Analytical method for Halauxifen-methyl (XDE-729 methyl) and its metabolite, XDE-729 acid, in soil

Reports: ECM: EPA MRID No.: 49702101 Hill, R. C. 2015. Method Validation Study for the Determination of Residues of XDE-729 Methyl and Its Acid Metabolite in Soil by Liquid Chromatography with Tandem Mass Spectrometry. Dow AgroSciences Study No.: 150877. Report prepared, sponsored, and submitted by Regulatory Sciences and Government Affairs – Indianapolis Lab, Dow AgroSciences LLC, Indianapolis, Indiana; 84 pages. Final report issued August 26, 2015.

ILV: EPA MRID No. 50215501. Wiltshire, K. 2016. Independent Laboratory Validation of an Analytical Method for the Determination of XDE-729 Methyl and XDE-729 Acid in Soil using LC-MS/MS. DAS PCTR No.: 10002074-012-40402-0002 and Study ID: 151206. Study ID: CEMS-7432. Report prepared by CEM Analytical Services Ltd. (CEMAS), Berkshire, United Kingdom, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 89 pages. Final report issued June 27, 2016.

Document No.: MRIDs 49702101 & 50215501

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49702101). 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 and UK GLP standards, (p. 3; Appendix 3, p. 81 of MRID 50215501). 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).

This analytical method is classified as **Supplemental**. In the ILV and ECM, no samples were prepared at 10×LOQ. The ILV soil was not characterized or described.

PC Code: 117501

Final EPA Reviewer: Rochelle F. H. Bohaty, PhD
Senior Chemist

Signature: ROCHELLE BOHATY Digitally signed by ROCHELLE BOHATY Date: 2019.06.10 08:58:07 -04'00'

Date: June 10, 2019

CDM/CSS-Dynamac JV Reviewers:

Lisa Muto,
Environmental Scientist

Signature: Lisa Muto

Date: 5/22/16

Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: Kathleen P. Ferguson

Date: 5/22/16

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

The analytical method, Dow AgroSciences Study No. 150877, is designed for the quantitative determination of XDE-729 methyl and its metabolite XDE-729 acid, in soil at the LOQ of 0.0015 ng/g[1] using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil of 7.8 x 10^{-6} µg/kg [2] (IC_{05}) for listed dicot (MRID 49053301). The ECM performed the method using characterized silt loam soil, sandy loam soil and two loam soils; the ILV validated the method using one uncharacterized, undescribed soil. Analytes were identified using two ion transitions. No samples were prepared at 10×LOQ in the ECM or ILV. In the ILV, the method for XDE-729 methyl and its metabolite was validated in the third trial with insignificant modifications to sample preparation and the analytical parameters and instrumentation. The first trial was unsuccessful due to an error with the XDE-729 acid internal standard preparation and contamination of the control samples. The second trial was unsuccessful due to contamination of the control samples. The ILV study author noted that it was critical to avoid any contamination of the control samples due to the low limit of quantitation and special precautions should be taken during sample processing.

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						
XDE-729 methyl	49702101	50215501		Soil[1,2]	26/08/2015	Dow AgroSciences LLC	LC/MS/MS	0.0015 ng/g
XDE-729 acid								

1 In the ECM, Silt loam soil (SGN 001; 39% sand 53% silt 8% clay, pH 5.6, 4.5% organic carbon) obtained from Brierlow, Derbyshire, United Kingdom, sandy loam soil (SGN 002; 65% sand 25% silt 10% clay, pH 7.9, 0.6% organic carbon) obtained from Longwoods, Lincolnshire, United Kingdom, loam soil (SGN 003; 37% sand 46% silt 17% clay, pH 6.1, 5.3% organic carbon) obtained from Schmallenberg, North Rhine, Germany, and loam soil (SGN 004; 43% sand 40% silt 17% clay, pH 7, 0.7% organic carbon) obtained from Stanislaus, California, United States were used in this study (USDA soil texture classification; p. 12; Table 2, p. 21 of MRID 49702101).

2 In the ILV, the soil (CCON/073/016) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota; however, the soil description and characterization was not included in the ILV study report (p. 12 of MRID 50215501).

---

[1] LOQ = 0.0015 ng/g (µg/kg) = 0.0000015 mg/kg;
0.0000015 mg/kg (s) = [x] kg/ha x 10^6 mg/kg / [0.15 m (depth) x 10^4 m^2/ha x 1.5 x 10^3 kg(s)/m^3(density)]
0.0000015 mg/kg(s) = [x] kg/ha x 10^6 mg/kg / 2.25 x 10^6 kg (s)/ha
= 3.4 x 10^{-6} kg/ha = LOQ = 3.0 x 10^{-6} lb/A

[2] Listed dicot IC_{05} = 9.5 x 10^{-8} lb/A (MRID 49053301) x 0.454 kg/lb / 2.47 ha/A] x 10^6 mg/kg / [0.15 m (depth) x 10^4 m^2/ha x 1.5 x 10^3 kg(s)/m^3(density)] = 1.7 x 10^{-8} kg/ha x 10^6 mg/kg / 2.25 x 10^6 kg (s)/ha = 4.3 x 10^{-9} mg/kg = 7.8 x 10^{-6} µg/kg (s) (IC_{05})

I. Principle of the Method

Samples (5.0 ± 0.05 g) of soil were weighed into 50 mL centrifuge tubes and fortified, as necessary (p. 11; Appendix I, pp. 74-75, 80-82 of MRID 49702101). The samples were extracted twice with 15 mL of acetonitrile:water (80:20, v:v) containing 0.1% H_3PO_4 by shaking for 30 minutes on a reciprocating shaker at *ca*. 280 excursions/minute. After centrifugation for 5 minutes at 3000 rpm, the supernatant was transferred to a clean 45 mL vial. The solvent of the combined extracts was removed using a TurboVap evaporator set at 40°C and a nitrogen pressure of *ca*. 15 psi. To the remaining liquid, 3 mL of HPLC water was added. The sample was thoroughly mixed via vortex and sonication for *ca*. 30 seconds. The Strata SCX solid phase extraction (SPE) cartridge (100 mg, 3 mL) was prepared by conditioning with 3 mL of methanol containing 0.1% H_3PO_4 followed by 3 mL of water containing 0.1% H_3PO_4. After the cartridge was dried under vacuum for 5-10 seconds, the extract mixture was applied to the column and pulled through at *ca*. 0.5 mL/minute. After the cartridge was dried under vacuum for *ca*. 5-10 seconds, the sample vial was rinsed with 2 mL of water containing 0.1% H_3PO_4 and transferred to the SPE column. After the cartridge was dried under vacuum for *ca*. 5-10 seconds, the cartridge was washed with two 1 mL aliquots of methanol containing 0.1% H_3PO_4. The analytes were eluted with two 1.5 mL aliquots of acetonitrile:methanol (90:10, v:v) containing 0.1% NH_4OH into an 12-mL culture tubes. Full vacuum was applied to the SPE for about 10 seconds after SPE stopped dripping to ensure complete recovery. Mixed internal standard (15 µL of 2.5 ng/mL) was added to each sample then the sample was evaporated to dryness using a TurboVap evaporator set at 40°C and a nitrogen pressure of *ca*. 15 psi. Coupling reagent (100 µL) was added with vortex mixing for 2-3 seconds then 100 µL of acetonitrile:butyl chloroformate (90:10, v:v) reagent was added with vortex mining for 2-3 seconds. The samples sat at room temperature for *ca*. 5 minutes before evaporating to dryness using a TurboVap evaporator set at 40°C and a nitrogen pressure of *ca*. 15 psi. To the remaining liquid, 500 µL of acetonitrile:water (70:30, v:v) containing 0.1% H_3PO_4 was added with vortex mixing for 2-3 seconds. Samples were transferred to autosampler vials for analysis by LC/MS/MS. Higher concentration samples were further diluted as necessary.

Supplemental notes were added to the ECM which indicated that modifications of the equipment, glassware, materials, reagents and chemicals with equivalent items were allowed, as well as modifications of solution volumes and analytical conditions (Appendix I, p. 82 of MRID 49970204).

Samples were analyzed for XDE-729 and XDE-729 acid (as XDE-729 butyl ester) by Agilent 1290 Infinity HPLC (Acquity UPLC HSS T3 column, 2.1 mm x 50 mm, 1.8 µm column; column temperature 35°C) using a mobile phase of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid [percent A:B at 0:0.5 min. 70:30, 5.5 min. 20:80, 6.0-7.0 min. 0:100] with AB SCIEX QTRAP 5500 MS using MS/MS-ESI (electrospray ionization) detection in positive polarity and multiple reaction monitoring (MRM; p. 11; Appendix I, pp. 83-84 of MRID 49970204). Injection volume was 10 µL. Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 344.9→250.1 and *m/z* 344.9→235.0 for XDE-729 and *m/z* 387.0→250.1 and *m/z* 387.0→207.1 for XDE-729 butyl ester. Observed retention times were *ca*. 3.25 and 4.7 minutes for XDE-729 methyl and XDE-729 butyl ester, respectively

(Figures 22-25, pp. 65-68). Ion transitions were also monitored for the internal standards of each analyte.

In the ILV, the ECM was performed as written, except for the use of sample concentrator set at 40°C with a flow of nitrogen instead of a TurboVap and insignificant modifications to the analytical method (pp. 11, 18; Appendix 2, pp. 79-80 of MRID 50215501). The modifications to the analytical method included: an Agilent 1260/1290 HPLC coupled with AB SCIEX QTRAP 5500 MS using MS/MS-ESI was used; the flow rate was reduced from 550 to 450 l/min; and 3 minutes of equilibration time was added to the end of the gradient program [percent A:B at 0:0.5 min. 70:30, 5.5 min. 20:80, 6.0-7.0 min. 0:100, 7.10-10.00 min. 70:30]. The same ion transitions were monitored in the ILV as the ECM. No other modifications to the ECM were reported.

In the ILV, the study authors noted the following critical step: due to the low limit of quantitation, it was critical to avoid any contamination of the control samples by having the sample concentration of the controls occur before fortified samples or thoroughly cleaning the concentrators with iso-propyl alcohol or having dedicated concentrator equipment for controls (p. 18 of MRID 50215501).

The Limit of Quantification (LOQ) for XDE-729 methyl and XDE-729 acid in soil was 0.0015 ng/g in the ECM and ILV (pp. 11, 15, 17; Table 27, p. 40 of MRID 49702101; pp. 11, 16 of MRID 50215501). The Limit of Detection (LOD) for XDE-729 methyl and XDE-729 acid in soil was reported as 0.00045 ng/g in the ECM and ILV. In the ECM, the LOQ and LOD were calculated as 0.0005-0.0015 ng/g and 0.0002-0.0005 ng/g, respectively, for XDE-729 methyl and 0.0011-0.0017 ng/g and 0.0003-0.0005 ng/g, respectively, for XDE-729 acid.

II. Recovery Findings

ECM (MRID 49702101): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of XDE-729 methyl and XDE-729 acid (as XDE-729 butyl ester) in soil matrices at the fortification level of 0.0015 ng/g (LOQ) and 0.100 ng/g (*ca*. 67×LOQ; Tables 7-26, pp. 23-39; DER Attachment 2); however, individual samples recoveries fell outside this range for XDE-729 acid in two soils. No samples were prepared at 10×LOQ. Both analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Recoveries for the 0.00045 ng/g samples (LOD) were reviewer-calculated; mean recovery, s.d. and RSDs could not be determined since n = 2. The LOD statistics were calculated using 0.0005 ng/g instead of 0.00045 ng/g as the fortification since that was what was reported as "added" in the tables of the study report. LOD recoveries were 100-260% for XDE-729 methyl and 60-260% for XDE-729 acid (matrices/ions combined). Four soil matrices were used in the ECM: silt loam soil (SGN 001; 39% sand 53% silt 8% clay, pH 5.6, 4.5% organic carbon) obtained from Brierlow, Derbyshire, United Kingdom, sandy loam soil (SGN 002; 65% sand 25% silt 10% clay, pH 7.9, 0.6% organic carbon) obtained from Longwoods, Lincolnshire, United Kingdom, loam soil (SGN 003; 37% sand 46% silt 17% clay, pH 6.1, 5.3% organic carbon) obtained from Schmallenberg, North Rhine, Germany, and loam soil (SGN 004; 43% sand 40% silt 17% clay,

pH 7, 0.7% organic carbon) obtained from Stanislaus, California, United States (USDA soil texture classification; p. 12; Table 2, p. 21). The soils were referred to by their international texture classifications of sandy loam, sandy loam, clay loam and clay loam, respectively, in the tables of the study report.

ILV (MRID 50215501): Mean recoveries and RSDs were within guideline requirements for analysis of XDE-729 methyl and XDE-729 acid in one soil matrix at fortification levels of 0.0015 ng/g (LOQ) and 0.100 ng/g (*ca*. 67×LOQ; 8-15, pp. 23-27; DER Attachment 2); however, individual samples fell outside this range sometime by a large margin for XDE-729 acid. No samples were prepared at 10×LOQ. Both analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Recoveries for the 0.00045 ng/g samples (LOD) were reviewer-calculated; mean recovery, s.d. and RSDs could not be determined since n = 1. LOD recoveries were only calculated for the quantitation ion of XDE-729 methyl (58%) since all other recovery values were reported as negatives. The test soil (CCON/073/016) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota; however, the soil description and characterization was not included in the ILV study report (p. 12). The method for XDE-729 methyl and its metabolite was validated in the third trial with insignificant modifications to sample preparation and the analytical parameters and instrumentation (pp. 18-19). The first trial was unsuccessful due to an error with the XDE-729 acid internal standard preparation and contamination of the control samples. The second trial was unsuccessful due to contamination of the control samples. The ILV study author noted that it was critical to avoid any contamination of the control samples due to the low limit of quantitation and special precautions should be taken during sample processing.

Table 2. Initial Validation Method Recoveries for XDE-729 methyl and Its Metabolite, XDE-729 acid, in Soil[1,2,3]

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Silt Loam Soil SGN 001					
	Quantitation Ion Transition					
XDE-729 methyl	0.00045 (LOD)	**2**	100, **160**	--	--	--
	0.0015 (LOQ)	5	98-115	104	6	6
	0.100	6	74-81	78	2	3
XDE-729 acid[4]	0.00045 (LOD)	**2**	100, **220**	--	--	--
	0.0015 (LOQ)	5	79-99	94	8	9
	0.100	6	85-91	88	2	2
	Confirmatory Ion Transition					
XDE-729 methyl	0.00045 (LOD)	**2**	100, **160**	--	--	--
	0.0015 (LOQ)	5	101-125	109	9	8
	0.100	6	74-81	78	2	3
XDE-729 acid[4]	0.00045 (LOD)	**2**	100, **200**	--	--	--
	0.0015 (LOQ)	5	81-102	95	8	9
	0.100	6	85-91	88	2	2
	Sandy Loam Soil SGN 002					

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation Ion Transition					
XDE-729 methyl	0.00045 (LOD)	2	100	--	--	--
	0.0015 (LOQ)	5	94-106	100	5	4
	0.100	6	86-92	90	2	3
XDE-729 acid[4]	0.00045 (LOD)	2	80, 100	--	--	--
	0.0015 (LOQ)	5	87-109	99	9	9
	0.100	6	85-101	96	6	6
	Confirmatory Ion Transition					
XDE-729 methyl	0.00045 (LOD)	2	100, 120	--	--	--
	0.0015 (LOQ)	5	98-108	103	4	4
	0.100	6	86-93	91	3	3
XDE-729 acid[4]	0.00045 (LOD)	2	80, 100	--	--	--
	0.0015 (LOQ)	5	90-106	97	7	8
	0.100	6	86-103	97	6	6
	Loam Soil SGN 003					
	Quantitation Ion Transition					
XDE-729 methyl	0.00045 (LOD)	2	100, 120	--	--	--
	0.0015 (LOQ)	5	92-104	96	5	5
	0.100	6	83-87	85	2	2
XDE-729 acid[4]	0.00045 (LOD)	2	60, 100	--	--	--
	0.0015 (LOQ)	5	60-89	76	11	15
	0.100	6	60-81	71	7	10
	Confirmatory Ion Transition					
XDE-729 methyl	0.00045 (LOD)	2	100, 120	--	--	--
	0.0015 (LOQ)	5	91-111	101	8	8
	0.100	6	83-86	85	1	2
XDE-729 acid[4]	0.00045 (LOD)	2	60, 80	--	--	--
	0.0015 (LOQ)	5	72-91	83	7	9
	0.100	6	60-81	71	7	10
	Loam Soil SGN 004					
	Quantitation Ion Transition					
XDE-729 methyl	0.00045 (LOD)	2	100, 240	--	--	--
	0.0015 (LOQ)	5	98-111	105	5	5
	0.100	5	91-94	93	2	2
XDE-729 acid[4]	0.00045 (LOD)	2	80, 260	--	--	--
	0.0015 (LOQ)	5	77-100	89	10	11
	0.100	5	70-81	77	4	5
	Confirmatory Ion Transition					
XDE-729 methyl	0.00045 (LOD)	2	100, 260	--	--	--
	0.0015 (LOQ)	5	99-116	109	7	6
	0.100	5	92-95	93	1	2
XDE-729 acid[4]	0.00045 (LOD)	2	80, 220	--	--	--
	0.0015 (LOQ)	5	78-103	88	10	11
	0.100	5	70-84	77	5	7

Data (uncorrected recovery results, pp. 52-53) were obtained from Tables 7-26, pp. 23-39 of MRID 49702101 and DER Attachment 2.

1 Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 344.9→250.1 and *m/z* 344.9→235.0 for XDE-729 and *m/z* 387.0→250.1 and *m/z* 387.0→207.1 for XDE-729 acid (as XDE-729 butyl ester).
2 Silt loam soil (SGN 001; 39% sand 53% silt 8% clay, pH 5.6, 4.5% organic carbon) obtained from Brierlow, Derbyshire, United Kingdom, sandy loam soil (SGN 002; 65% sand 25% silt 10% clay, pH 7.9, 0.6% organic carbon) obtained from Longwoods, Lincolnshire, United Kingdom, loam soil (SGN 003; 37% sand 46% silt 17% clay, pH 6.1, 5.3% organic carbon) obtained from Schmallenberg, North Rhine, Germany, and loam soil (SGN 004; 43% sand 40% silt 17% clay, pH 7, 0.7% organic carbon) obtained from Stanislaus, California, United States were used in this study (USDA soil texture classification; p. 12; Table 2, p. 21). The soils were referred to by their international texture classifications of sandy loam, sandy loam, clay loam and clay loam, respectively, in the tables of the study report.
3 Recoveries for the 0.00045 ng/g samples (LOD) were reviewer-calculated based on data from Tables 7-22, pp. 23-38 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. The LOD statistics were calculated using 0.0005 ng/g instead of 0.00045 ng/g as the fortification since that was what was reported as "added" in the tables of the study report.
4 As XDE-729 butyl ester.

Table 3. Independent Validation Method Recoveries for XDE-729 methyl and Its Metabolite, XDE-729 acid, in Soil[1,2,3]

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[4]	Relative Standard Deviation (%)
	Soil					
	Quantitation ion					
XDE-729 methyl	0.00045 (LOD)	1	58	--	--	--
	0.0015 (LOQ)	6	82-96	90	5	5.1
	0.100	6	80-89	83	4	4.2
XDE-729 acid[5]	0.00045 (LOD)	1	--[6]	--	--	--
	0.0015 (LOQ)	6	60-84	77	9	11.6
	0.100	6	53-83	71	13	18.2
	Confirmatory ion					
XDE-729 methyl	0.00045 (LOD)	1	--[6]	--	--	--
	0.0015 (LOQ)	6	86-93	90	2	2.8
	0.100	6	76-88	81	4	5.4
XDE-729 acid[5]	0.00045 (LOD)	1	--[6]	--	--	--
	0.0015 (LOQ)	6	60-89	82	11	13.3
	0.100	6	52-83	72	13	18.6

Data (uncorrected recovery results,) were obtained from Tables 8-15, pp. 23-27 of MRID 50215501 and DER Attachment 2.
1 Analytes were identified using two ion transitions (quantitative and confirmatory, respectively): *m/z* 344.9→250.1 and *m/z* 344.9→235.0 for XDE-729 and *m/z* 387.0→250.1 and *m/z* 387.0→207.1 for XDE-729 acid (as XDE-729 butyl ester).
2 The soil (CCON/073/016) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota; however, the soil description and characterization was not included in the ILV study report (p. 12).
3 Recoveries for the 0.00045 ng/g samples (LOD) was reviewer-calculated based on data from Tables 8-11, pp. 23-26 since the study author did not calculate these recoveries (DER Attachment 2). Mean recovery, s.d. and RSDs could not be determined since n = 1.
4 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.
5 As XDE-729 butyl ester.
6 A negative recovery value was reported in the tables in the study report.

III. Method Characteristics

The LOQ for XDE-729 methyl and XDE-729 acid in soil was 0.0015 ng/g in the ECM and ILV (pp. 11, 15, 17-18; Table 27, p. 40 of MRID 49702101; pp. 11, 16 of MRID 50215501). The Limit of Detection (LOD) for XDE-729 methyl and XDE-729 acid in soil was reported as 0.00045 ng/g in the ECM and ILV. Following the method of Keith, L. H., *et al*. (see section V. References below), the LOD and LOQ for determination of XDE-729 methyl and XDE-729 acid in soil were calculated in the ECM using the standard deviation from the 0.0015 ng/g 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 LOQ and LOD were calculated as 0.0005-0.0015 ng/g and 0.0002-0.0005 ng/g, respectively, for XDE-729 methyl and 0.0011-0.0017 ng/g and 0.0003-0.0005 ng/g, respectively, for XDE-729 acid. The calculated values support the LOQ and LOD established for the study. No justifications of the LOQ or LOD were reported in the ILV.

Table 4. Method Characteristics

Analyte		**XDE-729 methyl**	**XDE-729 acid**
Limit of Quantitation (LOQ)	ECM	0.0015 ng/g	
		0.0008-0.0009 ng/g (Q, calculated) 0.0005-0.0015 ng/g (C, calculated)	0.0011-0.0017 ng/g (Q, calculated) 0.0011-0.0013 ng/g (C, calculated)
	ILV	0.010 ppm g/g)	
Limit of Detection (LOD)	ECM	0.00045 ng/g	
		0.0003 ng/g (Q, calculated) 0.0002-0.0005 ng/g (C, calculated)	0.0003-0.0005 ng/g (Q, calculated) 0.0003-0.0004 ng/g (C, calculated)
	ILV	0.00045 ng/g	
Linearity (calibration curve r^2 and concentration range)	ECM[1]	r^2 = 0.9992-0.9994 (Q) r^2 = 0.9984-0.9994 (C)	r^2 = 0.9988-0.9990 (Q) r^2 = 0.9988-0.9992 (C)
	ILV	r^2 = 0.9990 (Q) r^2 = 0.9991 (C)	r^2 = 0.9997 (Q) r^2 = 0.9999 (C)
	Range	0.0045-2.5 ng/mL (equivalent to 0.00045-0.25 ng/g)	
Repeatable	ECM[2]	Yes at LOQ and *ca*. 67×LOQ, but **no** samples prepared at 10×LOQ.	
	ILV[3,4]		
Reproducible		Yes at LOQ and *ca*. 67×LOQ. **Could not be determined for 10×LOQ**.	
Specific		10×LOQ chromatograms were **not** presented.	
	ECM	Yes, no matrix interferences were observed.	
	ILV	Yes, no matrix interferences were observed.	Yes, matrix interferences were *ca*. 23% (Q) and *ca*. 14% (C) of the LOQ (based on peak area).[5]

Data were obtained from pp. 11, 15, 17; Tables 3-6, p. 22 (calibration data); Tables 7-26, pp. 23-39 (recovery data); Table 27, p. 40 (calculated LOQ/LOD); Figures 17-29, pp. 60-72 (chromatograms) of MRID 49702101; pp. 11, 16; Tables 6-7, p. 22 (calibration data); Tables 8-15, pp. 23-27 (recovery data); Figures 15-28, pp. 44-57 (chromatograms) of MRID 50215501. Q = Quantitation ion transition; C = Confirmatory ion transition.

1 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Tables 3-6, p. 22 of MRID 49702101; DER Attachment 2). Solvent standards were used.

2 In the ECM, Silt loam soil (SGN 001; 39% sand 53% silt 8% clay, pH 5.6, 4.5% organic carbon) obtained from Brierlow, Derbyshire, United Kingdom, sandy loam soil (SGN 002; 65% sand 25% silt 10% clay, pH 7.9, 0.6%

organic carbon) obtained from Longwoods, Lincolnshire, United Kingdom, loam soil (SGN 003; 37% sand 46% silt 17% clay, pH 6.1, 5.3% organic carbon) obtained from Schmallenberg, North Rhine, Germany, and loam soil (SGN 004; 43% sand 40% silt 17% clay, pH 7, 0.7% organic carbon) obtained from Stanislaus, California, United States were used in this study (USDA soil texture classification; p. 12; Table 2, p. 21 of MRID 49702101). The soils were referred to by their international texture classifications of sandy loam, sandy loam, clay loam and clay loam, respectively, in the tables of the study report.

3 In the ILV, the soil (CCON/073/016) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota; however, the soil description and characterization was not included in the ILV study report (p. 12 of MRID 50215501).

4 In the ILV, the method for XDE-729 methyl and its metabolite was validated in the third trial with insignificant modifications to sample preparation and the analytical parameters and instrumentation (pp. 18-19 of MRID 50215501). The first trial was unsuccessful due to an error with the XDE-729 acid internal standard preparation and contamination of the control samples. The second trial was unsuccessful due to contamination of the control samples.

5 Based on Figures 23-26, pp. 52-55 of MRID 50315501.

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM and ILV, no samples were prepared at 10×LOQ (Tables 7-26, pp. 23-39 of MRID 49702101; Tables 8-15, pp. 23-27 of MRID 50215501). OCSPP guidelines state that a minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

2. In the ILV, the soil (CCON/073/016) was supplied by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota; however, the soil description and characterization was not included in the ILV study report (p. 12 of MRID 50215501). It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.

3. The reviewer noted that the failures of the first two trials was due to contamination of the control samples (pp. 18-19 of MRID 50215501). The ILV study author noted that it was critical to avoid any contamination of the control samples due to the low limit of quantitation and special precautions should be taken during sample processing.

4. While the mean recoveries are within the guideline requirement, individual recoveries were outside the range sometimes by a large margin for the specified LOQ for XDE-729 acid [see **Table 2** (ECM) and Table **3** (ILV)] and LOD for XDE-729 [see **Table 2** (ECM) as well as the 0.100 fortification levels [see **Table 2** (ECM) and Table **3** (ILV)].

5. In ILV representative chromatograms of XDE-729 acid, matrix interferences were observed to be *ca*. 23% (Q) and *ca*. 14% (C) of the LOQ, based on reported peak area (Figures 23-26, pp. 52-55 of MRID 50215501). These interferences were <LOD (30% of the LOQ). The reviewer did not understand why the recovery values in Tables 10 and 11 did not match these observed matrix interferences (Tables 10-11, pp. 25-26).

6. The matrix effects were found to be insignificant (<20%) for XDE-729 methyl and XDE-729 acid in the ECM and ILV (quantitation and confirmatory transitions; p. 16; Tables 28-31, p. 41 of MRID 49702101; pp. 17-18; Tables 16-19, pp. 28-29 of MRID 50215501).

7. In the ECM, the satisfactory extraction efficiency of this analytical method was demonstrated by extracting samples of soil containing incurred residues from Dow AgroSciences Study 101621 (terrestrial field dissipation; pp. 17-18; Table 32, pp. 42-43 of MRID 49702101).

   In other Dow AgroSciences studies, the analytical standards and fortifications solutions were found to be stables up to 295 days of storage at ambient temperature and the final sample extracts were found to be stable up to 2 days at 4°C (p. 17; Tables 8-10, pp. 26-28 of MRID 50215501).

8. The ILV detailed the communications between the ILV Study Director and the ECM Study Monitor (p. 18; Appendix 4, pp. 82-89 of MRID 50215501). The communication mainly involved the communication of the failed ILV trials and investigation and contamination elimination work. The Study Monitor was quite involved in finding a solution to the contamination problem, including asking for videos or pictures of the ILV sample processing.

9. It was reported for the ILV that one sample set (1 reagent blank, 2 controls, 1 LOD sample, 5 LOQ samples and 5 *ca.* 67×LOQ samples) required *ca*. 14 hours for one technician to prepare with LC/MS/MS performed unattended overnight (p. 16 of MRID 50215501). Evaluation of the LC/MS/MS results was completed the following day, so a set of 14 samples was completely processed and evaluated in *ca*. 2 working 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. 18 of MRID 49702101).

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

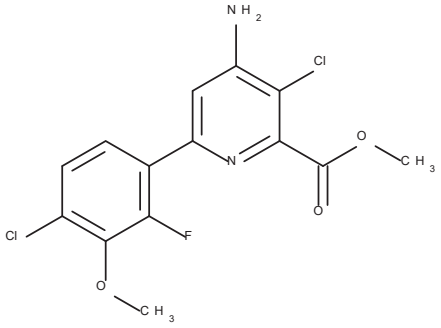
Halauxifen-methyl (XDE-729 methyl, XDE-729 ME, X11393729)

IUPAC Name: Methyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)pyridine-2-carboxylate

CAS Name: Methyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-2-pyridinecarboxylate

CAS Number: 943831-98-9

SMILES String: COc1c(ccc(c1F)c2cc(c(c(n2)C(=O)OC)Cl)N)Cl



XDE-729 acid (X11393729)

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

CAS Name: 2-Pyridinecarboxylic acid, 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-

CAS Number: 943832-60-8

SMILES String: COc1c(ccc(c1F)c2cc(c(c(n2)C(=O)O)Cl)N)Cl