120	105	9.8	9.4
	1.5	10	92-106	99	4.9	4.9
	7.5	9	81-98	88	5.7	6.5
X11438848	0.15 (LOD)	2	84, 99	--	--	--
	0.45 (LOQ)	10	85-103	92	4.8	5.2
	4.5	10	90-100	96	3.2	3.4
	22.5	9	92-106	96	4.1	4.3
X11966341	3.0 (LOD)	2	89, 94	--	--	--
	9.0 (LOQ)	10	88-97	93	3.1	3.3
	90	10	93-101	97	2.7	2.8
	450	9	82-90	86	2.6	3.0
Confirmatory Ion Transition						
Florpyrauxifen-benzyl	0.05 (LOD)	2	90, 116	--	--	--
	0.15 (LOQ)	10	99-116	105	5.3	5.1
	1.5	10	97-111	104	4.2	4.1
	7.5	9	86-115	94	8.8	9.4
X11438848	0.15 (LOD)	2	64 , 111	--	--	--
	0.45 (LOQ)	10	84-98	92	5.1	5.6
	4.5	10	88-97	92	3.6	3.9
	22.5	9	91-102	97	3.4	3.5
X11966341	3.0 (LOD)	2	85, 90	--	--	--
	9.0 (LOQ)	10	76-86	80	2.6	3.3
	90	10	78-85	82	2.4	3.0
	450	9	77-83	80	2.1	2.6
Pasture Compost						
Quantitation Ion Transition						
Florpyrauxifen-benzyl	0.05 (LOD)	2	84, 174	--	--	--
	0.15 (LOQ)	10	96-120	107	9.0	8.4
	1.5	10	106-124	115	5.7	4.9
	7.5	10	87-100	94	4.7	5.1
X11438848	0.15 (LOD)	2	95, 107	--	--	--
	0.45 (LOQ)	10	77-96	89	5.2	5.9
	4.5	10	88-96	93	2.5	2.7
	22.5	10	94-102	98	3.1	3.1
X11966341	3.0 (LOD)	2	95, 130	--	--	--
	9.0 (LOQ)	10	97-111	104	4.7	4.6
	90	10	102-109	105	2.5	2.4
	450	10	79-92	88	3.5	4.0

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Confirmatory Ion Transition						
Florpyrauxifen-benzyl	0.05 (LOD)	2	82, 130	--	--	--
	0.15 (LOQ)	10	73-109	91	10.9	11.9
	1.5	10	92-109	101	5.9	5.9
	7.5	10	89-109	100	6.2	6.2
X11438848	0.15 (LOD)	2	111, 141	--	--	--
	0.45 (LOQ)	10	80-104	95	8.0	8.4
	4.5	10	85-95	89	3.3	3.7
	22.5	10	91-103	98	3.8	3.9
X11966341	3.0 (LOD)	2	64 , 89	--	--	--
	9.0 (LOQ)	10	75-81	78	2.2	2.8
	90	10	80-84	81	1.5	1.8
	450	10	77-82	79	1.6	2.1

Data (recovery results were corrected for residues quantified in the controls, Tables 3-14, pp. 23-34; Figure 13, p. 53) were obtained from Tables 3-16, pp. 23-35 of MRID 51025001 and DER Attachment 2.

Bold red text indicates values not within guideline requirements.

- 1 Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 439→91 and *m/z* 441→91 for florpyrauxifen-benzyl, *m/z* 349→268 and *m/z* 349→253 for X11438848, and *m/z* 335→254 and *m/z* 337→256 for X11966341.
- 2 Pasture and manure compost were obtained from Woods End Laboratories Inc. (p. 11). Fresh cut grass and alfalfa were mixed and composted for 96 days to form the pasture compost. Cow manure and rye straw were mixed and composted for 95 days to create the manure compost. Composting procedures were cited by DAS Study ID 170933 (pp. 11, 19).
- 3 Recoveries for the LOD samples were reviewer-calculated based on data from Tables 3-14, pp. 23-34 since the study author did not calculate these recoveries (DER Attachment 2). Mean recovery, s.d. and RSDs could not be determined since n = 2.

Table 3. Independent Validation Method Recoveries for Florpyrauxifen-benzyl and its metabolites X11438848 and X11966341 in Compost^{1,2}

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Compost						
Quantitation ion						
Florpyrauxifen-benzyl	0.15 (LOQ)	5	107-111	109	2	2
	1.5	5	102-116	107	5	5
	7.5	5	93-105	98	4	5
X11438848	0.45 (LOQ)	5	79-88	84	4	5
	4.5	5	86-93	88	3	3
	22.5	5	80-85	83	2	3
X11966341	9.0 (LOQ)	5	69-71	70	1	1
	90	5	69-74	71	2	3
	450	5	66-73	70	3	4
Confirmatory ion						
Florpyrauxifen-benzyl	0.15 (LOQ)	5	101-107	104	3	3
	1.5	5	93-103	97	4	4
	7.5	5	82-89	85	3	3
X11438848	0.45 (LOQ)	5	81-90	85	4	5
	4.5	5	83-91	86	3	4
	22.5	5	79-84	82	3	3
X11966341	9.0 (LOQ)	5	71-75	73	1	2
	90	5	67-73	70	2	3
	450	5	72-75	74	1	2

Data (uncorrected recovery results, pp. 27-28) were obtained from pp. 27-28 of MRID 51025002 and DER Attachment 2.

1 Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): m/z 439→91 and m/z 441→91 for florpyrauxifen-benzyl, m/z 349→268 and m/z 349→253 for X11438848, and m/z 335→254 and m/z 337→256 for X11966341. These are the same as those of the ECM.

2 The compost used in this study was untreated material purchased from a local market; the compost source was not further specified (p. 24). The sample material was thoroughly homogenized in a cutter with dry ice and stored at ≤ -18°C in the dark until fortification and extraction.

3 Standard deviations were reviewer-calculated since these values were not calculated in the study report (see DER Attachment 2). The rules of significant figures were followed.

III. Method Characteristics

The LOQs in the ECM and ILV were reported as 0.15 ng/g, 0.45 ng/g, and 9.0 ng/g for florpyrauxifen-benzyl, X11438848, and X11966341, respectively (pp. 10, 14-15, 19; Table 17, p. 36 of MRID 51025001; pp. 28-29, 33 of MRID 51025002). The LODs in the ECM and ILV were reported as 0.05 ng/g, 0.15 ng/g, and 3.0 ng/g for florpyrauxifen-benzyl, X11438848 and X11966341, respectively, which were equivalent to 30% of the LOQ. Following the method of Keith, L. H., *et al.* (see section V. References below), the LOD and LOQ for determination of florpyrauxifen-benzyl in compost were calculated in the ECM and ILV using the standard deviation from the LOQ recovery results (Q&C; florpyrauxifen-benzyl/X11438848/X11966341), 0.008-0.016/0.021-0.035/0.210-0.421 ng/g (ECM) and 0.00268-0.00436/0.0167-0.1086/0.0976-0.128 ng/g (ILV). The LOD was calculated as three times the standard deviation ($3s$), and the LOQ was calculated as ten times the standard deviation ($10s$) of the recovery results. In the ECM, the LOQs and LODs for manure compost were calculated as 0.080-0.144 ng/g and 0.024-0.043 ng/g, respectively, for florpyrauxifen-benzyl, 0.214-0.227 ng/g and 0.064-0.068 ng/g, respectively, for X11438848, and 2.28-2.73 ng/g and 0.685-0.819 ng/g, respectively, for X11966341. In the ECM, the LOQs and LODs for pasture compost were calculated as 0.139-0.164 ng/g and 0.042-0.049 ng/g, respectively, for florpyrauxifen-benzyl, 0.231-0.355 ng/g and 0.069-0.106 ng/g, respectively, for X11438848, and 2.10-4.21 ng/g and 0.629-1.263 ng/g, respectively, for X11966341. In the ILV, the LOQs and LODs for compost were calculated as 0.0268-0.0436 ng/g and 0.00805-0.0131 ng/g, respectively, for florpyrauxifen-benzyl, 0.167-0.186 ng/g and 0.0502-0.0559 ng/g, respectively, for X11438848, and 0.976-1.28 ng/g and 0.293-0.384 ng/g, respectively, for X11966341. The calculated LOQ and LOD values supported the Method LOQ and LOD values.

Table 4. Method Characteristics

Analyte		Florpyrauxifen-benzyl	X11438848	X11966341
Limit of Quantitation (LOQ)	ECM/ILV	0.15 ng/g	0.45 ng/g	9.0 ng/g
	ECM (calc)	0.080-0.144 ng/g (Q/C, manure)	0.214-0.227 ng/g (Q/C, manure)	2.28-2.73 ng/g (Q/C, manure)
		0.139-0.164 ng/g (Q/C, pasture)	0.231-0.355 ng/g (Q/C, pasture)	2.10-4.21 ng/g (Q/C, pasture)
	ILV (calc)	0.0268 ng/g (Q) 0.0436 ng/g (C)	0.186 ng/g (Q) 0.167 ng/g (C)	0.976 ng/g (Q) 1.28 ng/g (C)
Limit of Detection (LOD)	ECM/ILV	0.05 ng/g	0.15 ng/g	3.0 ng/g
	ECM (calc)	0.024-0.043 ng/g (Q/C, manure)	0.064-0.068 ng/g (Q/C, manure)	0.685-0.819 ng/g (Q/C, manure)
		0.042-0.049 ng/g (Q/C, pasture)	0.069-0.106 ng/g (Q/C, pasture)	0.629-1.263 ng/g (Q/C, pasture)
	ILV (calc)	0.00805 ng/g (Q) 0.0131 ng/g (C)	0.0559 ng/g (Q) 0.0502 ng/g (C)	0.293 ng/g (Q) 0.384 ng/g (C)
Linearity (calibration curve r and concentration range)	ECM	r = 0.9987 (Q) r = 0.9981 (C)	r = 0.9992 (Q) r = 0.9993 (C)	r = 0.9993 (Q) r = 0.9995 (C)
	ILV	r = 0.9987 (Q) r = 0.9990 (C)	r = 0.9998 (Q) r = 0.9999 (C)	r = 0.9997 (Q & C)
	Range	0.045-9 ng/g	0.135-27 ng/g	2.7-540 ng/g
Repeatable	ECM ¹	Yes at the LOQ, 10×LOQ, and 50×LOQ in pasture and manure compost matrices.		
	ILV ^{2,3}	Yes at the LOQ, 10×LOQ, and 50×LOQ in unspecified compost matrices.		
Reproducible	Yes at the LOQ, 10×LOQ, and 50×LOQ.			
Specific	ECM	Representative 10×LOQ chromatograms were not presented.		
		Yes, matrix interferences were <3% (Q) and <20% (C, pasture) of the LOQ (based on peak area and quantified residues). LOQ peak was small compared to baseline noise. ⁴	Yes, matrix interferences were <8% of the LOQ (based on peak area).	No matrix interferences were observed. Yes for manure compost. No for pasture compost; analyte peak was very broad in pasture compost. ⁵
	ILV	No , no matrix interferences were observed; however, LOQ peak was only distinguishable from baseline noise by RT. ⁶	Yes, no matrix interferences were observed.	

Data were obtained from pp. 10, 14-15, 19; Table 17, p. 36 (LOD/LOQ); p. 14; Figures 7-12, pp. 47-52 (calibration data and figures); Tables 3-16, pp. 23-35 (recovery data); Figures 14-22, pp. 54-71 (chromatograms) of MRID 51025001; pp. 28-29, 33 (LOD/LOQ); p. 26; Appendix C, pp. 61-72 (calibration data and figures); pp. 27-28 (recovery data); Appendix D, pp. 74-121 (chromatograms) of MRID 51025002. Q = Quantitation ion transition; C = Confirmation ion transition.

- In the ECM, pasture and manure compost were obtained from Woods End Laboratories Inc. (p. 11 of MRID 51025001). Fresh cut grass and alfalfa were mixed and composted for 96 days to form the pasture compost. Cow manure and rye straw were mixed and composted for 95 days to create the manure compost. Composting procedures were cited by DAS Study ID 170933 (pp. 11, 19).
- In the ILV, compost used in this study was untreated material purchased from a local market; the compost source was not further specified (p. 24 of MRID 51025002). The sample material was thoroughly homogenized in a cutter with dry ice and stored at ≤-18°C in the dark until fortification and extraction.

- 3 The ILV validated the method for florpyrauxifen-benzyl in compost the first trial with insignificant modifications to the analytical instrumentation and the substitution of Phenomenex roQ QuEChERS kit with an unspecified QuEChERS Citrat kit (pp. 16, 24, 19-20, 31 of MRID 51025002).
- 4 Based on Figure 19, p. 64, and Figure 20, p. 66, of MRID 51025001.
- 5 Based on Figure 20, p. 67, of MRID 51025001.
- 6 Based on Appendix D, Figure 27, p. 80, and Figure 43, p. 88, of MRID 51025002.

IV. Method Deficiencies and Reviewer's Comments

1. The specificity of the method for florpyrauxifen-benzyl in compost at the LOQ was not supported by ILV representative chromatograms since the LOQ peak was only distinguishable from baseline noise by retention time (Appendix D, Figure 27, p. 80, and Figure 43, p. 88, of MRID 51025002). The reviewer noted that the ILV representative chromatograms of the 10×LOQ fortification for florpyrauxifen-benzyl in compost were acceptable; however, significant baseline noise was observed at that fortification level (Appendix D, Figure 29, p. 81, and Figure 45, p. 89).
2. Only one compost which was unspecified was used in the ILV; pasture and manure compost were used in the ECM (p. 11 of MRID 51025001; p. 24 of MRID 51025002).
3. The specificity of the method for X11966341 in pasture compost was not supported by ECM representative chromatograms since the LOQ analyte peak was very broad and undefined (Figure 20, p. 67 of MRID 51025001). The specificity of the method for florpyrauxifen-benzyl in manure/pasture compost was not well-supported by ECM representative chromatograms since the LOQ peak was small compared to baseline noise (Figure 19, p. 64; Figure 20, p. 66).
4. In the ECM, representative 10×LOQ chromatograms were not provided. Representative chromatograms from all matrices and fortifications should be provided for review.
5. In the ECM, recovery results were corrected for residues quantified in the controls (Tables 3-14, pp. 23-34; Figure 13, p. 53 of MRID 51025001). Residues in the controls accounted for *ca.* 1.3-6.4% of the LOQ for all three analytes (both ions/matrices), except for the confirmation ion analysis of florpyrauxifen-benzyl in pasture compost (*ca.* 10-17%).
6. The reviewer noted that ILV recoveries for X11966341 were acceptable but low (*ca.* 70%) for all fortifications/ions (pp. 27-28 of MRID 51025002). The ILV study report did not comment on this issue. The reviewer noted that X11966341 appeared to be the most water soluble of the three analytes based on chemical structure.

The reviewer noted that ECM performance data (recovery results) from primary and confirmatory analyses were not comparable for X11966341 where confirmation ion recoveries were notably lower (Tables 3-16, pp. 23-35 of MRID 51025001).

7. In the ILV, the Phenomenex roQ QuEChERS kit (EN Method; 4.0 g MgSO₄, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS, Phenomenex catalog number KS0-8909) was replaced with QuEChERS Citrat kit (no source/item No. reported; Appendix I, pp. 74, 80 of MRID 51025001; p. 16; Appendix A, pp. 35-36, 41, 48 of MRID 51025002). The reviewer assumed that the QuEChERS Citrat kit used by the ILV was also suitable for QuEChERS Method EN 15662:2008; however, the QuEChERS Citrat kit should have been listed in the ILV equipment list with details.
8. The matrix effects were found to be insignificant ($\leq 20\%$) for the analytes in the test matrices in the ECM (quantitation and confirmatory transitions) with the use of an internal standard, except for X11966341 (Q; *m/z* 335/254) transition in pasture compost (23.5%; pp. 16-17; Tables 21-22, p. 40 of MRID 51025001). The report noted that significant matrix effects (-41.6% and -52.4%) were observed for the X11966341 (Q; *m/z* 335/254 and 337/256) transition in pasture compost without the internal standard ratio. Solvent-based standards were used in the ECM and ILV; the reviewer noted that matrix-matched standards should have been used for X11966341 in pasture compost.
9. Carryover was assessed in the ECM and ILV (p. 15 of MRID 51025001; p. 30 of MRID 51025002). Carryover equivalent to $<30\%$ of the LOQ was observed in the ECM and ILV.
10. The reviewer noted a typographical error in the titles of Figures 93-94, p. 113, and Figures 109-110, p. 121 of MRID 51025002, which should have a fortification level of 0.09 mg/kg for the 10 \times LOQ fortification.
11. Since stable-isotope labeled internal standards were used, isotopic cross-over was evaluated in the ECM (pp. 11-12; Table 2, p. 22 of MRID 51025001). The concentration range of calibration curve and concentration of internal standard were chosen to minimize cross-over. No significant mass spectral isotopic crossover was observed.
12. In the ECM, the calibration solutions in 0.1% formic acid and methanol were found to be stable up to 3 days of refrigerated storage (*ca.* 4°C; pp. 15-16; Table 18, p. 37 of MRID 51025001). The stock solutions were found to be stable in methanol up to 158 days of storage in a separate study. The final sample extracts were found to be stable up to 1 day (pasture) and 2 days (manure) at *ca.* 4°C (p. 16; Tables 19-20, pp. 38-39).

In the ILV, the stability of the calibration solutions in 0.1% formic acid and methanol was determined to be up to 7 days when stored refrigerated (1-10°C) in the dark (p. 18 of MRID 51025002). The final sample extracts were found to be stable up to 5 days when stored refrigerated (1-10°C) in the dark (p. 19).
13. In the ECM, it was reported that the extraction efficiency of the method was previously studied (DAS Study ID 140594, 2016; pp. 13, 19 of MRID 51025001). The study also demonstrated the need for a hydrolysis step to hydrolyze conjugated residues of X11966341 and X11438848 to the free acid forms for determination of X11966341 and

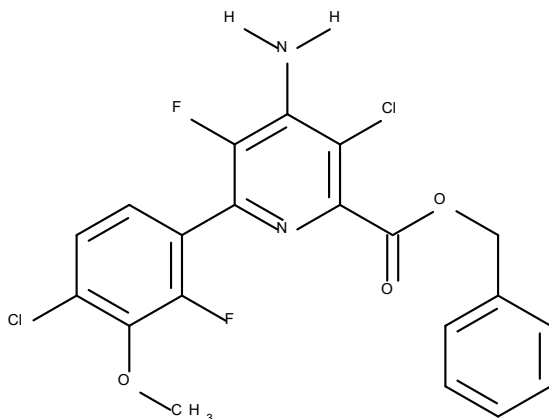
- X11438848. A hydrolysis step was included in the analytical method to hydrolyze the conjugated residues as described in nature of residue study.
14. The ILV reported that no communications occurred between the ILV and method developers (pp. 19, 31 of MRID 51025002). The ILV and ECM were carried-out at different laboratories with different personnel, instrumentation, materials, and equipment.
 15. In the ILV, it was reported that 12 samples required 11 hours of work, with LC/MS/MS unattended (Appendix A, p. 47 of MRID 51025002). It was reported for the ECM that one sample set (1 reagent blank, 1 control, a minimum of three fortified controls and associated samples) required *ca.* 1.5 working days (p. 13 of MRID 51025001). Evaluation of the LC/MS/MS results was completed the day following the sample processing.

V. References

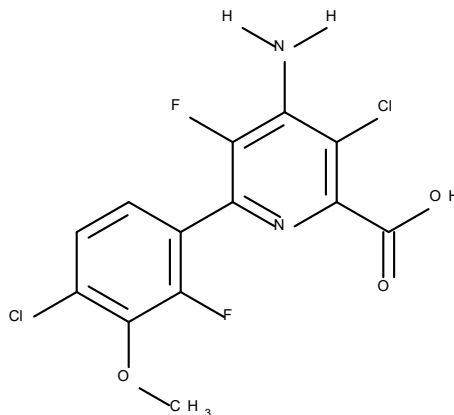
- Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, 55, 2210-2218 (p. 19 of MRID 51025001; p. 33 of MRID 51025002).
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods 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**Florpyrauxifen-benzyl (XDE-848 Benzyl Ester, or XDE-848 BE)**

IUPAC Name: Benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate
CAS Name: Not reported
CAS Number: Not reported
SMILES String: [H]N([H])c1c(c(nc(c1Cl)C(=O)OCc2ccccc2)c3ccc(c(c3F)OC)Cl)F

**X11438848**

IUPAC Name: 4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid
CAS Name: Not reported
CAS Number: Not reported
SMILES String: [H]N([H])c1c(c(nc(c1Cl)C(=O)O)c2ccc(c(c2F)OC)Cl)F



X11966341

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

CAS Name: Not reported

CAS Number: Not reported

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

