Independent Laboratory Validation of the Analytical Methodology Used for the Determination of XDE-208 (X11422208) and its Metabolites (X11519540, X11579457, and X11719474) in Sandy Loam and Clay Loam Soils

ABSTRACT

This study was conducted to provide independent laboratory validation data for the determination of residues of XDE-208 (sulfoxaflor) and its major metabolites (X11519540, X11579457, and X11719474) in soil - using Dow AgroSciences residue analytical method, "Method Validation Study for the Determination of Residues of XDE-208 and its Major Metabolites in Soil using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection", as well as to support the stated limit of quantitation (LOQ) established at 0.001 µg/g for XDE-208 and its major metabolites in soil. For the independent laboratory validation, one sandy loam and one clay loam soil was chosen. Untreated control samples were fortified at 0.001 µg/g and at 0.01 µg/g with XDE-208 and its metabolites and analyzed by LC-MS/MS.

All individual recovery values for each fortified control sample were within the EPA acceptance range of 70-120 %. Average recoveries at each fortification level were within the EU acceptance range of 70-110 %. The relative standard deviation (RSD) per fortification level and analyte did not exceed the level of ± 20%, and interferences were negligible, i.e. below the LOD of 0.0003 µg/g.

This independent laboratory validation was conducted to satisfy the requirements of the European Council Directive 91/414/EEC, as amended by European Commission Directive 96/46/EC; the European Commission Guidance Document on Residue Analytical Methods; SANCO/825/00 rev. 7; SANCO/3029/99 rev. 4; and the PMRA Residue Chemistry Guidelines as Regulatory Directive Dir98-02. The study was also conducted to satisfy the requirements of U.S. EPA Guideline OPPTS 850.7100, PR Notice 96-1 and PR Notice 86-5.

INTRODUCTION

Dow AgroSciences (DAS) residue analytical method 091185, "Method Validation Study for the Determination of Residues of XDE-208 and its Major Metabolites in Soil using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection" (1) (Appendix A), was developed and validated at Dow AgroSciences LLC. The method was found to be suitable for the determination of residues of the XDE-208 (sulfoxaflor) and its major metabolites (X11519540, X11579457, and X11719474) in soil over the concentration range of 0.001 to 1.00 µg/g. The validated limit of quantitation of the method was 0.001 µg/g.

An independent laboratory validation following DAS Study Number 091185 was conducted on two soils to satisfy the requirements of the European Council Directive 91/414/EEC (2), as amended by European Commission Directive 96/46/EC, and the European Commission Guidance Document on Residue Analytical Methods, SANCO/3029/99 rev. 4 (3), and SANCO/825/00 rev. 7 (4). The study was also conducted to satisfy the requirements of U.S. EPA Guideline OPPTS 850.7100(c)(2) (5), PR Notice 86-5 (6), PR Notice 96-1 (7), as well as the Canadian Residue Chemistry Guidelines, Regulatory Directive Dir98-02 (8).

The independent laboratory, the Study Director, and the analysts chosen to conduct the ILV were unfamiliar with the method, both in its development and subsequent use in analyzing samples. The independent laboratory used all of its own equipment and supplies, so that there was no common link between Dow AgroSciences and the ILV analysts. Throughout the conduct of the study, any communications between Dow AgroSciences and the Study Director and/or the analyst were logged for inclusion in the report. No one from Dow AgroSciences was allowed to visit the independent laboratory during the ILV trial to observe, offer help, or assist the chemists or technicians. These steps successfully maintained the integrity of the ILV study.

ANALYTICAL

Preparation and Storage of Samples

Untreated sandy loam and clay loam soil samples were obtained from field sites in Fresno, California and Seymour, Illinois, respectively. Upon receipt at EPSL, the soil samples were assigned EPSL identification numbers, 100420-3H (for the clay loam), and 100430-10H (for the sandy loam). Characterization data for both soils is provided in Appendix B. Following arrival at EPSL, the soils were placed in a freezer set to maintain a temperature of 10 – 20 C°C where they were retained at all times unless removed for preparation or analysis. The soils were prepared frozen by homogenization with a mortar and pestle and dry ice (sandy loam) or with an industrial blender and dry ice (clay loam), and then sieved using a 0.125 inch sieve.

Preparation of Solutions and Standards

Reagents used were of equivalent specifications as described in DAS Study Number 091185.
The following analytical test substances/analytical standards were utilized during the independent laboratory method validation:

Description	Analytical Test Substances/Reference Standards				Internal Standards	
	X11422208	X11519540	X11579457	X11719474	X11843864*	X11944782
Name / Abbreviation	Sulfoxaflor, XDE-208	XDE-208 sulfone	XDE-208 sulfoximine	XDE-208 urea	XDE-208 (M+3)	XDE-208 urea (M+4)
Dow TSN #	TSN105878	TSN106498	TSN030941 -0002	TSN030626 -0003	TSN030721-0002	TSN031118 -0001
Dow Lot #	200602464-9	37307-15	SYN-5866-65	E2695-1	XE5-F1245-108	XS9-37950-82
EPSL #	100426-1H	100426-4H	100426-5H	100426-3H	100422-1H	100426-2H
Purity (wt %)	99.7	98.0**	99.0	99.5	100**	97.0
Appearance	solid	solid	solid	solid	clear liquid	solid
Storage Conditions	5ºC - Ambient	5ºC - Ambient	5ºC - Ambient	5ºC - Ambient	Ambient	5ºC - Ambient
Certification Date	June 19, 2008	July 27, 2009	Jan 6, 2010	April 5, 2010	Jan 3, 2010	Jan 23, 2009
Recertification Date	June 19, 2010	Oct. 23, 2011	Jan 7, 2012	Aug 14, 2012	Feb 17, 2012	Feb 3, 2011

* Provided as a stock solution of 100 µg/mL in acetonitrile. **see Appendix E for protocol amendments.

These materials were received from the Sponsor on April 22 and 26, 2010 and further identified with EPSL reference numbers. Documentation of the methods of synthesis and characterization are maintained by the Sponsor.

Stock standard solutions and calibration standard solutions were prepared as described in DAS Study Number 091185. Full details of these materials are included in the raw data package for the study along with the preparation of all analytical and fortification standards prepared from the primary reference items. The test/reference items will be retained until expiry and then disposed of after completion and issuance of the final study report, upon which disposal will be authorized by the Study Monitor.

Fortification of Recovery Samples

Two ILV trials following Study number 091185 were attempted with each trial consisting of the following treatments:

1 reagent blank (containing no matrix or analyte)

2 unfortified control samples for each of the sandy loam and clay loam soils

5 control samples of each soil type were fortified with a mixed solution of the test substances at 0.001µg/g (the LOQ of the method).

5 control samples of each soil type were fortified with a mixed solution of the test substances at 0.01µg/g (10X the LOQ of the method).

Sample Extraction, Purification and Analysis

Untreated and fortified soils were assayed according to the analytical method described in DAS Study Number 091185 with negligible variations due to slightly different laboratory equipment and practices.

In brief, residues of XDE-208 (sulfoxaflor), and its major soil metabolites were extracted from soil by shaking with an acetonitrile/1.0 N hydrochloric acid (90:10) solution. After centrifugation, the solution was decanted and the extraction was repeated. The second extraction solution was combined with the first and brought to a 40-mL volume. A 4.0-mL aliquot of the extraction solution was taken and combined with a glycerin/methanol (10:90) solution. The sample was then concentrated to minimize the organic content of the sample and brought to a 2.0-mL volume using 0.01 N hydrochloric acid. Next, the solution was purified using a reverse-phase polymeric solid-

phase extraction (SPE) column. After elution from the SPE column with a solution of acetonitrile/water (80:20) with 0.1 % formic acid, a stable isotope internal standard mixture was added and the eluate was diluted with a solution of water/acetonitrile (95:5) with 0.01 % formic acid. Samples were analyzed by liquid chromatography with positive-ion electrospray ionization tandem mass spectrometry (LC/MS/MS).

Samples generated during the course of this study will be stored frozen and/or in the refrigerator until acceptance of the final report by the Sponsor representative. These samples will then be discarded unless the Study Director receives prior written instructions regarding shipment of the samples to the Sponsor or continued storage at EPSL at the Sponsor's expense.

Analytical Instrumentation and Equipment

The instrumental conditions used during the ILV trial were as described in DAS Study Number 091185, with minor adaptations as given on the following pages:

Typical HPLC Operating Conditions

Instrumentation:	Perkin-Elmer Series 200 Micro Pumps (2), Perkin Elmer Series 200 auto-sampler
Column:	Agilent Zorbax SB-C8 4.6 x 75 mm, 3.5-µm
Column Temperature:	ambient (approximately 20 °C)
Injection Volume:	50 µL
Injection Wash	
Pre-inject flush	500 µL of acetonitrile/water/formic acid (80:20:0.1)
Post -inject flush(2)	500 µL of acetonitrile/water/formic acid (80:20:0.1)
Run Time:	approximately 14 minutes
Mobile Phase:	A – water containing 0.01% formic acid B – acetonitrile containing 0.01% formic acid
Mobile Phase Split:	approximately 400 µL/min split to source

Gradient:

Time (min)	Flow Rate (mL/min)	Solvent A (percent)	Solvent B (percent)
0:00	1.00	100	0
3:00	1.00	100	0
8:00	1.00	0	100
10:00	1.00	0	100
11:00	1.00	100	0
14:00	1.00	100	0

Flow Diverter

Flow to Waste	0.0 min → 5.0 min
Flow to Source	5.0 min → 8.6 min
Flow to Waste	8.6 min → end of run

Typical Mass Spectrometry Operating Conditions

Instrumentation:	Applied Biosystems API 4000 MS System Applied Biosystems Analyst 1.4.1 data system
Ionization Mode:	Turbospray
Polarity:	positive
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – unit
Curtain Gas (CUR)	10 psi
Collision Gas (CAD):	6.0 psi
Ion Source Gas 1 (GS1)	60 psi
Ion Source Gas 2 (GS2)	50 psi
Temperature (TEM):	450 °C
IonSpray Voltage (IS):	5300 volts
Acquisition Time Delay:	0.00 minutes
Period Duration:	8.50 minutes
Dwell Time:	50 ms

Analytes	Precursor Ion, Q1	Product Ion, Q3	Declustering Potential, v	Entrance Potential, v	Collision Energy, v	Cell Exit Potential, v
XDE-208						
quantitation	278.3	174.1	50	5	13	15
confirmation	278.3	154.1	40	10	37	15
X11719474						
quantitation	296.3	174.1	30	2	15	5
confirmation	296.3	154	30	2	35	30
X11519540						
quantitation	254.3	175.1	40	5	25	30
confirmation	254.3	154.1	40	5	60	10
X11579457						
quantitation	253.3	174.1	30	5	14	10
confirmation	253.3	154.1	60	5	38	15

X11843864 (M+3 ISTD) (XDE-208 stable isotope)						
quantitation	281.3	177.1	60	5	13	20
confirmation	281.3	156.1	60	5	37	20
X11944782 (M+4 ISTD) (X11719474 stable isotope)						
quantitation	300.3	178.1	60	5	16	20
confirmation	300.3	157.1	60	5	30	20

Adaptations to the Analytical Method

Additional adaptations to the analytical method described in DAS Study Number 091185 are listed below:

a) Soils were homogenized, and sieved in a frozen state using dry ice and a mortar and pestle for the sandy loam soil and using dry ice and an Oster blender for the clay loam soil.

b) For preparation of the primary standard solutions, 0.05 g of standard was diluted to a 50 mL volume instead of 0.100 g to a 100 mL volume.

c) One hundred (100) mL mixing cylinders were used instead of 50 mL mixing cylinders in step 7 of the procedure.

d) Thirteen (13) mL graduated glass centrifuge tubes were used instead of 7 mL vials at steps 11 and 16.e of the method.

e) In the second ILV trial, the samples were concentrated to a volume of approximately 100 µL instead of 500 µL in an effort to improve recovery of the sulfoxflor and its metabolites.

Statistical Treatment of Data

The mean recoveries for the fortified samples were calculated using the “AVERAGE” function of the Microsoft Excel spreadsheet computer program, which divides the sum of the selected cells by the number of determinations. The standard deviation of the recoveries for a fortification level of one matrix type was calculated using the “STDEV” function of the same spreadsheet program, which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom, and extracts the square root of the quotient. Percent relative standard deviation (% RSD), was calculated by dividing the standard deviation by the mean, and then multiplying by 100.