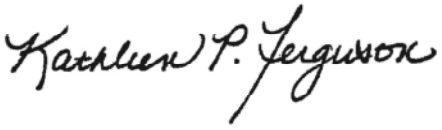
Test Material:	Halauxifen-methyl
MRID:	48557787
Title:	Method validation study for the determination of residues of XDE-729 and its metabolites in surface water, ground water and drinking water by liquid chromatography with tandem mass spectrometry, amended report.
MRID:	48557788
Title:	XDE-729 methyl ester - independent laboratory validation of analytical method 110718 for the determination of XDE-729 methyl ester and its metabolites residues in water.
EPA PC Code:	117501
OCSPP Guideline:	850.6100

For Cambridge Environmental

Primary Reviewer: Lynne Binari **Signature:** 

Date: 4/5/13

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 4/5/13

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

Date: 4/5/13

Analytical method for halauxifen-methyl, X11393729, X11406790 and X11449757 in water

Reports:	ECM: EPA MRID No. 48557787. Rodrigues Jr., A. and Q. Li. 2012. Method validation study for the determination of residues of XDE-729 and its metabolites in surface water, ground water and drinking water by liquid chromatography with tandem mass spectrometry, amended report. Study No.: 110718. Report prepared by Dow AgroSciences Ind., Ltda., São Paula, Brazil, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana (p. 3; Appendix B, p. 122); 135 pages. Final report issued December 23, 2011. Amended report issued August 30, 2012. ILV: EPA MRID No. 48557788. Gemrot, F. 2012. XDE-729 methyl ester - independent laboratory validation of analytical method 110718 for the determination of XDE-729 methyl ester and its metabolites residues in water. EAS Study No.: S11-04023. Dow Study Reference No.: 110827. Report prepared by Eurofins AgroScience Services Chem, Vergèze, France, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 160 pages. Final report issued March 28, 2012.
Document No.:	MRIDs 48557787 & 485577788
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 **UPGRADABLE** based on submission of additional data. The method calibration curves are inconsistent (see review comment 4) or are not provided. In addition, the independent laboratory recoveries could not be verified as supporting data were not provided (see review comment 6).
PC Code:	117501
Reviewer:	Rochelle F. H. Bohaty, Chemist U.S. EPA
Signature:	 **Date:** January 5, 2016

All page citations refer to MRID 48557787 (ECM) unless otherwise noted.

Executive Summary

This analytical method, Dow Study No.: 110718, is designed for the quantitative determination of halauxifen-methyl and its products X11393729, X11406790 and X11449757 in water using LC/MS/MS. The method is quantitative for halauxifen-methyl, X11393729, X11406790 and X11449757 at the proposed LOQ of 0.05 µg/L. 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 X11406790 X11449757	48557787	48557788	Water	30/08/2012	Dow AgroSciences LLC	LC/MS/MS	0.05 µg/L

I. Principle of the Method

Water (10 mL) is acidified with 10% formic acid (1.0 mL), then loaded onto a reverse-phase solid-phase extraction (SPE) cartridge (Strata X, 3 mL/60 mg), preconditioned with methanol, followed by 0.1% formic acid (p. 24). The loaded sample is rinsed with 0.1% formic acid, dried under vacuum, and then residues are eluted with methanol. Extract is fortified with a mixed stable-isotope internal standard solution (M+6 halauxifen-methyl, X11393729, X11406790 and X1144757), concentrated under nitrogen (*ca*. 7 psi, Turbo Vap, 40ºC), brought to volume (1.0 mL) with water:acetonitrile:formic acid (90:10:0.1, v:v;v), vortexed and sonicated to mix (pp. 22-23, 25).

Samples were analysed for halauxifen-methyl, X11393729, X11406790 and X11449757 by HPLC (Zorbax SB-C8, 4.6 x 75 mm, 3.5 µm column) using a mobile phase gradient of (A) 0.1% formic acid in methanol and (B) aqueous 0.1% formic acid [A:B at 0.00 min. 10:90 (v:v), 7.0-9.0 min. 100:0, 9.1-12.0 min. 10:90] 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 0.015 µg/L and LOQ of 0.05 µg/L were used in the ECM and ILV.

II. Recovery Findings

ECM (MRID 48557787): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%), except for X11406790 0.05 µg/L fortification in groundwater (RSD 22%; Tables 1-12, pp. 35-46). Confirmatory ion analyte/internal standard peak area ratios were compared to mean peak area ratios of the calibration standards, according to the method of Bethem, R.A., *et al*., *J. Am. Soc. Mass Spectrom.* 2003, 14: 523-541 (pp. 27-28, 34). All fortified sample ratios were within the specified range of ± 20% of the calibration standard mean peak area ratios (Tables 13-17, pp. 47-56).

ILV (MRID 48557788): Mean recoveries and RSDs were within guideline requirements (Tables 1-3, pp. 30-32). Quantitative ion and confirmatory ion recovery results were comparable. The method was validated with the first trial (p. 25).

Table 2. Initial Validation Method Recoveries for Analytes in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Groundwater (Well)					
Halauxifen-methyl	0.05 (LOQ)	6	72-109	92	15	16
	0.5	6	86-111	95	9	10
X11393729 (XDE-729 acid)	0.05 (LOQ)	6	78-117	100	17	17
	0.5	6	90-105	98	6	6
X11406790	0.05 (LOQ)	5	75-119	102	22	**22**
	0.5	6	87-98	96	4	5
X11449757	0.05 (LOQ)	6	78-119	103	15	15
	0.5	6	90-116	109	10	9
	Surface (River) Water					
Halauxifen-methyl	0.05 (LOQ)	6	72-105	86	15	17
	0.5	6	100-115	108	6	5
X11393729 (XDE-729 acid)	0.05 (LOQ)	5	76-115	98	18	18
	0.5	6	97-116	107	6	6
X11406790	0.05 (LOQ)	5	81-120	104	20	19
	0.5	6	94-109	103	6	6
X11449757	0.05 (LOQ)	5	78-119	96	18	19
	0.5	6	101-118	106	6	6
	Drinking (Tap) Water					
Halauxifen-methyl	0.05 (LOQ)	5	74-78	76	1	2
	0.5	5	104-110	108	3	2
X11393729 (XDE-729 acid)	0.05 (LOQ)	5	92-119	107	10	9
	0.5	6	95-114	106	7	6
X11406790	0.05 (LOQ)	5	70-82	78	5	6
	0.5	6	102-116	108	5	5
X11449757	0.05 (LOQ)	6	71-88	79	7	8
	0.5	6	98-115	103	6	6

Data were obtained from Tables 1-12, pp. 70-71 in MRID 48557787.

Table 3. Independent Validation Method Recoveries for Analytes in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Groundwater					
Halauxifen-methyl	0.05 (LOQ)	5	95-105	99	4	4
	0.5	5	104-106	105	1	1
X11393729 (XDE-729 acid)	0.05 (LOQ)	5	94-97	96	1	1
	0.5	5	85-95	90	4	4
X11406790	0.05 (LOQ)	5	92-98	94	3	3
	0.5	5	95-98	97	2	2
X11449757	0.05 (LOQ)	5	92-111	98	7	7
	0.5	5	87-93	90	3	3
	Surface (River) Water					
Halauxifen-methyl	0.05 (LOQ)	5	93-97	95	2	2
	0.5	5	100-107	103	3	3
X11393729 (XDE-729 acid)	0.05 (LOQ)	5	94-98	96	1	2
	0.5	5	100-106	103	2	2
X11406790	0.05 (LOQ)	5	93-103	98	4	4
	0.5	5	103-114	109	5	4
X11449757	0.05 (LOQ)	5	90-99	96	4	4
	0.5	5	97-106	102	4	4
	Drinking (Tap) Water					
Halauxifen-methyl	0.05 (LOQ)	5	95-101	98	2	2
	0.5	5	99-107	103	4	3
X11393729 (XDE-729 acid)	0.05 (LOQ)	5	93-95	94	1	1
	0.5	5	85-94	91	4	4
X11406790	0.05 (LOQ)	5	93-100	97	3	3
	0.5	5	90-94	92	2	2
X11449757	0.05 (LOQ)	5	91-98	94	3	3
	0.5	5	88-99	95	5	5

Data (quantitative ion results) were obtained from Tables 1-3, pp. 30-32 in MRID 48557788. Standard deviations not provided in ILV report were calculated by reviewer (DER Attachment 2).

III. Method Characteristics

The LOD and LOQ were proposed at 0.015 µg/L and 0.05 µg/L, respectively, for each analyte in the ECM (Appendix B, p. 124).

A set of eighteen samples and a reagent blank required *ca*. 1 ½ calendar days; *ca*. four person hours for laboratory work, followed by 5 hours of LC/MS/MS run time, and *ca*. 3 hours of data evaluation and transcription (p. 26 in MRID 48557788).

Table 4. Method Characteristics[1]

	Halauxifen-methyl	X11393729	X11406790	X11449757
Limit of Quantitation (LOQ)	0.05 µg/L	0.05 µg/L	0.05 µg/L	0.05 µg/L
Limit of Detection (LOD)	0.015 µg/L	0.015 µg/L	0.015 µg/L	0.015 µg/L
Linearity (calibration curve r^2 and concentration range)	r^2 = **0.9931-0.9934**[2] (0.15-20 µg/L)	r^2 = 0.9983-0.9990 (0.15-20 µg/L)	r^2 = 0.9979-0.9984 (0.15-20 µg/L)	r^2 = 0.9973-0.9979 (0.01-6.0 µg/L)
Repeatable	Yes	Yes	Yes	Yes
Reproducible	Yes	Yes	Yes	Yes

Specific	Yes	Yes	Yes	Yes

Data were obtained from p. 25; Tables 1-3, pp. 30-32; Figures 1-8, pp. 33-40 of MRID 48557788.
1 As verified by the ILV.
2 Satisfactory linearity is when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. It should be noted that the protocol provide in Appendix B was not executed as described; however, the environmental chemistry method that was actually used verified by the independent laboratory validation.

2. ECM performance data for X11406790 at the LOQ in ground water were not within guideline criteria (RSD 22%); however, all ILV performance data for X11406790 in groundwater, surface water and drinking water matrices met guideline criteria. *Therefore, this does not impact the classification of the study.*

3. ECM calibration curves for X11406790 and X1149757 could not be reconstructed because complete data were not provided. The data provided in Figures 3-4 (pp. 61-62) are the same peak area counts provided in Figure 1 (p. 59) for halauxifen-methyl. X11406970 and X1149757 calibration chromatograms were only provided for the 0.15, 0.5 and 5.0 ng/mL standards (Figures 7-8, pp. 65-66; Figures 11-12, pp. 69-70; Figures 15-16, pp. 73-74). *Additional data are needed to confirm the results of this study.*

4. For the ILV, linearity correlation coefficients (r^2) of the halauxifen-methyl calibration curves were 0.9934 and 0.9931 for the quantitative and confirmatory ions, respectively (Figures 1-2, pp. 33-34). Linearity is satisfactory when $r^2 \geq 0.995$; *however, the difference is minor and thus does not impact the study classification.*

5. ILV recoveries could not be verified because sufficient supporting data were not provided, *i.e.* peak area counts, measured concentrations. The provided calibration curves could not be correlated with the chromatograms to confirm any recovery results (DER Attachment 2: ILV chromatogram verification). *Additional data are needed to confirm the results of this study.*

6. Reagent blank samples were included in the ILV analyses, but the chromatograms were not provided for review (p. 13; Tables 1-3, pp. 30-32).

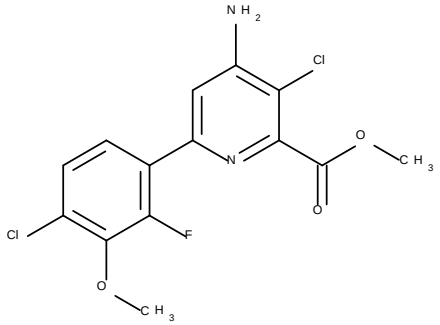
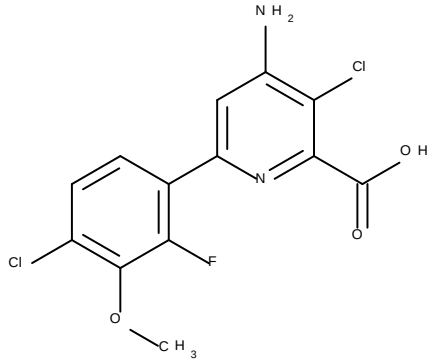
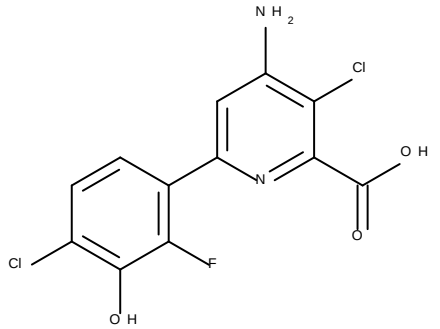
7. In the ECM, stability of halauxifen-methyl, XDE-729 acid (X11393729), X11406790 and X11449757 (0.05 and 0.50 µg/L) in extracts from the three water matrices was investigated after 6 days of refrigerator (temperature not specified) storage (p. 28). All recoveries were within 70-120% (Table 19. p. 58). Mean (n = 6) recoveries after 0 and 6 days of storage were 88% and 102%, respectively, for halauxifen-methyl, 108% and 100%, respectively, for X11393729, 88% and 103%, respectively, for X11406790, and 93% and 105%, respectively, for X11449757 (DER Attachment 2).

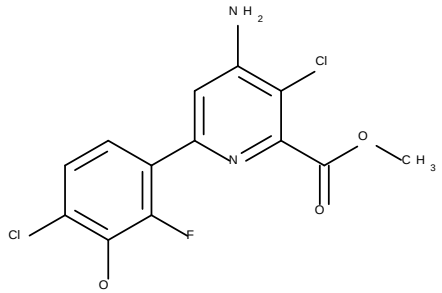
V. References

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.

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**	

Code Name/ Synonym	Chemical Name	Chemical Structure
X11406790 (X'790, X790)	CAS: **Methyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)pyridine-2-carboxylate** Formula: **$C_{13}H_9Cl_2FN_2O_3$** MW: **331.13 g/mol** SMILES: **COC(=O)c1c(c(cc(n1)c2ccc(c(c2F)O)Cl)N)Cl**	

MW means "molecular weight".