GRM: 01.31
EFFECTIVE: July 13, 2002
SUPERSEDES: NA

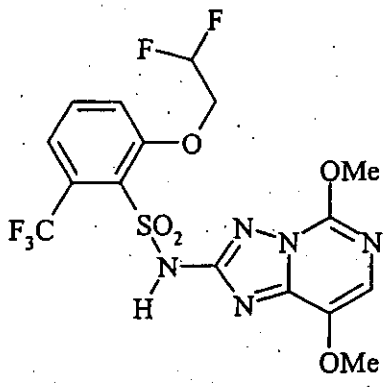
Dow AgroSciences

Determination of Residues of XDE-638 and Metabolites in Soil and Sediment by Liquid Chromatography with Tandem Mass Spectrometry

M. J. Hastings and G. E. Schelle

1. SCOPE

This method is applicable for the quantitative determination of XDE-638 and its metabolites in soil and sediment. The method measures XDE-638, the 5-OH, BSTCA, BSA, sulfonamide, and the 2-amino-TP metabolites over the concentration range 3.0-100 ng/g with a validated limit of quantitation of 3.0 ng/g. Chemical names and structures for these compounds are given in Table 1.



XDE-638
CAS No: 219714-96-2

2. PRINCIPLE

Residues of XDE-638 and its metabolites are extracted from soil or sediment using an acetonitrile:1.0 N hydrochloric acid solution (90:10 v/v). An aliquot of the extract is concentrated to remove the acetonitrile and then diluted with 0.1 N hydrochloric acid. The extract is purified using an HLB solid-phase extraction plate (SPE). The SPE plate is washed with a water:methanol solution (95:5 v/v) before XDE-638 and its metabolites are eluted from the SPE plate with an acetonitrile:methanol solution (80:20 v/v). The eluate is evaporated to dryness and reconstituted with a water:acetonitrile:methanol:acetic acid solution (90:5:5:0.1) containing a stable isotope of XDE-638 as internal standard (ISTD). The final solution is analyzed by liquid chromatography with tandem mass spectrometry (LC/MS/MS).

3. SAFETY PRECAUTIONS

3.1. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non Dow AgroSciences LLC products should be obtained from the container label or from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

3.2 Acetonitrile and methanol are flammable and volatile and should be used in well-ventilated areas away from ignition sources.

3.3 Acetic 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 chemicals.

4. EQUIPMENT (Note 12.1)

4.1. Laboratory Equipment

4.1.1. Balance, analytical, Model AE100, Mettler Instrument Corporation, Hightstown, NJ 08520.

4.1.2. Balance, analytical, Model P-1200, Mettler Instrument Corporation.

4.1.3. Centrifuge, with rotor to accommodate 40-mL vials, Model Centra-GP8, International Equipment Company, Needham Heights, MA 02194.

4.1.4. Evaporator, TurboVap LV, Zymark Corporation, Hopkinton, MA 01748.

4.1.5. Hammer mill, with 3/16-inch screen, Model 2001, AGVISE Laboratories, Inc., Northwood, ND 58267.

4.1.6. Oven, Model OV-490A-2, Blue M Electric Company, Blue Island, IL 60406.

4.1.7. Pipetter, adjustable, Eppendorf, 10-100 µL, catalog number 05-402-48, Fisher Scientific, Pittsburgh, PA 15275.

4.1.8. Pipetter, adjustable, Eppendorf, 50-1000 µL, catalog number 21-378-83, Fisher Scientific.

4.1.9. Shaker, Model 6010, Eberbach Corporation, Ann Arbor, MI 48106.

4.1.10. Ultrasonic cleaner, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.

4.1.11. Vacuum manifold, 96-Well, catalog number 121-9601, International Sorbent Technology Ltd, Hengoed, Mid Glamorgan UK and distributed by Jones Chromatography USA, Inc., Lakewood, CO 80228.

4.1.12. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.

4.2. Chromatographic Equipment (Note 12.1.)

4.2.1. Column, analytical, ZORBAX SB-C8, 4.6 x 75 mm, 3.5-µm, catalog number 866953-906, Agilent Technologies, Wilmington, DE 19808.

4.2.2. Liquid chromatograph autosampler, Model 1100, Agilent Technologies.

4.2.3. Liquid chromatograph binary pump, Model 1100, Agilent Technologies.

4.2.4. Liquid chromatograph degasser, Model 1100, Agilent Technologies.

4.2.5. Mass spectrometer, Model API 3000, Applied Biosystems, Foster City, CA 94404.

4.2.6. Mass spectrometer data system, Analyst 1.1, Applied Biosystems.

5. GLASSWARE AND MATERIALS (Note 12.1)

5.1. Adsorbent, drierite, indicating, catalog number 07-578-4A, Fisher Scientific.

5.2. Bottle, 1.0-L PyrexPlus media bottle, catalog number 06-423-3D, Fisher Scientific.

5.3. Bottle, 2.0-L PyrexPlus media bottle, catalog number 06-423-3E, Fisher Scientific.

5.4. Cylinder, graduated mixing, 50-mL catalog number 20039-0050, Kimble/Kontes, Vineland, NJ 08360.

5.5. Cylinder, graduated mixing, 100-mL catalog number 20039-0100, Kimble/Kontes, Vineland, NJ 08360.

5.6. Cylinder, graduated, 500-mL, catalog number C7000-500, National Scientific Company, Lawrenceville, GA 30243.

5.7. Cylinder, graduated, 1000-mL, catalog number C7000-1L, National Scientific Company.

5.8. Cylinder, graduated, 2000-mL, catalog number C7000-2L, National Scientific Company.

5.9. Deep-well collection plate, catalog number 121-5203, International Sorbent Technology Ltd.

5.10. Desiccator, Glass, 250 mm I.D, catalog number 08-595-E, Fisher Scientific.

5.11. Dish, 42-mL aluminum weighing, catalog number 08-732, Fisher Scientific.

5.12. Flask, volumetric, 10-mL, catalog number 161-8986, National Scientific Company, Duluth, GA 30097.

5.13. Flask, volumetric, 100-mL, catalog number 161-8987, National Scientific Company, Duluth, GA 30097.

5.14. Pipet, 5-mL disposable seriological, catalog number, 13-666-7D, Fisher Scientific.

5.15. Pipet, 3.2-mL disposable transfer, catalog number, 13-711-7, Fisher Scientific.

5.16. Pipet, volumetric, 0.5-mL, catalog number 261-6010, National Scientific Company.

5.17. Pipet, volumetric, 1.0-mL, catalog number 261-6011, National Scientific Company.

5.18. Pipet, volumetric, 2.0-mL, catalog number 261-6012, National Scientific Company.

5.19. Pipet, volumetric, 3.0-mL, catalog number 261-6013, National Scientific Company.

5.20. Pipet, volumetric, 5.0-mL, catalog number 261-6015, National Scientific Company.

5.21. Pipet, volumetric, 10.0-mL, catalog number 261-6020, National Scientific Company.

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

5.23. Vial, scintillation, 20-mL, catalog number 03-337-7, Fisher Scientific.

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

5.25. Vial, 40-mL, with PTFE-lined screw cap, catalog number B7800-6, National Scientific Company.

5.26. Waters HLB SPE plate, 30-mg packing, catalog number WAT 058951, Waters, Milford, MA 01757.

6. REAGENTS, STANDARDS, AND PREPARED SOLUTIONS (Note 12.1.)

6.1. Reagents

6.1.1. Acetic acid, catalog number A38S-500, Fisher Scientific.

6.1.2. Acetonitrile, HPLC grade, catalog number 2856, Mallinkrodt Baker, Inc., Paris, KY 40361.

6.1.3. Hydrochloric acid, 0.1 N, catalog number SA54-1, Fisher Scientific.

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

6.1.5. Methanol, HPLC grade, catalog number 3041, Mallinkrodt Baker Inc.

6.1.6. Water, HPLC grade, catalog number WX0004-1, Fisher Scientific.

6.2. Standards

6.2.1. 2-amino-TP metabolite of XDE-638: 5,8-dimethoxy[1,2,4]triazolo[1,5-*c*]pyrimidin-2-amine.

6.2.2. BSA triethylammonium salt of XDE-638: 2-(2,2-difluoroethoxy)-6-(trifluoromethyl) benzenesulfonic acid.

6.2.3. BSTCA triethylammonium salt of XDE-638: triethylammonium 5-[[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl) phenyl]sulfonyl]amino]-1*H*-1,2,4-triazole-3-carboxylic acid.

6.2.4. 5-OH metabolite of XDE-638: 2-(2,2-difluoroethoxy)-*N*-(5,6-dihydro-8-methoxy-5-oxo[1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)-6-(trifluoromethyl)-benzenesulfonamide.

6.2.5. Sulfonamide metabolite of XDE-638: 2-(2,2-difluoroethoxy)-6-(trifluoromethyl)-benzenesulfonamide.

6.2.6. XDE-638: 2-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy[1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)-6-(trifluoromethyl)-benzenesulfonamide.

Compounds can be obtained from Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1054.

6.2.7. Stable isotope of XDE-638: 2-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy [1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl-2-^{13}C-3,4-$^{15}N_2$)-6-(trifluoromethyl)-benzenesulfonamide.

Compound can be obtained from Specialty Synthesis Group, Dow AgroSciences LLC, 9330 Zionsville Road, Building 306, Indianapolis, IN 46268-1054.

6.3. Prepared Solutions

6.3.1. acetonitrile/1.0 N hydrochloric acid (90:10)

Measure 200 mL of 1.0 N hydrochloric acid into a 2.0-L bottle using a 500-mL graduated cylinder. Measure 1800 mL of acetonitrile using 2.0-L graduated cylinder and add to the bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature.

6.3.2. acetonitrile:methanol:acetic acid (50:50:0.1)

Add 1.0 mL of acetic acid into a clean 1.0-L bottle. Measure 500 mL of acetonitrile using a graduated cylinder and add to the bottle. Measure 500 mL of methanol using a graduated cylinder and add to the bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.3. acetonitrile:methanol (80:20)

Add 80 mL of acetonitrile and 20 mL of methanol to a 100-mL bottle using a measuring cylinder. Cap the bottle and mix the solution. Allow the solution to equilibrate to room temperature before use.

6.3.4. water:acetic acid (100:0.1)

Add 1.0 mL of acetic acid into a clean 1.0-L bottle. Measure 1.0 L of HPLC grade water using a graduated cylinder and add to the bottle. Cap the bottle and mix.

6.3.5. water:methanol solution (95:5 v/v)

Pipette 5.0 mL of methanol into a 100-mL volumetric flask and dilute to volume with water. Mix the solution and allow to equilibrate to room temperature before use.

6.3.6. water:acetonitrile:methanol:acetic acid (90:5:5:0.1)

Add 50 mL of methanol and 50.0 mL of acetonitrile into a clean 1.0-L bottle using a graduated cylinder. Pipette 1.0 mL of acetic acid into the bottle. Add 900 mL of water to the bottle using a graduated cylinder. Mix the solution and allow to equilibrate to room temperature before use.

7. PREPARATION OF STANDARD SOLUTIONS (Note 12.2.)

7.1. Preparation of Internal Standard Solution

7.1.1. Weigh 0.010 g of the stable-isotope labeled XDE-638 into a 20-mL vial and dissolve the compound in 10.0 mL of acetonitrile to obtain a 1000-µg/mL stock solution.

7.1.2. Pipette 1.0 mL from the 1000-µg/mL solution prepared in Section 7.1.1. into a 10-mL volumetric flask and dilute to volume with acetonitrile to obtain an XDE-638 stable-isotope solution at 100.0 µg/mL.

7.1.3. Pipet 1.0 mL of the 100-µg/mL XDE-638 stable-isotope solution prepared in Section 7.1.2 into a 10-mL volumetric flask and dilute to volume with acetonitrile to obtain an XDE-638 stable-isotope solution at 10.0 µg/mL.

7.1.4. Pipet 1.0 mL of the 10-µg/mL XDE-638 stable-isotope solution prepared in Section 7.1.3 into a 10-mL volumetric flask and dilute to volume with acetonitrile to obtain an XDE-638 stable-isotope solution at 1.0 µg/mL.

7.1.5. Pipet 5.0 mL of the 1.0 µg/mL XDE-638 stable-isotope solution prepared in Section 7.1.4 into a 1.0-L volumetric flask. Add 50 mL of acetonitrile, 50 mL of methanol and 1.0 mL of acetic acid into the flask. Add approximately 850 mL of water into the flask. Swirl the flask and allow the solution to equilibrate to room temperature before diluting to volume with water to obtain a working XDE-638 stable-isotope solution at 0.005 µg/mL .

7.2. Preparation of XDE-638 and Metabolites Spiking Solutions

7.2.1. Weigh 0.0100 g of 2-amino-TP of XDE-638 into a 20-mL scintillation vial and dissolve the compound in 10.0 mL of acetonitrile to obtain a 1000-µg/mL stock solution.

7.2.2. Weigh 0.0133 g of BSA triethylammonium salt of XDE-638 (equivalent to 0.01 g of the BSA metabolite) into a 20-mL scintillation vial and dissolve the compound in 10 mL of acetonitrile to obtain a 1000-µg/mL stock solution.

7.2.3. Weigh 0.0139 g of BSTCA triethylammonium salt of XDE-638 (equivalent to 0.01 g of the BSTCA metabolite) into a 20-mL scintillation vial and dissolve the compound in 10.0 mL of acetonitrile to obtain a 1000-µg/mL stock solution.

7.2.4. Weigh 0.0100 g of 5-OH of XDE-638 into a 20-mL scintillation vial and dissolve the compound in 10.0 mL of acetonitrile to obtain a 1000-µg/mL stock solution.

7.2.5. Weigh 0.0100 g of sulfonamide of XDE-638 into a 20-mL scintillation vial and dissolve the compound in 10.0 mL of acetonitrile to obtain a 1000-µg/mL stock solution.

7.2.6. Weigh 0.0100 g of XDE-638 into a 20-mL scintillation vial and dissolve the compound in 10.0 mL of acetonitrile to obtain a 1000-µg/mL stock solution.

7.2.7. Pipette 1.0 mL from each of the 1000-µg/mL solution prepared in Sections 7.2.1-7.2.6 into a 100-mL volumetric flask and dilute to volume with water:acetonitrile:methanol:acetic acid (90:5:5:0.1) to obtain a solution containing XDE-638, 5-OH, BSTCA, 2-amino-TP, BSA, and sulfonamide at 10.0 µg/mL.

7.2.8. Pipette 10.0 mL of the 10.0-µg/mL mixed standard prepared in section 7.2.7 into a 100-mL volumetric flask and dilute to volume water:acetonitrile:methanol:acetic acid (90:5:5:0.1) to obtain a solution containing XDE-638, 5-OH, BSTCA, 2-amino-TP, BSA, and sulfonamide at 1.0 µg/mL.

7.2.9. Pipette 10.0 mL of the 1.0-µg/mL mixed standard prepared in section 7.2.8 into a 100-mL volumetric flask and dilute to volume with water:acetonitrile:methanol:acetic acid (90:5:5:0.1) to obtain a solution containing XDE-638, 5-OH, BSTCA, 2-amino-TP, BSA, and sulfonamide at 0.1 µg/mL.

7.3. Preparation of XDE-638 and Metabolites Calibration Solutions

Prepare calibration standard solutions in water:acetonitrile:methanol:acetic acid (90:5:5:0.1) from the 10.0-µg/mL mixed standard (Section 7.1.7) over the range 0.00025–0.05 µg/mL as described below.

Original Standard Concentration µg/mL	Aliquot of Original Standard mL	Final Soln. Volume mL	Calib Soln. Final Conc. µg/mL	Equivalent Sample Conc. ng/g
10.00	10.0	100	1.00	N/A
1.00	10.0	100	0.10	N/A
1.00	5.0	100	0.05[a]	266.7
1.00	3.5	100	0.035[a]	186.7
1.00	2.0	100	0.02[a]	106.7
0.10	10.0	100	0.01[a]	53.3
0.10	5.0	100	0.005[a]	26.7
0.10	1.0	100	0.001[a]	5.3
0.10	0.5	100	0.0005[a]	2.7
0.10	0.25	100	0.00025[a]	1.4

[a] Add 0.5 mL of a 1.0-µg/mL XDE-638 stable isotope internal standard solution (prepared in Section 7.1.4.) to each volumetric flask prior to making to volume with mobile phase to give calibration standards containing 0.005 µg/mL of XDE-638 stable-isotope internal standard.

8. LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

8.1. Typical Liquid Chromatography Operating Conditions (Note 12.3.)

Instrumentation:	Agilent Model 1100 autosampler Agilent Model 1100 binary pump Agilent Model 1100 degasser PE SCIEX API 3000 LC/MS/MS System PE SCIEX Analyst 1.1 data system
Column:	Zorbax SB C8 4.6 x 75 mm, 3.5-µm
Column Temperature:	35 °C
Injection Volume:	40 µL
Run Time:	16 minutes
Mobile Phase:	A –methanol:acetonitrile with 0.1% acetic acid B –water with 0.1% acetic acid
Flow Rate:	900 µL/min, 150 µL/min split to ESI source

Gradient:

Time, min	A, %	B, %
0.0	10	90
0.5	10	90
13.0	90	10
13.1	10	90
16.0	10	90

8.2. Typical Mass Spectrometry Operating Conditions

API 3000:

Interface:	TurboIonSpray
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – low
Curtain Gas (CUR):	14
Collision Gas (CAD):	4.0
Temperature (TEM):	425 °C
Ion Source Gas 1 (GS1):	10
Ion Source Gas 2 (GS2):	7000
Period 1	
Time:	6.25 minutes

Polarity:	Positive
IonSpray Voltage (IS)	5500

Compound	Ion m/z Q1	Ion m/z Q3	Time, ms	CE, v
2-amino-TP	196.1	181.2	150	29

Period 2	
Time:	3.50 minutes
Polarity:	Negative
IonSpray Voltage (IS)	-4300

Compound	Ion m/z Q1	Ion m/z Q3	Time, ms	CE, v
5-OH	468.0	385.8	150	-26
BSTCA	415.0	329.0	150	-22
BSA	305.0	176.0	150	-40
Sulfonamide	304.0	78.0	150	-48

Period 3	
Time:	3.25 minutes
Polarity:	Positive
IonSpray Voltage (IS)	5500

Compound	Ion m/z Q1	Ion m/z Q3	Time, ms	CE, v
XDE-638	484.0	195.0	150	37
Stable Isotope of XDE-638	487.0	198.0	150	37

8.3. Typical Mass Spectra

Typical mass spectra and product ion spectra of XDE-638, metabolites of XDE-638, and internal standard are presented in Figures 1-7.

8.4. Typical Calibration Curve

Typical calibration curves for the determination of XDE-638 and metabolites of XDE-638 in soil are shown in Figures 8-13.

8.5. Typical Chromatograms

Typical chromatograms for a calibration standard, control soil extract, and control soil extracts fortified at limit of quantitation 3.0 ng/g (LOQ) and the highest fortified level are presented in Figures 14-17.

9. DETERMINATION OF RECOVERY OF XDE-638 AND METABOLITES IN SOIL AND SEDIMENT

9.1 Method Validation

Unless otherwise specified, a sample set should contain the following samples:

At least one reagent blank.
At least one control.
At least one control fortified at the limit of detection.
At least two controls fortified at the limit of quantitation.
At least one control fortified at a higher concentration.

9.2 Sample Preparation

Prepare soil samples for analysis by freezing the soil with dry ice and then grinding or chopping with a hammer mill equipped with a 3/16-inch screen size. For sediment samples, thoroughly stir the sediment prior to use.

9.3. Sample Analysis for XDE-638 and Metabolites

9.3.1. Weigh 5 ± 0.1g portions of the control soil or sediment into a series of 40-mL vials. For preparing fortified samples, add appropriate aliquots of the appropriate spiking solutions to obtain concentrations ranging from 1.0 to 100 ng/g.

9.3.2. Add 25 mL of the acetonitrile/1.0 N hydrochloric acid (90:10) extraction solution to each sample vial. Cap the vial with a PTFE-lined cap.

9.3.3. Shake the sample vial for a minimum of 60 minutes on a reciprocating shaker at approximately 180 excursions/minute.

9.3.4. Centrifuge the sample vial for approximately 5 minutes at 2000 rpm.

9.3.5. Decant the extraction solution into a clean 50-mL graduated cylinder.

9.3.6. Add an additional 15 mL of the acetonitrile/1.0 N hydrochloric acid (90:10) extraction solution to each sample vial.

9.3.7. Cap the vial with a PTFE-lined cap and vortex the sample to break loose the soil layer formed during centrifugation.

9.3.8. Shake the sample vial for a minimum of 30 minutes on a reciprocating shaker at approximately 180 excursions/minute. Centrifuge the sample vial for approximately 5 minutes at 2000 rpm.

9.3.9. Decant the soil extract to the cylinder from Step 9.3.5 and adjust final volume to 40.0 mL with additional extraction solution and mix thoroughly.

9.3.10. Transfer a 4.0-mL aliquot of the extraction solution from Step 9.3.9 into a clean 40-mL vial.

9.3.11. Evaporate the extracts to near dryness (approximately 50-100 µL should remain in the vial) using a TurboVap evaporator set at 40 °C and a nitrogen pressure of 20 psi.

Do not evaporate to dryness. Remove each vial from the evaporator as soon as evaporation is complete.

9.3.12. Add 4.0 mL of 0.1 N hydrochloric acid to each vial, vortex mix and sonicate for approximately 30 seconds.

9.3.12.1. Purify the samples using the following SPE procedure (Note 12.4.):

a. Condition a Waters HLB 96-well plate (30-mg) with 0.2 mL of methanol followed by 1.0 mL of 0.1 N hydrochloric acid (dry the plate under full vacuum for 10 seconds between solvents).

b. Transfer 1.50 mL of the sample solution from Step 9.3.10 to the 96-well plate. Draw the sample through the plate at approximately 1 mL/min, discarding the eluate. Dry the plate under full vacuum for 10 seconds after sample has eluted.

c. Wash the plate with 1.0 mL of water:methanol solution (95:5 v/v), discarding the eluate. Dry the plate under full vacuum for 10 seconds after the sample has all passed through the plate.

d. Elute the XDE-638 and metabolites from the plate at approximately 1 mL/min with two 1.0-mL aliquots of acetonitrile:methanol (80:20), collecting the eluate in a 2-mL deep well rack. (Allow the elution solvent to soak on the plate for approximately 30 seconds before elution.)

9.3.13. Using a transfer pipet, transfer the extracts to a clean 40-mL vial and evaporate the extracts to dryness using a TurboVap evaporator set at 40 °C and a nitrogen pressure of 10 psi.

Immediately remove the sample from the evaporator when completely dry.

9.3.14. Reconstitute the samples in 1.0 mL of 0.005-µg/mL XDE-638 stable-isotope internal standard in methanol:acetonitrile:water:acetic acid (5:5:90:0.1). Sonicate and vortex mix the sample vials for approximately 30 seconds.

9.3.15. Transfer the samples and standards to a 2-mL autosampler vial and seal with a cap.

9.3.16. Analyze the sample along with the calibration standards using the LC/MS/MS conditions listed in Section 8. For samples which contain XDE-638 and metabolite concentrations >0.05 µg/mL dilute with the 0.005-µg/mL XDE-638 stable-isotope internal standard in methanol:acetonitrile:water:acetic acid (5:5:90:0.1) to give a concentration <0.05 µg/mL. Determine the suitability of the chromatographic system using the following criteria:

a. Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration.

b. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 14-17 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 5:1 has been attained for the 0.00025-µg/mL calibration standard (equivalent to approximately 1.5 ng/g of analyte in soil or sediment).

10. CALCULATIONS

10.1. Calculation of Percent Recovery for XDE-638

10.1.1. Inject a series of calibration standards (Section 7.3.) as described in Section 8 and determine the peak areas for the analyte and the internal standard as indicated below.

XDE-638	*m/z* Q1/Q3 484/195 (quantitation)
XDE-638 stable isotope	*m/z* Q1/Q3 487/198 (internal standard)

10.1.2. For each standard, calculate the quantitation ratio. For example, using the data for XDE-638 from Figure 14 (f):

$$\text{Quantitation ratio} = \frac{\text{peak area of quantitation ion}}{\text{peak area of internal standard ion}}$$

$$\text{Quantitation ratio} = \frac{\text{peak area for } m/z \text{ Q1/Q3 484/195}}{\text{peak area for } m/z \text{ Q1/Q3 487/198}}$$

$$\text{Quantitation ratio} = \frac{9.89e3}{8.41e4}$$

$$\text{Quantitation ratio} = 0.118$$

10.1.3. Prepare a standard curve by plotting the ratio of the concentrations of the analyte/internal standard on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis) as shown in Figure 8. Using linear regression analysis (13.1.) forced through zero (Note 12.5.), determine the equation for the curve with respect to the abscissa.

For example, using linear regression with the XDE-638 data from Figure 8:

$$X = \left(\frac{Y - \text{intercept}}{\{\text{slope/int std conc}\}}\right)$$

$$\text{XDE - 638 conc. } (\mu g/mL) = \left(\frac{\text{XDE - 638 quantitation ratio} - \text{intercept}}{\{1.19/0.005\}}\right)$$

$$\text{XDE - 638 conc. } (\mu g/mL) = \left(\frac{\text{XDE - 638 quantitation ratio} - 0)}{238}\right)$$

10.2. Calculation of Percent Recovery for XDE-638

10.2.1. Determine the gross concentration in each recovery sample by substituting the quantitation ratio obtained into the above equation and solving for the concentration.

For example, using the data for XDE-638 from Figure 16f:

$$\text{XDE - 638 conc. (gross } \mu g/mL) = \left(\frac{\text{XDE - 638 quantitation ratio} - 0}{238}\right)$$

$$\text{XDE - 638 conc. (gross } \mu g/mL) = \left(\frac{0.11486 - 0)}{238}\right)$$

$$\text{XDE - 638 conc. (gross } \mu g/mL) = 0.000483 \ \mu g/mL$$

Convert the concentration of µg/mL to ng/g as follows:

$$\text{XDE - 638 conc. (gross ng/g)} = 0.000483 \ \mu g/mL \times \frac{(40 \ mL/1.5 \ mL) \times 1000}{5 \ g}$$

$$\text{XDE - 638 conc. (gross)} = 2.58 \ ng/g$$

10.2.2. Determine the net concentration in each recovery sample by subtracting the apparent XDE-638 concentration in the control sample from that of the gross XDE-638 concentration in the recovery sample.

For example, using the data from Figure 15f and 16f:

$$\text{XDE-638 conc. (net ng/g)} = \text{XDE-638 conc. (gross ng/g)} - \text{XDE-638 conc. (control ng/g)}$$

$$\text{XDE-638 conc. (net ng/g)} = 2.58 \text{ ng/g} - 0.0 \text{ ng/g}$$

$$\text{XDE-638 conc. (net)} = 2.58 \text{ ng/g}$$

10.2.3. Determine the percent recovery by dividing the net concentration of each recovery sample by the concentration added.

$$\text{Recovery} = \frac{\text{Conc. Found}}{\text{Conc. Added}} \times 100\%$$

$$\text{Recovery} = \frac{2.58}{3.00} \times 100\%$$

$$\text{Recovery} = 86\%$$

10.3. Determination of XDE-638 Residues in Soil or Sediment

10.3.1. Determine the gross concentration in each treated sample by substituting the quantitation ratio obtained into the equation for the standard calibration curve, and calculating the uncorrected residue result as described in Sections 10.2.1 and 10.2.2.

10.3.2. For those analytes that require correction for method recovery, use the average recovery of all the recovery samples from a given sample set to correct for method efficiency.

For example, using the XDE-638 data from Figure 16f and the average recovery from Table 2 for the sample analyzed on 04-Dec-2001:

$$\text{XDE-638 conc. (corrected ng/g)} = \text{XDE-638 conc. (gross ng/g)} \times \left(\frac{100}{\text{Average \% recovery}}\right)$$

$$\text{XDE-638 conc. (corrected ng/g)} = 2.58 \text{ ng/g} \times \frac{100}{83}$$

XDE-638 conc. (corrected ng/g) = 3.11 ng/g

10.4. Calculation of Percent Recovery for 5-OH Metabolite, Sulfonamide, BSTCA, BSA, and 2-Amino-TP

10.4.1. Inject a series of calibration standards as described in Section 8 and determine the peak areas for the analytes.

5-OH of XDE-638	*m/z* Q1/Q3 468/386
sulfonamide of XDE-638	*m/z* Q1/Q3 304/78
BSTCA of XDE-638	*m/z* Q1/Q3 415/329
BSA of XDE-638	*m/z* Q1/Q3 305/176
2-Amino-TP of XDE-638	*m/z* Q1/Q3 196/181

10.4.2. Prepare a standard curve by plotting the concentration of the analyte on the abscissa (x-axis) and the respective peak area on the ordinate (y-axis) as shown in Figure 9-13. Using linear regression analysis forced through zero, determine the equation for the curve with respect to the abscissa.

For example, using linear regression (13.1.) with the data from Figure 9:

$$X = \left(\frac{Y - \text{intercept}}{\text{slope}}\right)$$

$$\text{5-OH conc. } (\mu g/mL) = \left(\frac{\text{5-OH peak area} - \text{intercept}}{1.51e7}\right)$$

$$\text{5-OH conc. } (\mu g/mL) = \left(\frac{\text{5-OH peak area} - 0)}{1.51e7}\right)$$

10.5. Calculation of Percent Recovery for 5-OH Metabolite

10.5.1. Determine the gross concentration in each recovery sample by substituting the area obtained into the above equation and solving for the concentration.

For example, using the data for 5-OH metabolite from Figure 16e:

$$\text{5-OH conc. (gross } \mu g/mL) = \left(\frac{\text{5-OH peak area} - 0)}{1.51e7}\right)$$

$$\text{5-OH conc. (gross µg/mL)} = \left(\frac{7.75e3 - 0)}{1.51e7}\right)$$

$$\text{5-OH conc. (gross µg/mL)} = 0.000513 \text{ µg/mL}$$

Convert the concentration of µg/mL to ng/g as follows:

$$\text{5-OH conc. (gross ng/g)} = 0.000513 \text{ µg/mL} \times \frac{(40 \text{ mL}/1.5 \text{ mL}) \times 1000}{5 \text{ g}}$$

$$\text{5-OH conc. (gross)} = 2.74 \text{ ng/g}$$

10.5.2. Determine the net concentration in each recovery sample by subtracting the apparent 5-OH concentration in the control sample from that of the gross 5-OH metabolite concentration in the recovery sample.

For example, using the data from Figures 15e and 16e:

$$\text{5-OH conc. (net ng/g)} = \text{5-OH conc. (gross ng/g)} - \text{5-OH conc. (control ng/g)}$$

$$\text{5-OH conc. (net ng/g)} = 2.74 \text{ ng/g} - 0.0 \text{ ng/g}$$

$$\text{5-OH conc. (net ng/g)} = 2.74 \text{ ng/g}$$

10.5.3. Determine the percent recovery by dividing the net concentration of each recovery sample by the theoretical concentration added.

$$\text{Recovery} = \frac{\text{Conc. Found}}{\text{Conc. Added}} \times 100\%$$

$$\text{Recovery} = \frac{2.74 \text{ ng/g}}{3.0 \text{ ng/g}} \times 100\%$$

$$\text{Recovery} = 91\%$$

10.6. Determination of 5-OH Metabolite in Soil or Sediment

10.6.1. Determine the gross concentration in each treated sample by substituting the area obtained into the equation for the standard calibration curve, and calculating the uncorrected residue result as described in Sections 10.5.1 and 10.5.2.

For those analytes that require correction for method recovery, use the average recovery of all the recovery samples from a given sample set to correct for method efficiency.

For example, using 5-OH data from Figure 16e and the average recovery from Table 3 for the sample analyzed on 04-Dec-2001:

$$\begin{pmatrix}\text{5 - OH conc.}\\ \text{(corrected ng/g)}\end{pmatrix} = \begin{pmatrix}\text{5 - OH conc.}\\ \text{(gross ng/g)}\end{pmatrix} \times \left(\frac{100}{\text{Average \% recovery}}\right)$$

$$\begin{pmatrix}\text{5 - OH conc.}\\ \text{(corrected ng/g)}\end{pmatrix} = 2.74\ \text{ng/g} \times \frac{100}{95}$$

$$\begin{pmatrix}\text{5 - OH conc.}\\ \text{(corrected ng/g)}\end{pmatrix} = 2.88\ \text{ng/g}$$

10.7. Determination of Soil Moisture

10.7.1. Accurately weigh a 10-g portion of soil into a tarred aluminum weighing dish.

10.7.2. Place the sample in an oven at 110 °C and allow to dry for a minimum of 16 hours.

10.7.3. Remove the sample from the oven and place in a dessicator containing Drierite adsorbent. Re-weigh the sample when it has cooled to room temperature.

10.7.4. Calculate the percent moisture (dry weight basis) as follows:

$$\begin{pmatrix}\text{Percent Moisture}\\ \text{(dry weight basis)}\end{pmatrix} = \frac{\text{water, g}}{\text{dry soil, g}} \times 100$$

$$\begin{pmatrix}\text{Percent Moisture}\\ \text{(dry weight basis)}\end{pmatrix} = \frac{\begin{pmatrix}\text{sample weight}\\ \text{before drying, g}\end{pmatrix} - \begin{pmatrix}\text{sample weight}\\ \text{after drying, g}\end{pmatrix}}{\text{sample weight after drying, g}} \times 100$$

10.8. Determination of Dry Weight Concentrations of XDE-638 and Metabolites in Sediment and Soil

10.8.1. Determine the analyte concentrations in the sample as described in Sections 10.3. and 10.6.

10.8.2. Determine the soil moisture as described in Section 10.7.

10.8.3. Determine the dry weight analyte concentrations in the samples as follows:

$$\underset{\text{(dry weight ng/g)}}{\text{XDE-638 Conc.}} = \underset{\text{(ng/g)}}{\text{XDE-638 Conc.}} \times \left(1 + \frac{\%\ \text{Moisture}}{100}\right)$$

11.4. Standardization of HLB SPE Elution Profile

11.4.1. Add 100 µL of a 1.0-µg/mL mixed standard (Section 7.2.8) into 10.0 mL of 0.1 N hydrochloric acid.

a. Condition a Waters HLB 96-well plate (30-mg) with 0.2 mL of methanol followed by 1.0 mL 0.1 N hydrochloric acid (dry the plate at full vacuum for 10 seconds between solvents.)

b. Transfer 1.50 mL of the sample solution from Step 11.4.1 to the 96-well plate. Draw the sample through the plate at approximately 1 mL/min collecting the eluate in a deep well collection rack. Dry the plate at full vacuum for 10 seconds after sample has eluted.

c. Wash the plate with 1 mL of water:methanol (95: 5 v/v) solution, collecting the eluate in a deep well collection rack. Dry the plate at full vacuum for 10 seconds after the sample has all passed through the plate.

d. Elute the XDE-638 and metabolites from the plate at approximately 1 mL/min with three 1.0-mL aliquots of acetonitrile:methanol (80:20), collecting the eluate in separate 2-mL deep-well racks.

11.4.2. Using a transfer pipet, transfer the three 1.0-mL aliquots of acetonitrile:methanol (80:20) to a clean 40-mL vials and evaporate to dryness using a TurboVap evaporator set at 40°C and a nitrogen pressure of 10 psi.

11.4.3. Reconstitute the samples from Step 11.4.2. in 1.0 mL of 0.005-µg/mL XDE-638 stable-isotope internal standard in methanol:acetonitrile:water:acetic acid (5:5:90:0.1). Sonicate and vortex mix the sample vials for approximately 30 seconds.

11.4.4. Transfer the samples from Sections 11.4.1 b, 11.4.1.c and 11.4.3 to a 2-mL autosampler vial, and seal the vial with a cap. Analyze the samples along with the calibration standards. Calculate the percent recovery as described in Section 10.

Typical elution profiles are illustrated in Figure 18. If the elution profiles differ from those shown, adjust the volume of acetonitrile:methanol (80:20 v/v) to be collected in Step 9.3.13.d.

12. NOTES

12.1. 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 supplies are assumed to be readily available and are, therefore, not listed.

12.2. Section 7 provides suggested concentrations for calibration standard preparation. Other dilution schemes may be followed.

12.3. The data presented in this method was generated using a Sciex 3000 API 3000 in optimal condition. Operating conditions may be modified to obtain optimal separation or sensitivity. However, method performance will be compromised by increasing injection volume to compensate for low instrument sensitivity.

12.4. Before using each lot of HLB SPE Plates, determine the elution profile as described in section 11.4.

12.5. Linear Regression analysis using 1/x weighting or quadratic curve fit may also be used.

13. REFERENCES

13.1. Freund, J. E.; Williams, F. J. *Dictionary/Outline of Basic Statistics*; Dover: New York, 1991; p 170.

13.2. Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* **1983**, *55*, 2210-2218.

Table 1. Chemical Names and Structures for XDE-638, Metabolites, and Internal Standards

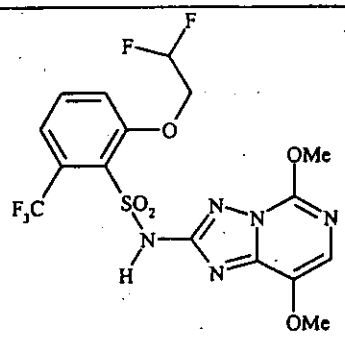
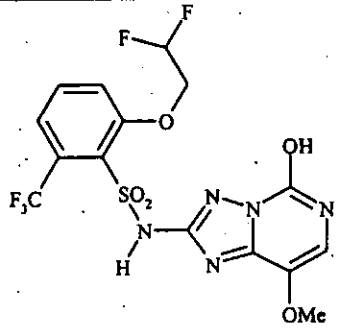
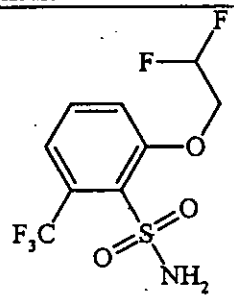
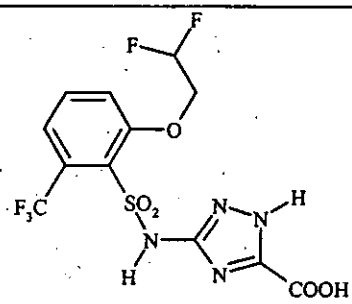
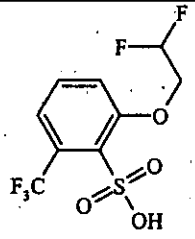
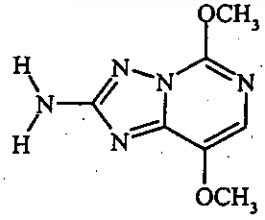
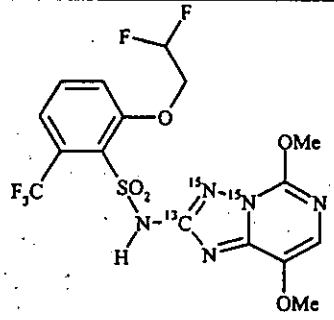
Common Name of Compound	Structure and CAS Name
XDE-638 Molecular Formula: $C_{16}H_{14}F_5N_5O_5S$ Molecular Weight: 483 CAS Number: 219714-96-2	 2-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy [1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)-6-(trifluoromethyl)-benzenesulfonamide
5-OH Metabolite of XDE-638 Molecular Formula: $C_{15}H_{12}F_5N_5O_5S$ Molecular Weight: 469 CAS Number: NA	 2-(2,2-difluoroethoxy)-*N*-(5,6-dihydro-8-methoxy-5-oxo[1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)-6-(trifluoromethyl)-benzenesulfonamide
Sulfonamide Metabolite of XDE-638 Molecular Formula: $C_9H_8F_5NO_3S$ Molecular Weight: 305 CAS Number: NA	 2-(2,2-difluoroethoxy)-6-(trifluoromethyl)-benzenesulfonamide

Table 1. (Cont.) Chemical Names and Structures for XDE-638, Metabolites, and Internal Standard

Common Name of Compound	Structure and CAS Name
BSTCA Metabolite of XDE-638 Molecular Formula: $C_{12}H_9F_5N_4O_5S$ Molecular Weight: 416 CAS Number: NA	 5-[[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl) phenyl]sulfonyl]amino]-1*H*-1,2,4-triazole-3-carboxylic acid
BSA Metabolite of XDE-638 Molecular Formula: $C_9H_7F_5O_4S$ Molecular Weight: 306 CAS Number: NA	 2-(2,2-Difluoroethoxy)-6-(trifluoromethyl) benzenesulfonic acid
2-Amino-TP Metabolite of XDE-638 Molecular Formula: $C_7H_9N_5O_2$ Molecular Weight: 195 CAS Number: NA	 5,8-Dimethoxy[1,2,4]triazolo[1,5-*c*]pyrimidin-2-amine
M+3 Stable Isotope of XDE-638 Molecular Formula: $^{13}CC_{15}H_{14}F_5{}^{15}N_2N_3O_5S$ Molecular Weight: 486 CAS Number: NA	 2-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy [1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl-2-^{13}C-3,4-^{15}N2)-6-(trifluoromethyl)-benzenesulfonamide