Test Material:	Florasulam
MRID:	49077901
Title:	Independent Laboratory Validation of Dow AgroSciences LLC Method ERC 96.21 - Determination of the Residues of XDE-570 and its 5-hydroxy Metabolite in Soil Using Organic Extraction
EPA PC Code:	129108
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 12/9/14

Secondary Reviewer: Lisa Muto **Signature:** 

Date: 12/9/14

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

Date: 12/9/14

Analytical method for florasulam (XDE-570) and its transformation product 5-OH florasulam (5-hydroxy XDE-570) in soil

Reports: **ECM**: EPA MRID No.: 49077901 (Appendix B, pp. 43-68). Butcher, S., D.R. Wright, B. Bratby, and M. Abrar (Appendix B, p. 44). 1997. Determination of Residues of XDE-570 and 5-hydroxy XDE-570 in Soil Using Organic Extraction. Report prepared by DowElanco Europe, Oxon, United Kingdom, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 25 pages. Lab Report Code ERC 96.21. Final report issued February 10, 1997.
ILV: EPA MRID No. 49077901. Beck, I.-C. and T. Class. 2008. Independent Laboratory Validation of Dow AgroSciences LLC Method ERC 96.21 - Determination of the Residues of XDE-570 and its 5-hydroxy Metabolite in Soil Using Organic Extraction. PTRL Europe Study No.: P 1485 G. Dow AgroSciences Protocol No.: 080131. Report prepared by PTRL Europe GmbH, Ulm, Germany, sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 77 pages. Final report issued August 15, 2008.

Document No.: MRID 49077901

Guideline: 850.6100

Statements: ECM: A statement pertaining to the conduct of the study in regards to Good Laboratory Practice (GLP) standards was not provided. Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were not provided.
ILV: The study was conducted in accordance with German Chemical Law (Chemikaliengesetz) GLP standards (§ 19a, 2002; p. 3; Appendix E, p. 77). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4; Appendix E, p. 77). A statement of the authenticity of the study report was included as part of the Quality Assurance Statement (p. 4).

Classification: This analytical method is classified as supplemental. An internal validation implementing major modifications to the original ECM was not provided. Sufficient ECM performance data at the LOQ and 10x LOQ for both analytes in all soil matrices were not provided. Sufficient chromatographic data were not provided to support validation of the ECM.
The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. Soil matrices used in the ECM were not adequately characterized, and the registrant did not specify that the soils used in the ILV were either an equivalent, or more difficult, analytical sample condition as those used in the ECM.

PC Code: 129108

Reviewer: James N. Carleton, Ph.D., Senior Fate Scientist **Date:** 12/16/15

JAMES CARLETON
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DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=JAMES CARLETON, dnQualifier=0000001907
Date: 2015.12.16 11:03:05 -05'00'

Page citations refer to the page numbers located in the upper right corner of MRID 49077901.

Executive Summary

This analytical method, Dow AgroSciences Method ERC 96.21, is designed for the quantitative determination of florasulam and its transformation product 5-OH florasulam in soil using LC/MS/MS. The method is quantitative for both analytes at the stated LOQ of 0.05 µg/kg. As no toxicological levels of concern have yet been established for florasulam or 5-OH florasulam in soil, it is not possible to determine whether the LOQ(s) is/are less than, equal to, or greater than the lowest toxicological level(s) of concern in soil. The independent laboratory validated the method for analysis of florasulam and 5-OH florasulam in sandy loam and clay soils after two trials. Major modifications were made to the ECM by the independent laboratory; however, an internal validation for the updated ECM implementing the ILV modifications was not provided. The registrant did not specify that the soils used in the ILV were either an equivalent, or more difficult, analytical sample condition as those used in the ECM.

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						
Florasulam	49077901 (Appendix B, pp. 43-68)	49077901		Soil	10/02/1997	Dow AgroSciences	LC/MS/MS	0.05 µg/kg
5-OH Florasulam								

I. Principle of the Method

Soil (25 ± 0.1 g) is fortified with a mixed standard of florasulam (XDE-570) and 5-OH florasulam (5-hydroxy XDE-570) in acetonitrile:water (1:1, v:v) for procedural recoveries, then extracted twice with acetone:1% aqueous acetic acid (9:1, v:v) by shaking (reciprocating shaker, speed not specified) for 60 minutes per extraction; extraction solvent volumes are 75 mL for the first extraction and 25 mL for the second extraction (Appendix B, pp. 48, 51). Soil and extract are separated by centrifugation (3,000 rpm, 10 minutes). Extracts are filtered through glass wool and combined. Acetone is removed from the combined extract by rotary evaporation (temperature not specified). The remaining aqueous sample is brought to 100 mL with water, acidified to <pH 3 (1M HCl, *ca*. 2 mL), then loaded onto a PolarPlus C18 solid-phase extraction (SPE) cartridge (2 g, 6 cc) preconditioned with methanol followed by 0.1M HCl. The loaded cartridge is rinsed with water, then residues are eluted with 3 mL acetonitrile. The eluate is brought to 30 mL with 0.01M sodium hydrogen carbonate ($NaHCO_3$), then loaded onto a SAX Bond Elute SPE cartridge (1 g, 6 cc) preconditioned with methanol followed by 0.01M $NaHCO_3$. The loaded cartridge is rinsed with 0.01M $NaHCO_3$ followed by acetone. Residues are eluted with 15 mL 0.1M HCl:methanol (9:1, v:v) and collected into a silanized glass vial. The eluate was partitioned twice with ethyl acetate (10 mL, followed by 5 mL). Organic phases are combined in a silanized glass tube, the residues are solvent exchanged into 1% aqueous acetic acid (2.0 mL), and the ethyl acetate removed by nitrogen evaporation (room temperature).

Samples are analyzed for florasulam and 5-OH florasulam by HPLC (Spherisorb ODS B, 4.6 mm x 250 mm, 5 µm column, column temperature not specified) using a mobile phase of (A) water:acetonitrile:acetic acid (60:40:1, v:v:v) and (B) 1% acetic acid in acetonitrile [percent A:B (v:v) at 0-10 min. 100:0, 11-15 min. 0:100, 16-21 min. 100:0; flow rate 1.0 mL/minute] with MS/MS-ESI (VG Quattro triple sector quadrupole MS, electrospray ionization, positive ion mode) detection and multiple reaction monitoring (MRM; Appendix B, pp. 49-50). Injection volume is 100 µL. Retention times are *ca*. 9 min. and 5 min. for florasulam and 5-OH florasulam, respectively. Florasulam and 5-OH florasulam are identified and quantified using one ion transition. Ion transitions monitored were as follows: *m/z* 360→129 for florasulam and *m/z* 346→129 for 5-OH florasulam. A confirmatory method was not reported.

The ILV performed the method as written with the following major modifications: the SAX Bond Elute SPE and subsequent phase partition extraction steps were eliminated as no analytes were detected in the SPE 0.1M HCl:methanol (9:1, v:v) eluate (p. 12). Following elution of residues from the PolarPlus C18 SPE cartridge with acetonitrile, an aliquot (1.5 mL) of the eluate was reduced to aqueous (*ca*. 200 µL) under nitrogen, brought to 1.0 mL with 1% aqueous acetic acid, and sonicated for LC/MS/MS analysis (p. 13). The following LC/MS/MS conditions were also modified: Phenomenex Luna C_{18} (4.6 mm x 250 mm, 5 µm) column with a Phenomenex C_{18} (3 mm x 4 mm) guard column, column temperature 30°C, flow rate 800 µL/minute, injection volume 80 µL (p. 14). An Applied Biosystems API 4000 LC/MS/MS was used under negative ion mode. Analytes were identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 358.1→166.9 (Q) and *m/z* 358.1→152 (C) for florasulam, and *m/z* 344.0→324 (Q) and *m/z* 344.0→104 (C) for 5-OH florasulam.

In the ECM and ILV, the LOQ for florasulam and 5-OH florasulam was 0.05 µg/kg (p. 12; Appendix B, p. 53). In the ECM, the LOD was set at 0.01 µg/kg. Although not specified, the ILV appeared to have also set the LOD at 0.01 µg/kg (20% of LOQ as defined by the ECM; pp. 9, 19; Appendix B, p. 53).

II. Recovery Findings

ECM (Appendix B, pp. 43-68 of 49077901): All fortifications were only performed in duplicate (n = 2); therefore, meaningful statistics for the recovery results per fortification level and soil matrix could not be generated. All individual recovery results were within 70-120% (Appendix B, Table 2, p. 57). All four soil matrices [clay loam, sandy loam (35% sand), sandy loam (70% sand), and loamy sand] were fortified with both analytes at 0.05 µg/kg (LOQ). Only the clay loam and sandy loam (70% sand) soils were fortified with both analytes at 0.5 µg/kg (10x LOQ). Additional fortification levels for both analytes were 5.0 µg/kg [100x LOQ, sandy loam (35% sand) and loamy sand soils] and 50.0 µg/kg (1,000x LOQ, clay loam and loamy sand soils). A confirmatory method was not utilized. Soil matrices were not characterized, other than percent sand in the two sandy loam soils.

ILV (MRID 49077901): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of florasulam and its transformation product 5-OH florasulam in a sandy loam soil and a clay soil at fortification levels of 0.05 µg/kg (LOQ) and 0.5 µg/kg (10x LOQ; Tables 1-2, pp. 22-23). Five replicates per fortification level/analyte/soil matrix were performed; however, for one clay soil replicate fortified with florasulam and 5-OH florasulam at 0.05 µg/kg (LOQ), low recoveries were considered outliers, as per Dixon test

(probability 99%), and were not used in the statistics (Table 2, p. 23). The method was validated for both analytes at both fortification levels in the two soil matrices after a second trial (p. 18). Due to complete retention of analytes on the SAX SPE cartridge during the first trial, the SAX SPE step and subsequent phase partition extraction steps of the original ECM were eliminated during the second trial (pp. 18-20). Additionally, LC/MS/MS detection was performed in negative ion mode due to signal suppression observed in the positive ion mode specified in the original ECM (p. 20). The independent laboratory also recommended that final extracts be analyzed by LC/MS/MS without delay due to possible instability of the analytes; supporting data were not provided. The foreign (Germany) soil matrices were characterized with USDA textural classifications (Appendix D, pp. 73-76). The sponsor, Dow AgroSciences, did not establish that the soils used in the ILV were either an equivalent, or more difficult, analytical sample condition as those used for the ECM.

Table 2. Initial Validation Method Recoveries for Florasulam (XDE-570) and Its Transformation Product 5-OH Florasulam (5-hydroxy XDE-570) in Soil[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Florasulam	**Clay loam soil**					
	0.05 (LOQ)	**2**	91, 87	*	*	*
	0.5	**2**	95, 96	*	*	*
	50.0	**2**	94, 97	*	*	*
	Sandy loam soil (35% sand)					
	0.05 (LOQ)	**2**	92, 104	*	*	*
	5.0	**2**	97, 92	*	*	*
	Sandy loam soil (70% sand)					
	0.05 (LOQ)	**2**	83, 86	*	*	*
	0.5	**2**	87, 79	*	*	*
	Loamy sand soil					
	0.05 (LOQ)	**2**	99, 97	*	*	*
	5.0	**2**	94, 89	*	*	*
	50.0	**2**	96, 87	*	*	*
5-OH Florasulam	**Clay loam soil**					
	0.05 (LOQ)	**2**	100, 89	*	*	*
	0.5	**2**	91, 87	*	*	*
	50.0	**2**	92, 94	*	*	*
	Sandy loam soil (35% sand)					
	0.05 (LOQ)	**2**	103, 104	*	*	*
	5.0	**2**	93, 89	*	*	*
	Sandy loam soil (70% sand)					
	0.05 (LOQ)	**2**	72, 73	*	*	*
	0.5	**2**	84, 78	*	*	*
	Loamy sand soil					
	0.05 (LOQ)	**2**	110, 88	*	*	*
	5.0	**2**	94, 94	*	*	*
	50.0	**2**	96, 96	*	*	*

Data (uncorrected recovery results) were obtained from Appendix B, Table 2, p. 57. No residues were detected in matrix control samples (Appendix B, Table 1, p. 56).

* = Not applicable, n = 2.

1 Soils were not characterized, other than percent sand in the sandy loam soils.

Table 3. Independent Validation Method Recoveries for Florasulam and Its Transformation Product 5-OH Florasulam in Soil[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Florasulam	**Sandy loam soil**					
	Quantitation ion					
	0.05 (LOQ)	5	82-91	88	4	5
	0.5	5	73-78	76	2	3
	Confirmation ion					
	0.05 (LOQ)	5	88-96	92	4	4
	0.5	5	69-75	73	2	3
	Clay soil					
	Quantitation ion					
	0.05 (LOQ)	**4**[2]	73-75	74	1	1
	0.5	5	70-73	72	2	2
	Confirmation ion					
	0.05 (LOQ)	**4**[2]	76-80	77	2	2
	0.5	5	70-74	72	2	3
5-OH Florasulam	**Sandy loam soil**					
	Quantitation ion					
	0.05 (LOQ)	5	72-82	76	4	5
	0.5	5	77-85	83	3	4
	Confirmation ion					
	0.05 (LOQ)	5	76-94	81	7	9
	0.5	5	77-84	80	3	4
	Clay soil					
	Quantitation ion					
	0.05 (LOQ)	**4**[2]	81-90	86	4	5
	0.5	5	77-101	89	9	10
	Confirmation ion					
	0.05 (LOQ)	**4**[2]	79-90	85	6	7
	0.5	5	80-103	91	10	11

Data (uncorrected recovery results) were obtained from Tables 1-2, pp. 22-23 and DER Attachment 2 (standard deviations).

1 Soils (Germany) were characterized with USDA textural classifications (Appendix D, pp. 73-76).

2 Five replicate fortifications were performed; however, low recoveries from one replicate (41% and 46% for quantitation and confirmation ions, respectively, for florasulam; 50% for both ions for 5-OH florasulam) were considered outliers as per Dixon test (probability 99%) and were not used in the statistics (Table 2, p. 23).

III. Method Characteristics

In the ECM and ILV, the LOQ for florasulam and 5-OH florasulam in soil was 0.05 µg/kg (p. 12; Appendix B, p. 53). The ECM defined the LOQ as the lowest validated fortification level, with the fortification level selected as "at least 4 times the average control value" (Appendix B, pp. 50, 53). Data supporting selection of the LOQ were not provided. The ECM defined the LOD as 20% of the lowest validated fortification level (0.01 µg/kg). Although not directly specified, the ILV appeared to have also set the LOD at 0.01 µg/kg (pp. 9, 19).

Table 4. Method Characteristics for Florasulam and Its Transformation Product 5-OH Florasulam in Soil

		Florasulam (XDE-570)	**5-OH Florasulam (5-hydroxy XDE-570)**
Limit of Quantitation (LOQ)		0.05 µg/kg	
Limit of Detection (LOD)		0.01 µg/kg	
Linearity (1/x weighting, calibration curve r^2 and concentration range)[1]	ECM:	Q ion: r^2 = 0.998807	Q ion: r^2 = 0.998586
	ILV:	Q ion: r^2 = 0.9988 C ion: r^2 = 1.0000	Q ion: r^2 = 0.9998 C ion: r^2 = 0.9998
	Range:	0.3-30 ng/mL (Figures 1-2, pp. 24-25; Appendix B, p. 48)	
Repeatable	ECM:	Insufficient performance data at all fortification levels (LOQ to 1,000x LOQ)	
	ILV:	Yes at LOQ and 10x LOQ[2]	
Reproducible		Yes; with major modifications to the ECM. Additionally, Dow AgroSciences did not establish that the soils used in the ILV (sandy loam and clay) were either an equivalent, or more difficult, analytical sample condition as those used for the ECM (clay loam, two sandy loams and a loamy sand).	
Specific	ECM:	Undetermined; chromatograms of matrix controls samples only provided for two of the four soil matrices [clay loam (96/295/1969) and loamy sand (96/295/2151)].	
	ILV:	Yes (modified ECM); interferences in matrix blank controls were ≤20% of LOQ (Figures 5-8, pp. 30-33; Figures 11-14, pp. 36-39).	

Data were obtained from pp. 9, 12, 19-20; Tables 1-2, pp. 22-23; Figures 1-2, pp. 24-25; Figures 5-8, pp. 30-33; Figures 11-14, pp. 36-39; Appendix B, pp. 48, 53, 57, 59-60, 62, 65; and DER Attachment 2.

1 Linearity of the ECM calibration curves could not be verified by the reviewer as individual calibration data were not provided (Appendix B, pp. 59-60). Linearity of the ILV calibration curves was verified by the reviewer (DER Attachment 2). ILV r^2 values are reviewer-generated from reported r values of 0.9994 (Q ion) and 1.0000 (C ion) for florasulam and 0.9999 (both ions) for 5-OH florasulam (Figures 1-2, pp. 24-25; DER Attachment 2).

2 Five replicates per fortification level/analyte/soil matrix were performed; however, for one clay soil replicate fortified with florasulam and 5-OH florasulam at 0.05 µg/kg (LOQ), low recoveries were considered outliers, as per Dixon test (probability 99%), and were not used in the statistics (Table 2, p. 23).

IV. Method Deficiencies and Reviewer's Comments

1. The independent laboratory made significant modifications to the original ECM to validate the method (pp. 19-20). Due to complete retention of analytes on the SAX SPE cartridge during the first trial, the SAX SPE step and subsequent phase partition extraction steps of the original ECM were eliminated during the second trial (pp. 18-20). Additionally, LC/MS/MS detection was performed in negative ion mode due to signal suppression observed in the positive ion mode specified in the original ECM (p. 20). Confirmation ion transitions were added to the LC/MS/MS method for both analytes. The independent laboratory also recommended that final extracts be analyzed by LC/MS/MS without delay due to possible instability of the analytes; supporting data were not provided. An internal validation implementing the changes to the original ECM was not provided.

2. Sufficient ECM performance data at the LOQ and 10x LOQ for both analytes in all soil matrices (a clay loam, two sandy loam and a loamy sand soils) were not provided. All fortifications were only performed in duplicate (n = 2); therefore, meaningful statistics for the recovery results per fortification level and soil matrix could not be generated. No performance data at 10x LOQ were reported for one sandy loam (35% sand) soil and the loamy sand soil.

3. Sufficient chromatographic data were not provided to support validation of the ECM. Chromatograms were not provided for reagent blanks, matrix blanks for the two sandy loam soils, the clay loam soil fortified at 10x LOQ, or for the two sandy loam soils and loamy sand soil fortified at the LOQ and 10x LOQ. Only chromatograms of the 5 ng/mL and 30 ng/mL standards for both analytes were provided (calibration standard range 0.3-30 ng/mL; p. 48). Standard curves for both analytes were provided, but the individual calibration standard data were not reported (Appendix B, Figures 1a-1b, pp. 59-60).

4. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. The ECM defined the LOQ (0.05 µg/kg) as the lowest validated fortification level, with the fortification level selected as "at least 4 times the average control value" (Appendix B, pp. 50, 53). Data supporting selection of the LOQ were not provided. The ECM defined the LOD as 20% of the lowest validated fortification level (0.01 µg/kg; Appendix B, p. 53). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, toxicological levels of concern in soil have not been determined. An LOQ above toxicological levels of concern will result in unacceptable method classification.

5. The registrant did not specify that the soils used in the ILV were either an equivalent to, or more difficult than, analytical sample conditions used in the ECM. The foreign (Germany) soil matrices (sandy loam and clay soils) used in the ILV were characterized with USDA textural classifications (Appendix D, pp. 73-76). The four soil matrices (one clay loam, two sandy loam, and one loamy sand soils) used in the ECM were not characterized, other than percent sand in the two sandy loam soils (Appendix B, Tables 1-2, pp. 56-57).

6. For the ILV, chromatograms of reagent blanks samples were not provided.

7. Separate ECM and ILV reports were not submitted. An ILV report (MRID 49077901) was submitted with the ECM report provided as Appendix B (pp. 43-68) of the ILV report.

8. The analytical purities of the florasulam (XDE-570) and 5-OH florasulam (5-hydroxy XDE-570) standards used for fortifications in the ECM were not reported (Appendix B, p. 47).

9. It was reported for the ILV that a single set of thirteen samples required *ca*. one and one-half working (calendar) days to complete; *ca*. 8 hours for sample preparation, followed by unattended overnight LC/MS/MS analysis, followed by *ca*. 2 hours of evaluation and data transcription (p. 20).

V. References

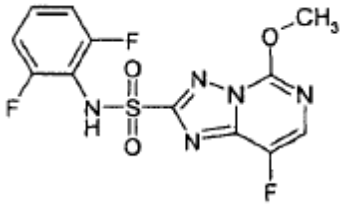
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Florasulam (XDE-570)

IUPAC Name: Not reported
CAS Name: N-(2,6-Difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-c]-pyrimidine-2-sulfonamide
CAS Number: 145701-23-1
SMILES String: Not found



5-Hydroxy florasulam (5-OH florasulam, 5-hydroxy XDE-570)

IUPAC Name: Not reported
CAS Name: N-(2,6-Difluorophenyl)-8-fluoro-5-hydroxy[1,2,4]triazolo[1,5-c]-pyrimidine-2-sulfonamide
CAS Number: N/A
SMILES String: Not found