Test Material:	Halauxifen-methyl
MRID:	48557785
Title:	Method validation study for the determination of residues of X11393728 (XDE-729 methyl), X11393729 (XDE-729 acid) and X11449757 (des-methyl XDE-729 acid) in soil using high performance liquid chromatography with positive-ion electrospray ionization mass spectrometry detection.
MRID:	48557862
Title:	Independent laboratory validation of analytical method study number 110716 for the determination of XDE-729 methyl ester and its metabolite residues in soil.
EPA PC Code:	117501
OCSPP Guideline:	850.6100

For Cambridge Environmental

Primary Reviewer: Lynne Binari **Signature:** Lynne Binari

Date: 4/5/13

Secondary Reviewer: Kathleen Ferguson **Signature:** Kathleen P. Ferguson

Date: 4/5/13

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

Date: 4/5/13

Analytical method for halauxifen-methyl, X11393729 and X11449757 in soil

Reports:	ECM: EPA MRID No. 48557785. Blakeslee, B. 2012. Method validation study for the determination of residues of X11393728 (XDE-729 methyl), X11393729 (XDE-729 acid) and X11449757 (des-methyl XDE-729 acid) in soil using high performance liquid chromatography with positive-ion electrospray ionization mass spectrometry detection. Study No.: 110716. Report prepared, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana (p. 3); 148 pages plus 2 cover pages. Final report issued August 27, 2012. ILV: EPA MRID No. 48557862. Gemrot, F. 2012. Independent laboratory validation of analytical method study number 110716 for the determination of XDE-729 methyl ester and its metabolite residues in soil. EAS Study No.: S12-03363. Dow Study Reference No.: 120894. Report prepared by Eurofins AgroScience Services Chem, Vergèze, France, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 107 pages. Final report issued August 16, 2012.
Document No.:	MRIDs 48557785 & 48557862
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report is included in the Quality Assurance statement (p. 4). ILV: The study was conducted in accordance with OECD Good Laboratory Practice (GLP) standards (p. 3). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report is included in the Quality Assurance statement (p. 4).
Classification:	This analytical method is classified as **ACCEPTABLE**; however, the established LOQ for **halauxifen-methyl and its products X11393729 and X11449757** in soil is **not** less than the lowest toxicological level of concern.
PC Code:	117501
Reviewer:	Rochelle F. H. Bohaty, Chemist U.S. EPA **Signature:** Rochelle F H Bohaty **Date:** April 23, 2014

All page citations refer to MRID 48557785 (ECM) unless otherwise noted.

Executive Summary

This analytical method, Dow Study No.: 110716, is designed for the quantitative determination of halauxifen-methyl and its products X11393729 and X11449757 in soil using LC/MS/MS. The method is quantitative for halauxifen-methyl, X11393729 and X11449757 at the stated LOQ of 0.05 µg/kg[1]; however, based on preliminary analysis of currently available data, the lowest level of concern 7.8 x 10^{-6} µg/kg [2] (IC_{05}) for listed dicot (MRID 49053301) provides the lowest level of concern for terrestrial organisms. Thus this method would **NOT** be able to detect concentrations in soil below the lowest level of concern. No major issues were discovered by the independent laboratory.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation					
Halauxifen-methyl X11393729 X11449757	48557785	48557862	Soil	27/08/2012	Dow AgroSciences LLC	LC/MS/MS	0.05 µg/kg

I. Principle of the Method

Soil (5 g) is combined with diatomaceous earth, then extracted with methanol:water (50:50, v:v) containing 0.1% phosphoric acid by Accelerated Solvent Extraction (ASE), two cycles at 90°C and 1,500-2,500 psi for 5 minutes (pp. 14, 19, 25). The extract is fortified with a mixed stable-isotope internal standard solution ([phenyl-U-^{13}C]-labeled halauxifen-methyl, XDE-729 acid and X1144757), concentrated to *ca*. 1 mL under nitrogen (*ca*. 7 psi , Turbo Vap, 40ºC), brought to volume (100 mL) with acetonitrile, briefly sonicated and vortexed, then an aliquot (500 µL) was filtered (45-µm; pp. 23, 26).

Samples were analysed for halauxifen-methyl, X11393729 and X11449757 by HPLC (Phenomenex Synergi Hydro-RP, 4.6 x 75 mm, 4.0 µm, 80Å column) using a mobile phase gradient of (A) aqueous 0.1% formic acid and (B) 0.1% formic acid in acetonitrile [A:B at 0.00 min. 90:10 (v:v), 7.00-9.00 min. 0:100, 9.15-13.00 min. 90:10] with MS/MS-ESI$^+$ detection and Multiple Reaction Monitoring (MRM; pp. 19-21). Two MRM parent-daughter ions were monitored (quantitative, confirmatory) per analyte. The LOD of 0.02 µg/kg and LOQ of 0.05 µg/kg were used in the ECM and ILV.

II. Recovery Findings

[1] LOQ = 0.05 µg/kg = 0.00005 mg/kg; LOD = 0.02 µg/kg
0.00005 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)]
0.00005 mg/kg(s) = [x] kg/ha x 10^6 mg/kg / 2.25 x 10^6 kg (s)/ha
= 1.1 x 10^{-5} kg/ha = LOQ = 1.0 x 10^{-5} lb/A; LOD = 8.6 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})

ECM (MRID 48557785): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of halauxifen-methyl and X11393729 in soil for quantitative ion results (Tables 30-33, pp. 70-71). For X11449757 (quantitative ion results), the following sample sets were not within guideline criteria, 0.05 µg/kg fortified clay loam, silt loam and clay (means 63%, 66% and 69%, respectively) and 8.0 µg/kg fortified clay loam (mean 62%; Tables 31-33, pp. 70-71). Overall, quantitative ion and confirmatory ion results were comparable (Tables 30-37, pp. 70-73).

ILV (MRID 48557862): Mean recoveries and RSDs were within guideline requirements (Tables 1-2, pp. 29-30). Quantitative ion and confirmatory ion results were comparable. The method was validated with the first trial (p. 24).

Table 2. Initial Validation Method Recoveries for Analytes in Soil

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sand Soil					
Halauxifen-methyl	0.05 (LOQ)	8	85-112	96	9	9
	0.10	3	95-98	96	2	2
	8.0	5	102-110	105	3	3
XDE-729 acid (X11393729)	0.05 (LOQ)	8	72-83	77	4	6
	0.10	3	75-85	79	5	7
	8.0	5	70-78	75	3	4
des-methyl XDE-729 acid (X11449757)	0.05 (LOQ)	8	59-81[1]	73	7	10
	0.10	3	76-84	80	4	5
	8.0	5	78-85	83	3	3
	Clay Loam Soil					
Halauxifen-methyl	0.05 (LOQ)	9	81-106	98	8	8
	0.10	3	103-107	105	2	2
	8.0	5	92-99	95	3	3
XDE-729 acid (X11393729)	0.05 (LOQ)	9	64-89	75	8	11
	0.10	3	76-82	79	3	4
	8.0	5	68-74	70	3	4
des-methyl XDE-729 acid (X11449757)	0.05 (LOQ)	9	56-76	**63**	6	10
	0.10	3	68-73	71	3	4
	8.0	5	58-68	**62**	5	8
	Silt Loam Soil					
Halauxifen-methyl	0.05 (LOQ)	12[3]	93-109	101	5	5
	0.10	4	99-104	102	3	3
	8.0	6	97-109	102	4	4
XDE-729 acid (X11393729)	0.05 (LOQ)	16	70-92	79	7	8
	0.10	4	72-78	76	3	4
	8.0	6	73-79	76	2	3
des-methyl XDE-729 acid (X11449757)	0.05 (LOQ)	16	56-85	**66**	7	11
	0.10	4	72-75	74	1	2
	8.0	6	64-76	71	5	7
	Clay Soil					
Halauxifen-methyl	0.05 (LOQ)	8	94-111	103	5	5
	0.10	3	97-107	102	5	5
	8.0	5	92-97	95	2	2
XDE-729 acid (X11393729)	0.05 (LOQ)	8	78-93	84	5	6
	0.10	3	80-84	81	2	3
	8.0	5	76-80	78	2	2
des-methyl XDE-729 acid (X11449757)	0.05 (LOQ)	8	55-78	**69**	9	13
	0.10	3	73-84	78	6	7
	8.0	5	73-74	73	1	1

Data (quantitative ion results) were obtained from Tables 30-33, pp. 70-71 in MRID 48557785.
n.p. = Not performed.
1 Range incorrectly reported as 59-74% in Table 30 (p. 70); see Table 22 (p. 62).
2 In study report, 81% recovery deemed outlier by Grubbs' test and was excluded from summary table (Table 8, p. 48; Table 31, p. 70). Mean, SD and RSD calculated by reviewer (DER Attachment 2).
3 Results from four recovery samples were excluded due to obvious fortification error (Table 32, p. 71).

Table 3. Independent Validation Method Recoveries for Analytes in Soil

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Clay Soil					
Halauxifen-methyl	0.05 (LOQ)	5	89-103	97	6	6
	0.5	5	97-116	106	7	6
XDE-729 acid (X11393729)	0.05 (LOQ)	5	84-98	90	5	6
	0.5	5	94-111	100	6	6
des-methyl XDE-729 acid (X11449757)	0.05 (LOQ)	5	89-114	102	11	11
	0.5	5	92-108	100	6	6
	Sand Soil					
Halauxifen-methyl	0.05 (LOQ)	5	89-104	98	6	6
	0.5	5	103-109	106	2	2
XDE-729 acid (X11393729)	0.05 (LOQ)	5	87-104	97	7	7
	0.5	5	103-112	106	4	3
des-methyl XDE-729 acid (X11449757)	0.05 (LOQ)	5	79-109	95	12	13
	0.5	5	92-102	99	4	4

Data (quantitative ion results) were obtained from Tables 1-2, pp. 29-30 in MRID 48557862. Standard deviations not provided in ILV report were calculated by reviewer (DER Attachment 2).

III. Method Characteristics

The LOD and LOQ were proposed at 0.02 µg/kg and 0.05 µg/kg, respectively, for each analyte at ECM initiation (p. 35). The LOD and LOQ were then verified through calculation of 3x and 10x, respectively, the standard deviation from the 0.05 µg/kg recovery (measured in fortified sample less matrix blank) results from each soil type (pp. 35-36; Tables 38-41, pp. 74-75). The determinations of the LOD/LOQ were based on scientifically acceptable procedures as defined in Keith, L.H., *et al*., *Anal. Chem*., 1983, 55: 2210-2218 (pp. 35, 38).

A set of eighteen samples and a reagent blank required *ca*. 1 ½ calendar days; *ca*. eight person hours for laboratory work, followed by 5 hours of unattended LC/MS/MS run time, and *ca*. 3 hours of data evaluation and transcription (p. 25 in MRID 48557862).

Table 4. Method Characteristics[1]

	Halauxifen-methyl	X11393729	X11449757
Limit of Quantitation (LOQ)	0.05 µg/kg	0.05 µg/kg	0.05 µg/kg
Limit of Detection (LOD)	0.02 µg/kg	0.02 µg/kg	0.02 µg/kg
Linearity (calibration curve r^2 and concentration range)	r^2 = 0.9996-1.0000 (0.01-6.0 µg/L)	r^2 = 0.9994 (n = 2) (0.01-6.0 µg/L)	r^2 = 0.9986-0.9992 (0.01-6.0 µg/L)
Repeatable	Yes	Yes	Yes
Reproducible	Yes	Yes	Yes
Specific	Yes	Yes	Yes

Data were obtained from p. 24; Tables 1-2, pp. 29-30; Figures 1-6, pp. 32-37 of MRID 48557862.
1 As verified by the ILV.

IV. Method Deficiencies and Reviewer's Comments

1. The estimation of the LOD was based on scientifically acceptable procedures (Reference 2 below).

2. The method is quantitative for halauxifen-methyl, X11393729 and X11449757 at the stated LOQ of 0.05 µg/kg[3]; however, based on preliminary analysis of currently available data, the lowest level of concern 4.3 x10^{-3} µg/kg[4] (NOAEC) for vascular plant (MRID 48557770) provides the lowest level of concern for terrestrial organisms

3. ECM performance data at 10 x LOQ were not reported.

4. All ECM performance data from clay loam, silt loam and clay soils were not within guideline criteria; however, all ILV performance data using sand and clay soils met guideline criteria.

5. Reagent blank samples were included in the ILV analyses, but the chromatograms were not provided for review (p. 11; Tables 1-2, pp. 29-30).

6. In the ECM, stability of halauxifen-methyl, XDE-729 acid and X11449757 (0.05-8.0 µg/kg) in clay loam soil extracts was investigated after 4 and 10 days of refrigerator (temperature not specified) storage (p. 34). Mean (n = 13) recoveries after 0, 4 and 10 days of storage were 99%, 98% and 100%, respectively, for halauxifen-methyl, 74%, 76% and 80%, respectively, for XDE-729 acid, and 64%, 66% and 68%, respectively, for X11449757 (Table 3, pp. 42-43).

7. In the ILV, stability of halauxifen-methyl, XDE-729 acid and X11449757 (0.05 µg/kg) in clay soil extracts was investigated after 18 days of refrigerator (0-9ºC) storage (Table 3, p. 31). Mean (n = 5) recoveries after 0 and 18 days of storage were 97% and 91%, respectively, for halauxifen-methyl, 90% and 97%, respectively, for XDE-729 acid, and 102% and 85%, respectively, for X11449757 (Table 3, p. 31).

V. References

1. 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, D.C. EPA 712-C-001.

2. Keith, L.H.; Crummett; Deegan, J., Jr.; Libby, R.A.; Taylor, J.K; Wentler, G.; "Principles of Environmental Analysis", *Anal. Chem.*, 1983, 55: 2210-2218.

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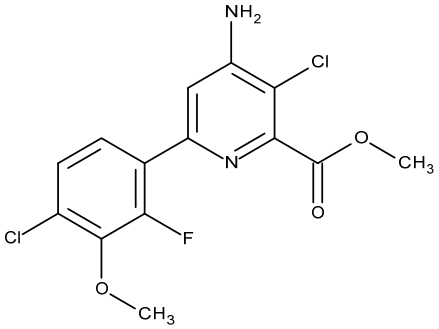
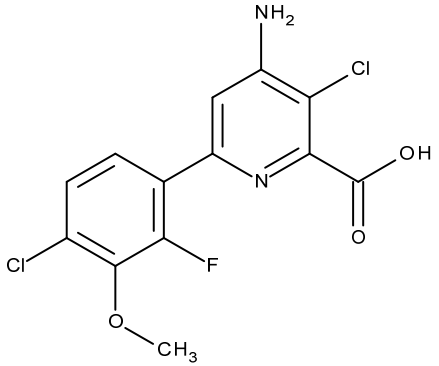
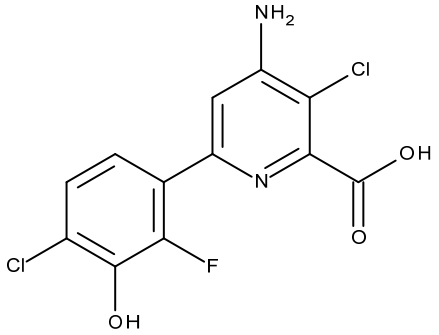
[3] LOQ = 0.05 µg/kg = 0.00005 mg/kg
0.00005 mg/kg (s) = [x] kg/ha x 10^6 mg/kg / [0.15 m (depth) x 10^4 m^2/ha x 1.5 x 10^3 kg(s)/m^3(density)]
0.00005 mg/kg(s) = [x] kg/ha x 10^6 mg/kg / 2.25 x 10^6 kg (s)/ha
= 1.1 x 10^{-4} kg/ha = **1.0 x 10^{-4} lb/A**

[4] **[**8.7 x 10^{-6} lb/A (NOAEC) x 0.454 kg/lb x 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)]
9.76 x 10^{-6} kg/ha x 10^6 mg/kg / 2.25 x 10^6 kg (s)/ha
= 4.3 x 10^{-6} mg/kg = **4.3 x 10^{-3} µg/kg (s) (NOAEC)**

DER ATTACHMENT 1. Halauxifen-methyl and Its Transformation Products.

Code Name/ Synonym	Chemical Name	Chemical Structure
Halauxifen-methyl (XDE-729 methyl, X11393728, XR-279 methyl, XDE-729 ME)	IUPAC: **Methyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)pyridine-2-carboxylate** CAS: **Methyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-2-pyridinecarboxylate** CAS No.: **943831-98-9** Formula: **$C_{14}H_{11}Cl_2FN_2O_3$** MW: **345.16 g/mol** SMILES: **COc1c(ccc(c1F)c2cc(c(c(n2)C(=O)OC)Cl)N)Cl**	
X11393729 (X'729, XDE-729 acid)	IUPAC: **4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)pyridine-2-carboxylic acid** CAS: **2-Pyridinecarboxylic acid, 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-** CAS No.: **943832-60-8** Formula: **$C_{13}H_9Cl_2FN_2O_3$** MW: **331.13 g/mol** SMILES: **COc1c(ccc(c1F)c2cc(c(c(n2)C(=O)O)Cl)N)Cl**	
X11449757 (X'757, Des-methyl-XR-729, Des-methyl XDE-729, X757)	IUPAC: **4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)picolinic acid** CAS: **4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-pyridine-2-carboxylic acid** Formula: **$C_{12}H_7Cl_2FN_2O_3$** MW: **317.11 g/mol** SMILES: **c1cc(c(c(c1c2cc(c(c(n2)C(=O)O)Cl)N)F)O)Cl**	

MW means "molecular weight".