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

INTRODUCTION

Scope

This method is applicable for the quantitative determination of residues of XDE-208 (sulfoxaflor), and its major metabolites in soil. The method was validated over the concentration range of 0.001-1.00 µg/g with a validated limit of quantitation of 0.001 µg/g. Common and chemical names, molecular formulas, and the nominal masses for the analyte and related compounds are given in Table 1.

This study was conducted to fulfill data requirements outlined in the U. S. EPA Residue Chemistry Test Guidelines, OPPTS 850.7100 (1), the European Commission Guidance Document on Residue Analytical Methods, SANCO/825/00 rev.7 (2) and SANCO/3029/99 (3), and PMRA Residue Chemistry Guidelines as Regulatory Directive Dir98-02 (4).

Method Principle

Residues of XDE-208 (sulfoxaflor), and its major soil metabolites are extracted from soil by shaking with an acetonitrile/1.0 N hydrochloric acid (90:10) solution. After centrifugation, the solution is poured off and the extraction is performed again. The second extraction solution is then combined with the first and brought to a fixed volume. A 4.0-mL aliquot of the extraction solution is taken and combined with a glycerin/methanol (10:90) solution. The sample is then concentrated to decrease the organic content of the sample and brought to a 2.0-mL volume with 0.01 N hydrochloric acid. Next, the solution is purified using a reverse-phase polymeric solid-phase extraction (SPE) column. After elution from the SPE column with an acetonitrile/water (80:20) solution with 0.1 % formic acid, a stable isotope internal standard mixture is added and the eluate is diluted with a water/acetonitrile (95:5) with 0.01 % formic acid. The sample is analyzed by liquid chromatography with positive-ion electrospray ionization tandem mass spectrometry (LC/MS/MS).

Safety Precautions

Each analyst must be acquainted with the potential hazards of the equipment, reagents, products, solvents, and procedures used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: OPERATION MANUALS, MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information should be obtained from the supplier. Disposal of waste materials, reagents, reactants, and solvents must

be in compliance applicable governmental requirements.

Acetonitrile and methanol are flammable and should be used in well-ventilated areas away from ignition sources. Formic acid and hydrochloric acid are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling these reagents

Test Substance/Analytical Standard and Internal Standard

Test Substance/ Analytical Standard	TSN Number	Percent Purity	Certification Date	Reference
XDE-208 (sulfoxaflor)[a]	105878	99.7	10-Feb-2009	ML-AL-2008-003623REV
X11519540[a]	106498	98	05-Oct-2009	FA&PC 09-228627
X11579457[a]	030941-0001	97	18-Jun-2008	FA&PC 08-184890
X11719474[a]	030626-0003	99.5	14-May-2008	FA&PC 08-181812
X11843864[b]	030721-0002	100	30-Apr-2008	FA&PC 08-167391
X11944782[b]	031118-0001	97	16-Dec-2009	FA&PC 09-203293

[a] Test substance/analytical standard
[b] Internal standard

The above standards may be obtained from the Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1054.

Equipment, Glassware, and Materials

Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory glassware and supplies are assumed to be readily available. Unless specified otherwise, class A volumetric glassware is used to prepare analytical standards, fortification solutions, and calibration standards. Suggested equipment and materials are listed in the following sections.

Laboratory Equipment

Balance, analytical, Model AE100, Mettler-Toledo, Inc.

Balance, pan, Model BB2440, Mettler-Toledo, Inc.

Centrifuge, with rotor to accommodate 45-mL vials, Model Centra-GP8, Thermo International Equipment Company.

Evaporator, TurboVap LV, Caliper Life Sciences.

Hammer mill, with 1/8- through 1/4-inch screens, Model 2001, AGVISE Laboratories, Inc.

Pipet, positive-displacement, 20-50 µL capacity, catalog number M50, Gilson Inc.

Pipet, positive-displacement, 100-1000 µL capacity, catalog number M1000, Gilson Inc.

Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation.

Ultrasonic cleaner, Model 1200, Branson Cleaning Equipment Co.

Vacuum manifold, Model spe-12G, Mallinckrodt Baker, Inc.

Vortex mixer, Model G-560, Scientific Industries, Inc.

Chromatographic System

Column, analytical, Zorbax SB-C8, 4.6 mm x 75 mm, 3.5-µm particle size, catalog number 866953-906, Agilent Technologies.

Liquid chromatograph, Symbiosis Pharma, Spark Holland Inc.

Mass spectrometer, Model API QTRAP 5500, MDS/Sciex.

Mass spectrometer data system, Model Analyst 1.5.1, MDS/Sciex.

Glassware and Materials

Column, Oasis HLB SPE, 30-mg sorbent, 1-mL reservoir, catalog number WAT094225, Waters.

Pipet tip, positive-displacement, 50-µL capacity, catalog number CP50, Gilson Inc.

Pipet tip, positive-displacement, 1000-µL capacity, catalog number CP1000, Gilson Inc.

Vial, autosampler, 2-mL, catalog number C4000-1W, National Scientific Company.

Vial, 45-mL, catalog number 60958A-11, Kimble Glass Co.

Vial, 7-mL, with PTFE-lined screw cap, catalog number 03-340-60B, Fisher Scientific.

Vial cap, PTFE-lined, for 45-mL vial, catalog number 5205, Qorpak.

Vial cap, for autosampler vial, catalog number C4000-54B, National Scientific Company.

Reagents

Acetonitrile, HPLC grade, catalog number 2856-10, Mallinckrodt Baker.

Formic acid, 96%, ACS grade, catalog number 251364-500G, Sigma-Aldrich.

Glycerin, certified ACS grade, catalog number G33-500, Fisher Scientific.

Hydrochloric acid, 0.01 N, certified concentration, catalog number SA62-1, Fisher Scientific.

Hydrochloric acid, 1.0 N, certified concentration, catalog number SA48-1, Fisher Scientific.

Methanol, HPLC grade, catalog number 3041-10, Mallinckrodt Baker.

Nitrogen, refrigerated liquid, catalog number LQNI, BOC Gases.

Water, OmniSolv grade, catalog number WX-0004-1, EMD Chemicals.

Prepared Solutions

acetonitrile containing 0.01% formic acid (mobile phase B)

Pipet 0.20 mL of formic acid into a 2000-mL graduated mixing cylinder containing approximately 1900 mL of acetonitrile. Dilute to volume with acetonitrile. Cap the cylinder and invert it multiple times to mix well prior to use.

acetonitrile/1.0 N hydrochloric acid (90:10) (v/v)

Measure 100 mL of 1.0 N hydrochloric acid into a 1000-mL graduated cylinder. Add approximately 850 mL of acetonitrile and allow to equilibrate to room temperature. Dilute to volume with acetonitrile.

acetonitrile/water (80:20) (v/v) containing 0.1% formic acid

Measure 800 mL of acetonitrile using a 1-L graduated cylinder. Add 1.0 mL of formic acid and approximately 150 mL of water into the cylinder and allow to equilibrate to room temperature. Dilute to volume with water.

glycerin/methanol (10:90) (w/v)

Weigh 1.0 g of glycerin into a 45-mL vial. Measure 9.0 mL of methanol and transfer to the 45-mL vial containing the glycerin. Cap the vial and mix. Allow the solution to equilibrate to room temperature before use.

water containing 0.01% formic acid (mobile phase A)

Pipet 0.20 mL of formic acid into a 2000-mL graduated mixing cylinder containing approximately 1900 mL of water. Dilute to volume with water. Cap the cylinder and invert it multiple times to mix well prior to use.

water/ acetonitrile (95/5) (v/v) containing 0.01% formic acid

Measure 50 mL of acetonitrile containing 0.01% formic acid (prepared above as mobile phase B) into a 1000-mL graduated cylinder. Add 900 mL of water containing 0.01% formic acid (prepared above as mobile phase A) and allow to equilibrate to room temperature. Dilute to volume with water containing 0.01% formic acid.

EXPERIMENTAL

Instrumental Conditions

Typical HPLC Operating Conditions

Instrumentation:	Spark Holland Symbiosis Pharma
Column:	
Primary	Agilent Zorbax SB-C8 4.6 x 75 mm, 3.5-µm
Alternate	Phenomenex Synergi Hydro-RP 80A 4.6 x 75 mm, 4-µm
Column Temperature:	ambient (approximately 22 °C)
Injection Volume:	10 µL
Injection Wash	
Wash Port 1	700 µL of acetonitrile/water/formic acid (80:20:0.1)
Wash Port 2	700 µL of methanol
Wash Port 3	700 µL of water
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 200 µL/min split to source

Gradient:

Time (min)	Flow Rate (mL/min)	Solvent A (percent)	Solvent B (percent)
0:01	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 QTRAP 5500 MS System Applied Biosystems Analyst 1.5.1 data system
Ionization Mode:	electrospray
Polarity:	positive
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – unit
Curtain Gas (CUR)	40 psi
Collision Gas (CAD):	medium
Ion Source Gas 1 (GS1)	40 psi
Ion Source Gas 2 (GS2)	60 psi
Temperature (TEM):	475 °C
Entrance Potential (EP):	10 volts
IonSpray Voltage (IS):	5000 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	Collision Energy, v	Cell Exit Potential, v
X11422208					
quantitation	278.1	174.1	60	13	22
confirmation	278.1	154.1	60	38	18
X11719474					
quantitation	296.1	174.1	51	14	20
confirmation	296.1	105.1	51	21	14

X11519540					
quantitation	254.1	175.1	86	26	22
confirmation	254.1	154.1	86	55	20
X11579457					
quantitation	253.1	174.1	52	12	22
confirmation	253.1	154.1	52	35	20
X11843864 (M+3 ISTD) (XDE-208 stable isotope)					
quantitation	281.1	177.1	63	13	24
X11944782 (M+4 ISTD) (X11719474 stable isotope)					
quantitation	300.1	178.1	61	16	22

Representative spectra, calibration curves, and chromatograms are shown in Figures 1-24. Typical chromatograms for the determination and the confirmation of XDE-208 (sulfoxaflor), and its major soil metabolites in soil are illustrated in Figures 13-24.

Preparation of Standard Solutions

Preparation of XDE-208 (Sulfoxaflor) and Metabolite Stock Solutions

Weigh 0.1000 g of X11422208 (XDE-208) analytical standard and quantitatively transfer to a 100-mL volumetric flask with acetonitrile. Dilute to volume to obtain a 1000-µg/mL stock solution.

Weigh 0.1000 g of X11519540 analytical standard and quantitatively transfer to a 100-mL volumetric flask with acetonitrile. Dilute to volume to obtain a 1000-µg/mL stock solution.

Weigh 0.1000 g of X11579457 analytical standard and quantitatively transfer to a 100-mL volumetric flask with acetonitrile. Dilute to volume to obtain a 1000-µg/mL stock solution.

Weigh 0.1000 g of X11719474 analytical standard and quantitatively transfer to a 100-mL volumetric flask with acetonitrile. Dilute to volume to obtain a 1000-µg/mL stock solution.

Pipet 5.0 mL of the 1000-µg/mL solutions above into a single 200-mL volumetric flask and dilute to volume with acetonitrile to obtain a mixed solution containing 25.0 µg/mL of each compound.

Pipet 20.0 mL of the 25.0-µg/mL solution above into a 200-mL volumetric flask and dilute to volume with acetonitrile to obtain a mixed solution containing 2.50 µg/mL of each compound.

Pipet 20.0 mL of the 2.50-µg/mL solution above into a 200-mL volumetric flask and dilute to volume with acetonitrile to obtain a mixed solution containing 0.250 µg/mL of each compound.

Pipet 20.0 mL of the 0.250-µg/mL solution above into a 200-mL volumetric flask and dilute to volume with acetonitrile to obtain a mixed solution containing 0.025 µg/mL of each compound.

Pipet 20.0 mL of the 0.025-µg/mL solution above into a 200-mL volumetric flask and dilute to volume with acetonitrile to obtain a mixed solution containing 0.0025 µg/mL of each compound.

Prepare solutions for spiking samples by diluting the above stock solutions with acetonitrile as follows:

Concentration of Stock Soln.	Aliquot of Stock Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]
µg/mL	mL	mL	µg/mL	µg/g
0.0025	60.0	100	0.0015	0.0003
0.0025	--	--	0.0025	0.0005
0.025	20.0	100	0.0050	0.0010
0.025	50.0	100	0.0125	0.0025
0.025	--	--	0.025	0.0050
0.250	20.0	100	0.050	0.010
0.250	50.0	100	0.125	0.025
0.250	--	--	0.250	0.050
2.50	20.0	100	0.500	0.100
2.50	50.0	100	1.25	0.250
2.50	--	--	2.50	0.500
25.0	20.0	100	5.00	1.00
25.0	50.0	100	12.50	2.50
25.0	--	--	25.0	5.00

[a] The equivalent sample concentration is based on fortifying a 5.0-g soil sample with 1.0 mL of spiking solution.

Preparation of the Mixed Staple-Isotope Internal Standard Solution

Weigh 0.0050 g of the X11843864 (XDE-208 M+3) stable-isotope internal standard and quantitatively transfer to a 50-mL volumetric flask with acetonitrile. Dilute to volume with acetonitrile to obtain a 100-µg/mL stock solution.

Weigh 0.0050 g of the X11944782 (X11719474 M+4) stable-isotope internal standard and quantitatively transfer to a 50-mL volumetric flask with acetonitrile. Dilute to volume with acetonitrile to obtain a 100-µg/mL stock solution.

Pipet 1.00 mL each of the 100-µg/mL solutions above into a single 100-mL volumetric flask and dilute to volume with acetonitrile to obtain a mixed solution containing 1.00 µg/mL of each compound.

Pipet 10.0 mL of the 1.00 µg/mL mixed stable-isotope solution above into a 100-mL volumetric flask containing approximately 50 mL of a glycerin/methanol solution (10:90, w/v). Swirl the

flask and allow the solution to equilibrate to room temperature. Dilute to volume with additional glycerin/methanol solution. The resulting solution contains 0.100 µg/mL (100 ng/mL) of each stable isotope.

Preparation of Calibration Standards for Samples

Prepare calibration standards by dispensing 250 µL of the 1.00 µg/mL mixed stable-isotope internal standard solution and 1000 µL of the 0.0015-1.25 µg/mL spiking solutions into a series of 20-mL volumetric flasks and dilute to volume with a water/acetonitrile solution (95:5) containing 0.01% formic acid.

The concentrations of the calibration standards are as follows:

Concentration of Spiking Soln.	Aliquot of Spkg. Soln.	Final Soln. Volume	Calib Soln. Final Conc.	Equivalent Sample Conc.
µg/mL	mL	mL	ng/mL	µg/g[a]
0.0015	1.00	20.0	0.075	0.0003
0.0025	1.00	20.0	0.125	0.0005
0.0050	1.00	20.0	0.250	0.0010
0.0125	1.00	20.0	0.625	0.0025
0.0250	1.00	20.0	1.25	0.0050
0.0500	1.00	20.0	2.50	0.010
0.125	1.00	20.0	6.25	0.025
0.250	1.00	20.0	12.5	0.050
0.500	1.00	20.0	25.0	0.100
1.25	1.00	20.0	62.5	0.250

[a] Conversion from equivalent sample concentration to final concentration is outlined in the example calculations (Figures 25-26).

Preparation of XDE-208 (X11422208) Standards to Determine Isotopic Crossover

Using a 1000-µL syringe or positive-displacement pipet, dispense 1000 µL of the 0.250 µg/mL X11422208 mixed fortification solution into a 20-mL volumetric flask and dilute to volume with a water/acetonitrile solution (95:5) containing 0.01% formic acid. The resulting solution contains 12.5 ng/mL of a mixed X11422208 solution.

Preparation of X11843864 Internal Standards to Determine Isotopic Crossover

Using a 250-µL syringe or positive-displacement pipet, dispense 250 µL of the 1.00 µg/mL XDE-208 mixed internal standard solution into a 20-mL volumetric flask and dilute to volume with a water/acetonitrile solution (95:5) containing 0.01% formic acid. The resulting solution contains 12.5 ng/mL of a mixed X11843864 internal standard solution.

Sample Origin, Numbering, Preparation and Storage

Untreated control samples of the soil were obtained from the Dow AgroSciences LLC Control Soil Database. Unique sample numbers were assigned to the samples to track them during receipt, storage, and analysis. Characterization data for the soil samples used in the validation study are given in the table below. Complete source documentation is included in the study file.

Sample Number	Matrix (USDA textural classification)	pH	% Organic Carbon
M763	Silt Loam Soil	6.0	3.5
M764	Sandy Loam Soil	7.2	1.2
M768	Clay Loam Soil	5.5	1.8
M771	Loam Soil	6.1	1.1

The samples were prepared by sieving. To ensure homogeneity and sample integrity for all soil types, it is recommended to freeze samples with liquid nitrogen and then grind or chop using a hammer mill with a 1/8-inch screen size.

During the course of the study, the samples were stored in refrigerated, except when removed for analysis.

Analysis Procedure

1. Weigh 5.0-g portions of the prepared soil sample into a series of 45-mL glass vials.
2. For preparing fortified samples, dispense 1000-µL aliquots of the appropriate spiking solutions into the sample vial to encompass the necessary concentration range.
3. Add 25 mL of the acetonitrile/1.0 N hydrochloric acid (90:10) extraction solution to the sample vial.
4. Cap the vial with a PTFE-lined cap, and sonicate the sample for approximately 10 minutes.
5. Shake the sample vial for a minimum of 30 minutes on a reciprocating shaker at approximately 180 excursions/minute.
6. If necessary, centrifuge the sample vial for 5 minutes at 2000 rpm.
7. Transfer the extraction solution into a clean 50-mL graduated mixing cylinder.
8. Repeat Steps 3-6 with 14 mL of the acetonitrile/1.0 N hydrochloric acid (90:10) extraction solution.
9. Combine the extraction solution from Step 8 with the 25 mL from Step 7.

10. Adjust the volume in the graduated mixing cylinder to 40.0 mL with additional acetonitrile/1.0 N hydrochloric acid (90:10) extraction solution. Stopper the cylinder and mix thoroughly.

11. Pipet 4.0 mL of the sample solution from Step 10 into a clean 7-mL vial.

12. Using a positive-displacement pipet, dispense 50 µL of a glycerin/methanol solution (10:90, w/v) into the sample vial. Cap the vial with a PTFE-lined cap, and vortex mix the sample for 1-2 seconds.

13. Concentrate the sample extract using a TurboVap evaporator set at 40 °C and a nitrogen pressure of approximately 10 psi. (Note: There will be approximately 500 µL of liquid remaining in the vial.)

14. Adjust the volume in the sample vial to 2.00 mL with 0.01 N hydrochloric acid by comparing the height of the solution in the sample vial to identical vials containing 2.00 mL of 0.01 N hydrochloric acid. Cap the vial with a PTFE-lined cap, vortex mix the sample for 1-2 seconds, and then sonicate the sample for 1-2 seconds.

15. Centrifuge the sample vial for 5 minutes at 2000 rpm.

16. Purify the sample using the following SPE procedure:
    a. Place an OASIS® HLB SPE column (1-mL, 30 mg) on the vacuum manifold.
    b. Condition the SPE column with 1 mL of acetonitrile followed by 1 mL of 0.01 N hydrochloric acid. Dry the SPE column under full vacuum (≈-10 inches Hg) for 5 seconds between solvents.
    c. Transfer 1.0 mL of the sample from Step 15 to the SPE column (if dilutions are needed, a smaller aliquot may be used as appropriate). Draw the sample through the column at a flow rate of approximately 1 mL/min, discarding the eluate.
    d. Rinse the SPE column with 1 mL of 0.01 N hydrochloric acid. Draw the solvent through the column at a flow rate of approximately 1 mL/min, discarding the eluate. Dry the column under full vacuum (≈-10 inches Hg) for approximately 60 seconds.
    e. Elute the XDE-208 and metabolites from the SPE column with two 250-µL aliquots of an acetonitrile/water solution (80:20) containing 0.10% formic acid, collecting the eluate in a 7-mL vial.

17. Using a positive-displacement pipet, dispense 125 µL of the 0.100 µg/mL mixed internal standard solution into the sample vial.

18. Adjust the volume in the sample vial to 1.00 mL with a water/acetonitrile solution (95:5) containing 0.01% formic acid by comparing the height of the solution in the sample vial to identical vials containing 1.00 mL of the water/acetonitrile solution (95:5) containing 0.01% formic acid.

19. Transfer the sample to a 2-mL autosampler vial or a 96-well plate.

20. Analyze the samples and calibration standards by LC/MS/MS with positive-ion electrospray tandem mass spectrometry.

Calculations

For XDE-208 and X11719474

Inject the series of calibration standards described in the calibration standard preparation section using the conditions listed in the instrument section and determine the peak areas for the analyte and internal standard.

For each sample and standard calculate the quantitation ratio (analyte peak area/internal standard peak area).

Prepare a standard curve using linear regression analysis with 1/x weighting by plotting the equivalent analyte concentration on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis) as shown in Figures 7-8. Power regression or quadratic curve fit may also be used if appropriate.

Determine the concentration (µg/g) and/or recovery (%) from the sample as described in the example calculation outlined in Figure 25.

For X11519540 and X11579457

Inject the series of calibration standards described in the calibration standard preparation section using the conditions listed in the instrument section and determine the peak areas for the analyte.

Prepare a standard curve using linear regression analysis with 1/x weighting by plotting the equivalent analyte concentration on the abscissa (x-axis) and the respective peak area on the ordinate (y-axis) as shown in Figures 9-10. Power regression or quadratic curve fit may also be used if appropriate.

Determine the concentration (µg/g) and/or recovery (%) from the sample as described in the example calculation outlined in Figure 26.

Confirmation of Residue Identity

The method is specific for the determination of XDE-208 (sulfoxaflor), and its major soil metabolites X11519540, X11579457, and X11719474 by virtue of the chromatographic separation and selective detection system used. To demonstrate confirmation, a total of two MS/MS ion transitions are monitored for each analyte. The following example is given for the analyte XDE-208.

Inject the series of calibration standards described in the calibration standard preparation section using the conditions listed in the instrument section and determine the peak areas for the analyte as indicated below.

XDE-208 (X11422208)	Q1/Q3 *m/z* 278/174 (quantitation)
	Q1/Q3 *m/z* 278/154 (confirmation)

For each standard, calculate the respective confirmation ratios.

$$\text{Confirmation Ratio} = \frac{\text{peak area of confirmation ion transition}}{\text{peak area of quantitation ion transition}}$$

$$\text{Confirmation Ratio} = \frac{\text{XDE - 208 peak area at } m/z \text{ 278/154}}{\text{XDE - 208 peak area at } m/z \text{ 278/174}}$$

For example, using the data for XDE-208 (X11422208) from the 0.125-ng/mL standard, from set 091185 S01, found in Figure 12:

$$\text{Confirmation Ratio} = \frac{4080}{5538} = 0.7367$$

Confirmation of the presence of the analyte is indicated when the retention time of the samples matches that of the standards and the confirmation ratio is in the range of ±20% of the average found for the standards. The confirmation MS/MS ion transition area ratios for each recovery sample at or above the LOQ were all within the range of ±20% of the average found for the standards within the sets with only two exceptions, both LOQ recovery samples. Example chromatograms in Figures 12-24 include both quantitation and confirmation transitions.

Determination of Isotopic Crossover

In this assay, analytes and internal standards are quantitated using MS/MS transitions characteristic of each compound. When using stable-isotope labeled internal standards, there is a

possibility that isotopic contributions will occur between the transitions used for quantitation of the unlabeled and labeled compounds. This isotopic overlap between the analyte and the internal standard is determined empirically by analyzing standard solutions of each compound and should be addressed for accurate determination of concentrations (5, 6).

To determine the isotopic crossover for each analyte and respective stable isotope internal standard, inject the crossover standards described in the Preparation of Standard Solutions section, and determine the peak areas for the analyte and internal standard.

For example, to determine the contribution of the unlabeled XDE-208 to X11843864 (XDE-208-[M+3] stable isotope) using the XDE-208 standard crossover data from Figure 11 for the quantitation ion:

$$\text{Crossover Factor (analyte}\rightarrow\text{ISTD)} = \frac{\text{peak area of internal standard transition}}{\text{peak area of analyte transition}}$$

$$\text{Crossover Factor (analyte}\rightarrow\text{ISTD)} = \frac{\text{peak area at } m/z \text{ 281/177}}{\text{peak area at } m/z \text{ 278/174}}$$

$$\text{Crossover Factor (analyte}\rightarrow\text{ISTD)} = \frac{0}{583981} = 0$$

In a similar manner, to determine the contribution of X11843864 to the unlabeled XDE-208 using the internal standard crossover data from Figure 11 for the quantitation ion:

$$\text{Crossover Factor (ISTD}\rightarrow\text{ analyte)} = \frac{\text{peak area of analyte transition}}{\text{peak area of internal standard transition}}$$

$$\text{Crossover Factor (ISTD}\rightarrow\text{ analyte)} = \frac{\text{peak area at } m/z \text{ 278/174}}{\text{peak area at } m/z \text{ 281/177}}$$

$$\text{Crossover Factor (ISTD}\rightarrow\text{ analyte)} = \frac{0}{557335} = 0$$

During the validation study, no significant mass spectral isotopic crossover was observed for quantitation or confirmation transitions for the two analytes with stable isotope internal standards and therefore no correction was performed. If isotopic crossover is encountered it should be assessed and the respective quantitation ratios corrected for accurate determination of concentrations.

Statistical Treatment of Data

Statistical treatment of data included the calculation of regression equations, coefficients of determination (r^2) for describing the linearity of calibration curves, and means, standard deviations, and relative standard deviations of the results for the fortified recovery samples.

Standardization of SPE Elution Profile

There is a possibility that variation in the Oasis HLB SPE columns may influence the elution profile of the analytes. If it is necessary to obtain an elution profile for each lot of SPE columns used to ensure optimum recovery and clean-up efficiency, the following procedure can be used:

1. To a 7-mL vial containing 2.0 mL of 0.01 N hydrochloric acid, add 80 µL of the 1.25-µg/mL mixed spiking solution and vortex mix.

2. Place an OASIS® HLB SPE column (1-mL, 30 mg) on the vacuum manifold.

3. Condition the SPE column with 1 mL of acetonitrile followed by 1 mL of 0.01 N

hydrochloric acid. Dry the SPE column under full vacuum (≈-10 inches Hg) for 5 seconds between solvents.

4. Transfer 1.0 mL of the sample from Step 1 to the SPE column. Draw the sample through the column at a flow rate of approximately 1 mL/min, discarding the eluate.

5. Rinse the column with 1 mL of 0.01 N hydrochloric acid. Draw the solvent through the column at a flow rate of approximately 1 mL/min, discarding the eluate. Dry the column under full vacuum (≈-10 inches Hg) for approximately 60 seconds.

6. Elute the XDE-208 and metabolites from the SPE column with four 250-µL aliquots of an acetonitrile/water solution (80:20) containing 0.10% formic acid, collecting each fraction in a separate 7-mL vial.

7. For each fraction collected, dispense 125 µL of the 0.100 µg/mL mixed internal standard solution into the sample vial.

8. Adjust the volume in the sample vial to 1.00 mL with a water/acetonitrile solution (95:5) containing 0.01% formic acid by comparing the height of the solution in the sample vial to identical vials containing 1.00 mL of the water/acetonitrile solution (95:5) containing 0.01% formic acid.

9. Transfer the sample to a 2-mL autosampler vial or a 96-well plate.

10. Analyze the samples and calibration standards by LC/MS/MS with positive-ion electrospray tandem mass spectrometry.

11. Calculate the percent recovery as described in the Calculations section.

A typical elution profile is illustrated in Figure 27. If the elution profile differs from that shown, adjust the volume of the acetonitrile/water solution (80:20) containing 0.10% formic acid solution to be collected in Step 16 of the Analysis Procedure section.

Supplemental Notes

1. The instrumental conditions may be modified to obtain optimal chromatographic separation and sensitivity.

2. Aliquot volumes may differ if different stock solution concentrations are available. Final volume of prepared solutions can vary accordingly with use demand and/or reagent availability.

3. Each instrument should be tested for carryover by injecting a reagent blank after the highest calibration standard solution. After evaluation of the result, the analyst may decide to avoid injecting high calibration standard solutions or treated samples before untreated samples.

4. The type of regression model can be chosen to give the best fit (coefficient of determination) for the data.

5. In Step 7 of the Analysis Procedure section, a 45-mL glass vial may be substituted for the 50-mL graduated mixing cylinder. If this option is chosen, then in Step 10, adjust the volume in the 45-mL sample vial to 40.0 mL with additional acetonitrile/1.0 N hydrochloric acid (90:10) extraction solution by comparing the height of the solution in the sample vial to identical vials containing 40.0 mL of acetonitrile/1.0 N hydrochloric acid (90:10) extraction solution.

6. If the sample extracts contain analyte concentrations that exceed the linear range of the standard calibration curve (approximately 62.5 ng/mL, equivalent to 0.250 µg/g in the original sample), dilute those samples with an appropriate amount of water/acetonitrile solution (95:5) containing 0.01% formic acid to obtain responses at least 20% less than the highest calibration standard.

CONCLUSIONS

This method is applicable for the quantitative determination of residues of XDE-208 (sulfoxaflor), and its major soil metabolites X11519540, X11579457, and X11719474 in soil. The method was validated over the concentration ranges of 0.001-1.00 µg/g with a validated limit of quantitation of 0.001 µg/g.

ARCHIVING

The protocol, raw data, and the original version of the final report are all filed in the Dow AgroSciences LLC archives at 9330 Zionsville Road in Indianapolis, Indiana 46268-1054.

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Table 1. Identity and Structures of XDE-208 and Related Compounds

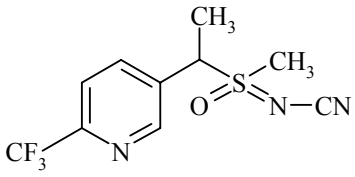
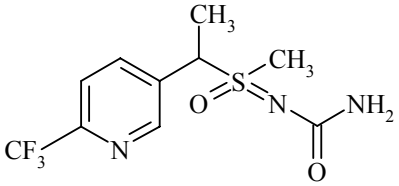
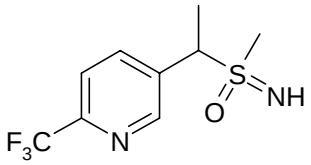
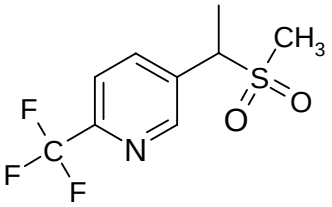
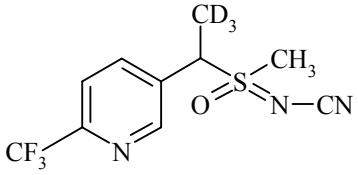
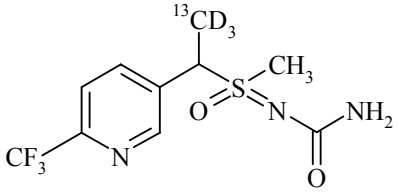
Common Name of Compound	Structural Formula and Chemical Name
XDE-208 (sulfoxaflor) Molecular Formula: $C_{10}H_{10}F_3N_3OS$ Formula Weight: 277.27 Nominal Mass: 277 CAS Number 946578-00-3	 [1-(6-trifluoromethylpyridin-3-yl)ethyl](methyl)-oxido-λ^4-sulfanylidenecyanamide
X11719474 Molecular Formula: $C_{10}H_{12}F_3N_3O_2S$ Formula Weight: 295.29 Nominal Mass: 295 CAS Number not available	 N-(methyl)oxido){1-[6-(trifluoromethyl)pyridine-3-yl]ethyl}-λ^4-sulfanylidene) urea
X11579457 Molecular Formula: $C_9H_{11}F_3N_2OS$ Formula Weight: 252.26 Nominal Mass: 252 CAS Number not available	 [5-[1-(S-methylsulfonimidoyl)ethyl]-2-(trifluoromethyl)pyridine
X11519540 Molecular Formula: $C_9H_{10}F_3NO_2S$ Formula Weight: 253.24 Nominal Mass: 253 CAS Number not available	 5-(1-methylsulfonyl)ethyl)-2-(trifluoromethyl)pyridine

Table 1 (Cont.) Identity and Structures of XDE-208 and Related Compounds

Common Name of Compound	Structural Formula and Chemical Name
X11843864 (XDE-208 M+3) stable-isotope Molecular Formula: $C_{10}D_3H_7F_3N_3OS$ Formula Weight: 280.29 Nominal Mass: 280 CAS Number not available	 [1-(6-trifluoromethylpyridin-3-yl)-2,2,2-^{2}H3-ethyl](methyl)-oxido-λ^4-sulfanylidenecyanamide
X11944782 (X11719474 M+4) stable-isotope Molecular Formula: $^{13}CC_9D_3H_9F_3N_3O_2S$ Formula Weight: 299.30 Nominal Mass: 299 CAS Number not available	 N-(methyl)oxido){2-^{13}C-2,2,2-^{2}H3-1-[6-(trifluoromethyl)pyridine-3-yl]ethyl}-λ^4-sulfanylidene) urea