ANALYTICAL

Sample Numbering, Preparation, and Storage

One soil sample and one sediment sample were collected at the Mogi Mirim Field Station and tracked into the Mogi Mirim Regulatory Laboratory sample management system using unique sample numbers. About 300 g of each sample was shipped to Laborsolo at Londrina, PR, Brazil, for physical-chemical characterization (Appendix B), and the remaining amount was frozen (at

approximately -20°C) in a temperature-monitored freezer room. Samples were stored in this condition except when removed for preparation and analysis.

Sample preparation consisted of grinding them in a Stephan mill with dry ice to keep the samples frozen.

Preparation of Solutions and Standards

The following analytical reference standards/internal standards were utilized during the independent laboratory method validation:

Test Items/ Analytical Standards	AGR/TSN No.	Percent Purity	Certification Date	Reference
XDE-175-J	TSN104472	97.6	22-Mar-2004	FA&PC 043028
XDE-175-L	TSN104480	96.1	24-Mar-2004	FA&PC 043036
XDE-175-*N*-demethyl J	TSN105114	98	11-Apr-2005	FA&PC 053239
XDE-175-*N*-demethyl L	TSN105124	99	14- Apr -2005	FA&PC 053249
XDE-175-J stable isotope	TSN104657	96	17-Jun-2004	FA&PC 043195
XDE-175-J stable isotope	TSN104658	96	17-Jun-2004	FA&PC 043196
XDE-175-*N*-demethyl J stable isotope	TSN104663	96	17-Jun-2004	FA&PC 043203
XDE-175-*N*-demethyl L stable isotope	TSN104664	93	17-Jun-2004	FA&PC 043204

Standard solutions were prepared as described in Section 7 of method GRM 05.02.

Fortification of Recovery Samples

One ILV trial of the method was performed. The sample set consisted of the following:

1 reagent blank

2 (two) unfortified control samples (1 sediment sample and 1 soil sample)

10 (ten) control samples fortified at 0.005 µg/g - the LOQ of the method (5 sediment and 5 soil samples)

10 (ten) control samples fortified at 0.05 µg/g - ten times (10x) the LOQ of the method (5 sediment and 5 soil sample

Sample Extraction, Purification and Analysis

Residues of XDE-175 and its metabolites are extracted from soil samples by shaking with a methanol/0.1 N sodium hydroxide (90:10) solution. An aliquot of the extraction solvent is diluted with a water/acetic acid (99.5:0.5) solution. A mixed XDE-175 and metabolites stable isotope internal standard solution is added to each sample and the final solution is purified by on-line solid phase extraction using an SCX cartridge. The SPE cartridge is washed with methanol followed by a methanol:acetonitrile:water (4:4:2) solution containing 0.1 M ammonium acetate. The SPE cartridge is eluted with a methanol:acetonitrile:water (4:4:2) solution containing 0.1 M ammonium acetate onto the analytical column where XDE-175 and its metabolites are analyzed by liquid chromatography with positive ion atmospheric pressure chemical ionization (APCI) tandem mass spectrometry (LC/MS/MS).

The ILV trial was conducted as described in Section 9 of method GRM 05.02 with no exceptions.

Analytical Instrumentation

The following instruments were utilized and are considered equivalent to those described in Section 8 of method GRM 05.02. LC/MS/MS conditions were optimized to obtain the best response on this equipment:

Instrumentation

On-line SPE/Liquid Chromatograph, Symbiosis Pharma, Spark Holland Inc., equipment ID LAB 120, SN 40028/40031 (pumps), 40014 (stacker), 40014 (injector), 40028/40031 (HPD), 40018 (ACE)

Mass Spectrometer, Model API 4000, MDS/Sciex, LAB 117, SN V04890405

Mass Spectrometer Data System Analyst 1.4, MDS/Sciex, SN 166673-042904

N_2 generator, Peak Scientific, LAB 119, SN J4-04-38

Computer, Dell Precision 360, LAB 118, SN 4DCPP41

Printer, Hewlett Packard Laserjet 4000, LAB 056

Operating Conditions

Liquid Chromatography

Quantitation column: Waters YMC ODS-AM, 50 x 4.6 mm, 5-µm,
PN AM12S05-0546WT, SN 040510643

Confirmation column: Phenomenex Synergi Polar, 75 x 4.6 mm, 4-µm,
PN 00C-4336-E0, SN 274203-9

Column Temperature: Ambient

Stacker Temperature: 4°C

Injection Volume: 10-µL – partial loopfill – flexibility mode

Autosampler Wash Program:

700-µL acetonitrile/methanol (1:1) + 0.1% formic acid

700-µL methanol

700-µL acetonitrile/methanol (1:1) + 0.1% formic acid

Autosampler Needle Height: 5 mm

Autosampler Puncturing-Injection Needle: 5 mm

Autosampler Flush Volume: 45-µL

Mobile Phase:

A – acetonitrile/methanol (50:50) + 10 mM ammonium acetate.

B – water + 10 mM ammonium acetate.

Equilibration Time: 00:00:01 seconds

Max. Pressure.: 350 bar

Run Time: 06:15 minutes

Gradient:

Time, (min:secs)	Flow (mL/min)	A %	B %
00:01	1.0	70	30
00:05	0.8	70	30
01:00	0.8	70	30
01:05	1.0	70	30
03:05	1.0	100	0
05:00	1.0	100	0
05:15	1.0	70	30
06:15	1.0	70	30

Flow Diverter Program:

0 minute – position A (to waste)

3.0 minute – position B (to detector)

5.0 minute – position A (to waste)

Mass Spectrometry

Interface: APCI

Polarity: Positive

Scan Type: MRM

Resolution: Q1 – unit, Q3 – unit

Detector Parameters:

CAD	GS1	GS2	EP	TEMP	NC	CUR	Dwell (msec)	CXP
5	20	-	10	425°C	3	10	150	24

Compound:

Compound	Q1/Q3 m/z	DP	CE
XDE-175-J	748.5/142.2	86	43
XDE-175-L	760.5/142.2	81	41
XDE-175-*N*-demethyl J	734.5/128.2	81	59
XDE-175-*N*-demethyl L	746.5/128.2	56	69
XDE-175-J (ISTD)	757.5/146.2	71	47
XDE-175-L (ISTD)	769.6/146.3	71	43
XDE-175-*N*-demethyl J (ISTD)	739.6/128.2	71	67
XDE-175-*N*-demethyl L (ISTD)	751.4/128.3	66	59

On-Line Solid Phase Extraction

SPE Cartridge: Spark Holland BondElut SCX, 40-90 µm

ACE:

SSM A – acetonitrile

SSM B – water

SSM C – methanol

SSM D – acetonitrile/methanol/water (4:4:2) + 0.1 M ammonium acetate

HPD 2:

Syr port 1: acetonitrile/methanol/water (4:4:2) + 0.1 M ammonium acetate

Syr port 2: acetonitrile/methanol/water (4:4:2) + 0.1 M ammonium acetate

Syr port 4: water

ACE Program:

Step 1 – move cartridge (from ctdge left to other clamp)

Step 2 – elution (01:02 minutes at duration time), focusing (HPD elution), (acetonitrile/methanol/water (4:4:2) + 0.1 M ammonium acetate - 200-µL - 200-µL/min)

Step 3 – new cartridge (from plate to left clamp)

Step 4 – solvation (acetonitrile – 1000-µL – 5000-µL/min)

Step 5 – equilibration (water – 1000-µL – 5000-µL/min)

Step 6 – start autosampler

Step 7 – sample extraction (water – 2000-µL – 2000-µL/min)

Step 8 – wash cartridge (methanol – 2000-µL – 2500-µL/min)

Step 9 – wash cartridge (acetonitrile/methanol/water (4:4:2) + 0.1 M ammonium acetate – 200-µL – 200-µL/min)

Step 10 – clamp flush (acetonitrile/methanol/water (4:4:2) + 0.1 M ammonium acetate – 1000-µL – 5000-µL/min)

Step 11 – clamp flush (water – 2000-µL – 5000-µL/min)

Step 12 – move cartridge (from right clamp to tray)

Analytical Equipment and Materials

Equipment and materials were utilized in the conduct of the ILV as described in method GRM 05.02 with the following exceptions, which are considered to be equivalent substitutions:

(1) A bench-top Centra CL-3-R centrifuge (LAB 092, SN 37570117) was substituted for Centra GP-8 centrifuge.

(2) A 5-50-mL Brand dispenser was substituted for a dispenser bottle-top Brinkmann 5-25-mL.

(3) A Tecnal TE 240 Pendular model (LAB 073, SN 01099) was substituted for an Eberbach variable speed reciprocating shaker.

(4) A vortex mixer model Genie 2 (LAB 004, SN 2-271719) was substituted for a G-560 model.

Reagents

Reagents utilized in the conduct of this study are in accordance with those stated in method GRM 05.02 with the following exceptions, which are considered to be equivalent substitutions:

(1) J.T. Baker acetic acid, glacial, lot number V32C63, val. Aug/2011, was substituted for Fisher brand.

(2) J.T. Baker HPLC grade acetonitrile, lot number B02C52, val. Mar/2015 was substituted for Mallinckrodt ChromAR HPLC grade.

(3) J.T. Baker ammonium acetate, lot number V32472, val. Aug/2001, was substituted for Fisher HPLC grade brand.

(4) Methanol Ultimar grade from Mallinckrodt, lot number A21E31, val. May/2014, was substituted for ChromAR HPLC grade from the same brand.

(5) No liquid nitrogen was used because the MS/MS system is equipped with a N_2 generator.

(6) Fisher sodium hydroxide 0.1 N solution was substituted by Mallinckrodt solid sodium hydroxide p.a. grade, lot number A23D57, val. Jun/2014, and the solution was prepared with 0.4 g of sodium hydroxide dissolved in 100-mL of water.

(7) HPLC grade water from EM Science was substituted by deionized water produced by the Barnstead NANOpure II water purifier.

Additional equipment/material used

Analytical balance Oertling model NA 164, LAB 011, SN 893385

Balance, pan - Mettler model PB3002S, LAB 072, SN 1120263965

Pipettor, adjustable, Eppendorf, 0.5-10 µL (LAB 133, SN 4258143), 5-100 µL (LAB 126, SN 2025441), 50-1000 µL (LAB 130, SN 2398024), 100-5000 µL (LAB 132, SN 3407404)

Oven, model 315SE, LAB 050

Collection plate, 96-well, 2-mL, Masterblocks

Adsorbent, Silica

Desiccator, glass, 250-mm I.D

Glass dish, 90-mm

Pipet tips, Eppendorf polyethylene disposable transfer, 200-µL, 1000-µL, 5-mL

Calculations

The calculations were performed as described in Section 10 of method GRM 05.02. Calibration standards (0.1, 0.5, 1.0, 5.0, 10, 20, 35, and 50 ng/mL, each containing 5 ng/mL of mixed XDE-175 stable isotope internal standard) were analyzed with the sample set. Linear regression equations with 1/x weighting were generated for XDE-175-J and XDE-175-L and their metabolites XDE-175-*N*-demethyl J and XDE-175-*N*-demethyl L using the concentration of the analyte standard on the abscissa (x-axis) versus the quantitation ratio on the ordinate (y-axis).

1. Calculation of Residue Found

   Concentrations of XDE-175-J and XDE-175-L and their XDE-175-*N*-demethyl J and XDE-175-*N*-demethyl L metabolites were determined by substituting the peak area responses into their respective linear regression equations. For example, take soil sample 051329 + 0.005r1, analysis of XDE-175-J, dil. factor = 1 (Page 132):

   Peak area m/z 748.5/142.2 = 9640

   Peak area m/z 757.5/146.2 = 116000

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

$$\text{Quantitation Ratio} = \frac{9640}{116000} = 0.0831$$

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

$$\underset{(ng/mL)}{\text{Analyte concentration}} = \left(\frac{\text{quantitation ratio} - \text{intercept}}{\text{slope}}\right)$$

$$\underset{(ng/mL)}{XDE\text{-}175\text{-}J_{051329+0.005rl}} = \left(\frac{0.0831 - 0.0031}{0.2344}\right) = 0.3413$$

The concentration found is expressed in ng/mL, so it must be converted into µg/g using the following expressions:

$$\underset{(mL/g)}{\text{Method factor}} = \left(\frac{\text{final volume (mL)} \; x \; \text{extraction volume (mL)}}{\text{aliquot (mL)} \; x \; \text{nominal sample weight (g)}}\right)$$

$$\underset{(mL/g)}{\text{Method factor}} = \left(\frac{0.5\text{ mL} \; x \; 40\text{ mL}}{0.25\text{ mL} \; x \; 5\text{ g}}\right) = 16$$

$$\underset{(\text{gross } \mu g/g)}{\text{Analyte concentration}} = \text{analyte conc. - ng/mL } x \text{ method factor - mL/g } x\, 10^{-3} \, x \text{ dilution factor}$$

$$\underset{(\text{gross } \mu g/g)}{\text{Analyte concentration}} = 0.3413 \, x \, 16 \, x \, 10^{-3} \, x \, 1 = 0.0054$$

Determine the net concentration in each recovery sample by subtracting any contribution found at the expected retention time of each analyte in the untreated control sample from that of the gross analyte concentration found in the recovery sample:

Analyte concentration. = Analyte concentration – Analyte concentration
(net µg/g) (gross µg/g) (control µg/g)

$$\underset{(\text{net } \mu g/g)}{\text{Analyte concentration}} = 0.0054 - 0.0001 = 0.0053$$

2. Calculation of Percent Recovery

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

$$\text{Recovery} = \frac{\text{conc. found}}{\text{conc. added}} \times 100\%$$

For the given example:

$$\text{Recovery} = \frac{0.0053}{0.005} \times 100\% = 108\%$$

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 recovery for a sample 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.

GRM: 05.02
EFFECTIVE: 26-May-2005
SUPERSEDES: New

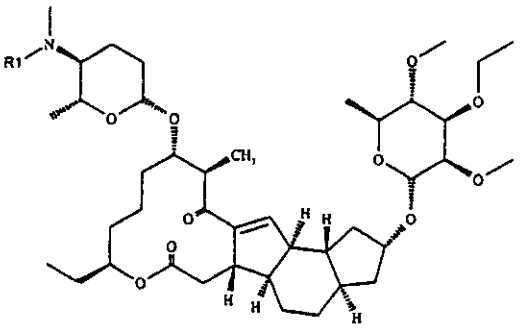
Dow AgroSciences

Determination of Residues of XDE-175 and its Metabolites in Soil and Sediment by On-Line Solid Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry

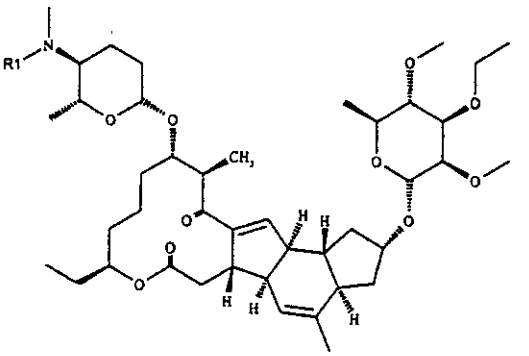
M. J. Hastings

1. SCOPE

This method is applicable for the quantitative determination of XDE-175-J and XDE-175-L and their metabolites XDE-175-*N*-demethyl J and XDE-175-*N*-demethyl L, in soil and sediment. The method was validated over the concentration range of 0.005-1.0 µg/g with a validated limit of quantitation of 0.005 µg/g.



XDE-175-J, R1 = CH_3
XDE-175-*N*-Demethyl-J, R1 = H



XDE-175-L, R1 = CH_3
XDE-175-*N*-Demethyl-L, R1 = H

Common and chemical names along with other identifying information are given in Table 1.

2. PRINCIPLE

Residues of XDE-175 and its metabolites are extracted from soil samples by shaking with a methanol/0.1 N sodium hydroxide (90:10) solution. An aliquot of the extraction solvent is diluted with a water/acetic acid (99.5:0.5) solution. A mixed XDE-175 and metabolites stable isotope internal standard solution is added to each sample and the final solution is purified by on-line solid phase extraction using an SCX cartridge. The SPE cartridge is washed with methanol followed by a methanol:acetonitrile:water (4:4:2) solution containing 0.1 M ammonium acetate. The SPE cartridge is eluted with a methanol:acetonitrile:water (4:4:2) solution containing 0.1 M ammonium acetate onto the analytical column where XDE-175 and its metabolites were analyzed by liquid chromatography with positive ion atmospheric pressure chemical ionization (APCI) 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. It is imperative that proper eye and personal protection equipment be worn when handling these chemicals.

3.3. Sodium hydroxide and acetic acid are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be used when handling all chemicals.

4. EQUIPMENT (Note 12.1.)

4.1. Laboratory Equipment

4.1.1. Balance, analytical, Model AE100, Mettler-Toledo, Inc., Hightstown, NJ 08520.

4.1.2. Balance, pan, Model P2002, Mettler-Toledo, Inc.

4.1.3. Centrifuge, with rotor to accommodate 40-mL vials, Model Centra-GP8, International Equipment Company, Needham Heights, MA 02494.

4.1.4. Dispenser, Bottle-Top, adjustable, Brinkmann, 5-25 mL, catalog number 13-688-134, Fisher Scientific, Pittsburgh, PA 15219.

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. Pipettor, adjustable, Gilson Microman M250, 50-250 µL, catalog number F148505, Gilson Inc., Middleton, WI 53562.

4.1.8. Pipettor, adjustable, Gilson Microman M1000, 100-1000 µL, catalog number F148506, Gilson Inc.

4.1.9. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.

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

4.2. Chromatographic System

4.2.1. Column, analytical, YMC ODS-AM, 50 x 4.6 mm, 5-µm, catalog number AM12S05-0546WT, Waters, Milford, MA 01757.

4.2.2. Column, confirmatory, Synergi Polar, 75 x 4.6 mm, 4-µm, catalog number 00C-4336-E0, Phenomenex, Torrance, CA 90501.

4.2.3. On-line SPE/Liquid chromatograph, Symbiosis Pharma, Spark Holland Inc., Plainsboro, NJ 08536.

4.2.4. Mass spectrometer, Model API 4000, MDS/Sciex, Foster City, CA 94404.

4.2.5. Mass spectrometer data system, Analyst 1.4, MDS/Sciex.

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, media bottle, catalog number 06-423-3D, Fisher Scientific.

5.3. Centrifuge tube, graduated, 50-mL, catalog number 05-539-9, Fisher Scientific.

5.4. Collection plate, 96-well, 2-mL, catalog number 121-5203, Argonaut Technologies, Inc., Redwood City, CA 94063.

5.5. Collection plate sealing cap, catalog number 121-5205, Argonaut Technologies, Inc.

5.6. Cylinder, graduated, 100-mL, catalog number C7000-100, National Scientific Company, Lawrenceville, GA 30243.

5.7. Cylinder, graduated, 500-mL, catalog number C7000-500, National Scientific Company.

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

5.9. Desiccator, glass, 250-mm I.D, catalog number 08-595-E, Fisher Scientific.

5.10. Dish, 42-mm aluminum weighing, catalog number 08-732, Fisher Scientific.

5.11. Flask, volumetric, 100-mL, catalog number 161-8987, National Scientific Company.

5.12. Pipet, polyethylene disposable transfer, 3-mL, catalog number, 13-711-7, Fisher Scientific.

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

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

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

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

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

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

5.19. Pipetter tips, Gilson Microman CP250, catalog number F148114, Gilson Inc.

5.20. Pipetter tips, Gilson Microman CP1000, catalog number F148560, Gilson Inc.

5.21. SPE cartridges, BondElut SCX, 40-90 µm, catalog number 0722.141, Spark Holland Inc.

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

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

6.1. Reagents

6.1.1. Acetic acid, glacial, HPLC Grade, catalog number A35-500, Fisher Scientific.

6.1.2. Acetonitrile, ChromAR HPLC grade, catalog number 2856, Mallinckrodt-Baker, Inc., Paris, KY 40361.

6.1.3. Ammonium acetate, HPLC grade, catalog number A639-500, Fisher Scientific.

6.1.4. Dry ice, Continental Carbonic, Decatur, IL 62526.

6.1.5. Methanol, ChromAR HPLC grade, catalog number 3041, Mallinckrodt-Baker Inc.

6.1.6. Nitrogen, refrigerated liquid, BOC Group Inc., Murray Hill, NJ 07974.

6.1.7. Sodium hydroxide, 0.1 N, certified ACS grade, catalog number SS276-1, Fisher Scientific.

6.1.8. Water, HPLC grade, catalog number WX0004-1, EM Science, Gibbstown, NJ 08027.

6.2. Standards

6.2.1. Analytical standard information for XDE-175-J, XDE-175-L, XDE-175-*N*-Demethyl-J, XDE-175-*N*-Demethyl-L is listed in Table 1.

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

6.2.2 Stable isotope labeled internal standards information for XDE-175-J, XDE-175-L, XDE-175-*N*-Demethyl-J, XDE-175-*N*-Demethyl-L is listed in Table 1.

Obtain from Specialty Synthesis Group, Dow AgroSciences LLC, 9330 Zionsville Road, Building 306, Indianapolis, IN 46268-1054.

6.3. Prepared Solutions

6.3.1. acetonitrile/methanol/water (4:4:2) containing 0.1 M ammonium acetate

Weigh 7.7 g of ammonium acetate into a 40-mL vial and quantitatively transfer with 200 mL of HPLC water into a 1-L bottle. Measure 400 mL of methanol and 400 mL of acetonitrile using a 500-mL graduated cylinder and transfer to the 1.0-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.2. acetonitrile/methanol (1:1) containing 10 mM ammonium acetate

Weigh 0.77 g of ammonium acetate into a 40-mL vial and quantitatively transfer with 100 mL of methanol into a 1-L bottle. Add a further 400 mL of methanol to the bottle. Measure 500 mL of acetonitrile using a 500-mL graduated cylinder and then transfer to the 1.0-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.3. acetonitrile/water (80:20)

Measure 800 mL of acetonitrile using a 1-L graduated cylinder and then transfer into a 1.0-L bottle. Measure 200 mL of acetonitrile using a 500-mL graduated cylinder and then transfer into the 1.0-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.4. methanol/0.1 N sodium hydroxide (90:10)

Measure 900 mL of methanol using a 1.0-L graduated cylinder and then transfer into a 1.0-L bottle. Measure 100 mL of a 0.1 N sodium hydroxide solution using a 100-mL graduated cylinder and transfer to the 1.0-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.5. water containing 10 mM ammonium acetate

Weigh 0.77 g of ammonium acetate into a 40-mL vial and quantitatively transfer with 100 mL of HPLC water into a 1-L bottle. Add a further 900 mL of HPLC water to the bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.6. water/glacial acetic acid (99.5:0.5 v/v)

Add approximately 90 mL of HPLC water to a 100-mL volumetric flask. Pipet 0.5 mL of glacial acetic acid into the volumetric flask. Stopper the flask and mix. Dilute to volume with HPLC water.

7. PREPARATION OF STANDARD SOLUTIONS

7.1. Preparation of XDE-175 Spiking Solutions

7.1.1. Weigh 0.0100 g of each XDE-175 analytical standard (XDE-175-J, XDE-175 -L, XDE-175-*N*-demethyl-J and XDE-175-*N*-demethyl-L) and quantitatively transfer each standard to separate 100-mL volumetric flasks with acetonitrile. Dilute to volume with acetonitrile to obtain a 100-µg/mL stock solution of each analyte.

7.1.2. Pipet 10.0 mL of each 100-µg/mL solution (Section 7.1.1.) into a 100-mL volumetric flask. Dilute to volume with acetonitrile to obtain a 10.0-µg/mL mixed XDE-175 spiking solution. Further dilute the 10.0-µg/mL mixed XDE-175 spiking solution with acetonitrile according to the following suggested scheme:

Concentration of Initial Stock Solution	Aliquot of Stock Solution	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc. [a]	Volume of Spiking Soln.
µg/mL	mL	mL	µg/mL	µg/g	µL
100.0	10.0	100	10.0	1.0	500
100.0	10.0	100	10.0	0.5	250
10.0	10.0	100	1.0	0.05	250
1.0	10.0	100	0.1	0.005	250
0.1	10.0	100	0.01	N/A	N/A

[a] The equivalent sample concentration is based on fortifying a 5-g soil or sediment

sample.

7.2. Preparation of XDE-175 Stable Isotope Internal Standard Solutions

7.2.1. Weigh 0.0100 g of each XDE-175 stable isotope standard (XDE-175-J IS, XDE-175-L IS, XDE-175-*N*-demethyl-J IS and XDE-175-*N*-demethyl-L IS) and quantitatively transfer each standard to separate 100-mL volumetric flasks with acetonitrile. Dilute to volume with acetonitrile to obtain a 100-µg/mL stock solution of stable isotope standard.

7.2.2. Pipet 10.0 mL of each 100-µg/mL solution (Section 7.2.1.) into a 100-mL volumetric flask. Dilute to volume with acetonitrile to obtain a 10.0-µg/mL mixed XDE-175 stable isotope internal standard solution.

7.2.3. Pipet 1.0 mL of the 10.0-µg/mL mixed XDE-175 stable isotope internal standard solution (Section 7.2.2.) into a 100-mL volumetric flask. Dilute to volume with acetonitrile to obtain a 0.1-µg/mL mixed XDE-175 stable isotope internal standard solution.

7.3. Preparation of Mixed XDE-175 Calibration Solutions

7.3.1. Prepare dilutions of the 1.0, 0.1 and 0.01 µg/mL mixed XDE-175 spiking solutions (Section 7.1.2.) in acetonitrile/water (80:20) containing 5 ng/mL mixed XDE-175 stable isotope internal standard to give calibration standards over the range 0.1–50 ng/mL. Calibration standards may be prepared following the suggested scheme:

Concentration of Stock Solution	Aliquot of Spiking Solution	Final Soln. Volume	Calibration Soln. Final Conc.	Equivalent Sample Conc.[a]
µg/mL	mL	mL	ng/mL	µg/g
1.0	5.0	100	50	0.80
1.0	3.5	100	35	0.56
1.0	2.0	100	20	0.32
1.0	1.0	100	10	0.16
0.1	5.0	100	5.0	0.08
0.1	1.0	100	1.0	0.016
0.01	5.0	100	0.5	0.008
0.01	1.0	100	0.1	0.0016

[a] The equivalent sample concentration is based on extracting a 5-g soil or sediment sample.

8. ON-LINE SPE/LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

8.1. Typical Liquid Chromatography Operating Conditions

Instrumentation:	Spark Holland Symbiosis Pharma MDS/Sciex API 4000 LC/MS/MS System MDS/Sciex Analyst 1.4 data system
Column:	YMC ODS-AM, 50 x 4.6 mm, 5-µm (Quantitation) Synergi Polar RP, 75 x 4.6 mm, 4-µm (Confirmation)
Column Temperature:	Ambient
Injection Volume:	30 µL
Autosampler Wash Program:	Autosampler loop and needle washed with: 1) 700 µL of acetonitrile/methanol (1:1) containing 0.1% formic acid 2) 700 µL of methanol 3) 700 µL of acetonitrile/methanol (1:1) containing 0.1% formic acid
Run Time:	Approx 6 minutes
Mobile Phase:	A –acetonitrile/methanol (1:1) containing 10 mM ammonium acetate B –water containing 10 mM ammonium acetate

Gradient:

Time, (min:secs)	Flow (mL/min)	A, %	B, %
00:01	1.0	70	30
00:05	0.8	70	30
01:00	0.8	70	30
01:05	1.0	70	30
03:05	1.0	100	0
05:00	1.0	100	0
05:15	1.0	70	30
06:15	1.0	70	30

Flow Diverter Program:
1) 0.0→3.0 min: flow to waste
2) 3.0→5.0 min: flow to source
3) 5.0→end of run: flow to waste

8.2. Typical On-Line Solid Phase Extraction Operating Conditions

SPE Cartridge:	SCX, 40-90 µm
SPE Solvation:	acetonitrile, 1 mL at 5 mL/min (SSM A)
SPE Equilibration:	water, 1 mL at 5 mL/min (SSM B)

Sample Extraction:	water, 2 mL at 5 mL/min (SSM B)
SPE Wash 1:	methanol, 2 mL at 2.5 mL/min (SSM C)
SPE Wash 2:	methanol:acetonitrile/water (4:4:2) containing 0.1 M ammonium acetate, 0.2 mL at 0.2 mL/min (HPD2)
SPE Elution:	focus mode, methanol/acetonitrile:water (4:4:2) containing 0.1 M ammonium acetate, 0.2 mL at 0.2 mL/min (HPD2)
Clamp Flush 1:	methanol:acetonitrile/water (4:4:2) containing 0.1 M ammonium acetate, 1 mL at 5.0 mL/min (HPD2)
Clamp Flush 2:	water, 2 mL at 5 mL/min (HPD2)

Additional information relating to the on-line SPE method can be found in Appendix 1.

8.3. Typical Mass Spectrometry Operating Conditions

Interface:	APCI
Polarity:	Positive
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – unit
Curtain Gas (CUR):	12 psi
Collision Gas (CAD):	4 psi
Temperature (TEM):	425 °C
Ion Source Gas 1 (GS1):	50
Ion Source Gas 2 (GS2):	N/A
Period 1	
Acquisition Time Delay:	3.0 mins
Period Duration:	2.0 mins
Nebulizer Current (NC):	5 µA

Compound:	Ion, *m/z*		Time, ms	Collision Energy, V
	Q1	Q3		
XDE-175-J	748.6	142.2	50	37
XDE-175-L	760.9	142.2	50	37
XDE-175-*N*-Demethyl-J	734.9	128.2	50	31
XDE-175-*N*-Demethyl-L	746.7	128.2	50	33
XDE-175-J IS	757.9	146.2	50	37
XDE-175-L IS	769.9	146.2	50	37
XDE-175-*N*-Demethyl-J IS	739.9	128.2	50	31
XDE-175-*N*-Demethyl-L IS	751.7	128.2	50	33

8.4. Typical Mass Spectra

Typical mass spectra and product ion spectra of XDE-175, its metabolites and stable isotope internal standards are presented in Figures 1-16.

8.5. Typical Calibration Curve

Typical calibration curves for the determination of XDE-175 and its metabolites in soil and sediment are shown in Figures 17-20.

8.6. Typical Chromatograms

Typical chromatograms of a 0. 5-ng/mL calibration standard, a control sediment sample, a control sediment sample fortified at 0.005 µg/g (limit of quantitation), and a control sediment sample fortified at 0.05 µg/g (10 times the limit of quantitation) are presented in Figures 21-24. Typical chromatograms generated using the confirmatory HPLC column are presented in Figures 25-28.

9. DETERMINATION OF RECOVERY OF XDE-175 AND ITS METABOLITES IN SOIL AND SEDIMENT

9.1. Method Validation Prior to Field Sample Analysis

Unless otherwise specified, a sample set should contain, at the minimum, 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 two controls 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. Sediment samples should be thoroughly stirred prior to use. Prepared soil samples should be stored frozen prior to analysis and sediment samples should be stored refrigerated prior to analysis.

9.3. Sample Analysis for XDE-175 and Metabolites in Soil and Sediment

9.3.1. Weigh 5 ± 0.05g portions of sample into amber 40-mL vials.

9.3.2. Add the required volume of the appropriate fortification solution to the recovery samples (Section 7.1.2.) using a positive displacement pipet.

9.3.3. Add 20 mL of methanol/0.1 N sodium hydroxide (90:10).

9.3.4. Cap the vial and shake the sample for 60 minutes on a flat-bed shaker at approximately 180 excursions/minute. **Note: If not using amber vials, protect the samples from bright light during extraction with paper towels or aluminum foil.**

9.3.5. Centrifuge the sample for 5 minutes at 2000 rpm and decant the extract into a 50-mL centrifuge tube. Store the centrifuge tubes containing the first soil extract in a dark location.

9.3.6. Add an additional 15-mL of extraction solution to each soil sample and shake for 30 minutes on a flat-bed shaker at approximately 180 excursions/minute.

9.3.7. Centrifuge the sample for 5 minutes at 2000 rpm and combine the extraction solvent with the first extract (Section 9.3.4.) in the 50-mL centrifuge tube.

9.3.8. Adjust the volume in the centrifuge tube to 40 mL with the methanol/0.1 N sodium hydroxide (90:10) extraction solvent. Cap the centrifuge tube and mix thoroughly.

9.3.9 Pipet 250 µL of the extraction solution into a 96-well plate.

9.3.10. Add 25 µL of the 0.1-µg/mL mixed XDE-175 stable isotope standard (Section 7.2.3.) and 250 µL of a water/glacial acetic acid (99.5:0.5 v/v) solution to the sample.

9.3.11. Add approximately 500 µL of each calibration standard to empty wells of the 96-well plate, cap and vortex mix for approximately 30 seconds.

9.3.12. Chromatograph the samples and standard using the conditions given in Section 8, injecting the calibration standards throughout the run.

9.3.13. For sample extracts which contain XDE-175 concentrations > 50 ng/mL (equivalent to >0.8 µg/g), dilute with methanol/water (90:10) containing 5 ng/mL mixed XDE-175 stable isotope standard. 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. Peak resolution: Determine visually that sufficient resolution has been achieved for the analyte relative to any background interferences.

c. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 21-24 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 20:1 has been attained for the 0.5-ng/mL calibration standard (equivalent to 0.008 µg/g of XDE-175 and or metabolites in a soil or sediment sample).

10. CALCULATIONS

10.1. Determination of Isotopic Crossover

In this assay, the analyte and internal standard 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 can be determined empirically by analyzing standard solutions of each compound and should be addressed for accurate determination of concentrations.

10.1.1. To determine the isotopic crossover for XDE-175 and its metabolites and their respective stable isotopes, inject a 5-ng/mL mixed XDE-175 and metabolite standard and a 5-ng/mL mixed XDE-175 stable isotope standard and determine the peak areas for the analyte and internal standard as indicated below. For example, to determine the contribution of the unlabeled XDE-175-J to the stable isotope labeled XDE-175-J internal standard:

XDE-175-J	*m/z* Q1/Q3 748.6/142.2
XDE-175-J IS	*m/z* Q1/Q3 757.9/146.2

To determine the contribution of the unlabeled XDE-175-J to the labeled XDE-175-J internal standard:

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

$$\text{Crossover Factor (analyte}\rightarrow\text{ISTD)} = \frac{\text{peak area at } m/z \text{ 757.9/146.2}}{\text{peak area at } m/z \text{ 748.6/142.2}}$$

In a similar manner, to determine the contribution of the labeled XDE-175-J stable isotope to the unlabeled XDE-175-J:

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

$$\text{Crossover Factor (ISTD}\rightarrow\text{ analyte)} = \frac{\text{peak area at } m/z \text{ 748.6/142.2}}{\text{peak area at } m/z \text{ 757.9/146.2}}$$

During method development, no significant mass spectral isotopic cross over was observed and therefore no correction of the measured quantitation ratio was performed. If isotopic cross over is encountered it should be assessed and the respective quantitation ratios corrected for accurate determination of concentrations (13.1, 13.2).

10.2. Calculation of Standard Calibration Curve for XDE-175 and its Metabolites

10.2.1. Inject a series of calibration standards (Section 7.3.) using the conditions described in Section 8 and determine the peak areas for XDE-175 and its metabolites and internal standards as indicated below:

XDE-175-J	*m/z* Q1/Q3 748.6/142.2
XDE-175-L	*m/z* Q1/Q3 760.9/142.2
XDE-175-*N*-Demethyl-J	*m/z* Q1/Q3 734.9/128.2
XDE-175-*N*-Demethyl-L	*m/z* Q1/Q3 746.7/128.2
XDE-175-J IS	*m/z* Q1/Q3 757.9/146.2
XDE-175-L IS	*m/z* Q1/Q3 769.9/146.2
XDE-175-*N*-Demethyl-J IS	*m/z* Q1/Q3 739.9/128.2
XDE-175-*N*-Demethyl-L IS	*m/z* Q1/Q3 751.7/128.2

10.2.2. For each standard, calculate the XDE-175 quantitation ratio.

For example, using the data for XDE-175-J from injection no. 6, Figure 17:

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

$$\text{Quantitation Ratio} = \frac{\text{XDE-175-J peak area}}{\text{XDE-175-J IS stable isotope internal standard peak area}}$$

$$\text{Quantitation Ratio} = \frac{7412}{60382}$$

$$\text{Quantitation Ratio} = 0.1228$$

10.1.3. Prepare a standard curve by plotting the concentration of the analytes on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis), as shown in Figures 17-20. Using linear regression analysis (13.3.) with a 1/x weighting (13.4.), determine the equation for the curve with respect to the abscissa.

For example, using the XDE-175-J data from Figure 17:

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

$$\text{XDE-175-J conc. (ng/mL)} = \left(\frac{\text{XDE-175-J quantitation ratio} - \text{intercept}}{\text{slope}}\right)$$

$$\text{XDE-175-J conc. (ng/mL)} = \left(\frac{\text{XDE-175-J quantitation ratio} - (-0.0017)}{0.2558}\right)$$

10.3. Calculation of Percent Recovery for XDE-175 and its Metabolites

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

For example, using the data for XDE-175-J data from injection no.15, Figure 17:

$$\text{XDE-175-J conc. (ng/mL)} = \left(\frac{\text{XDE-175-J quantitation ratio} - (-0.0017)}{0.2558}\right)$$

$$\text{XDE-175-J conc. (gross ng/mL)} = \left(\frac{0.075 - (-0.0017)}{0.2558}\right)$$

$$\text{XDE-175-J conc. (gross)} = 0.2998 \text{ ng/mL}$$

Convert the concentration of ng/mL of XDE-175-J found in the final sample extract prepared for analysis to µg/g of XDE-175-J in the original soil sample as follows:

$$\text{XDE-175-J conc. (gross ng/g)} = 0.2998 \text{ ng/mL} \times \frac{(40 \text{ mL}/0.5 \text{ mL}) \times 1.0 \text{ mL}}{5 \text{ g}} \times \text{DF}$$

$$\text{XDE-175-J conc. (gross)} = 4.7968 \text{ ng/g or } 0.0048 \text{ µg/g}$$

Where DF = final dilution factor for samples diluted at Step 9.3.13.

10.3.2. Determine the net concentration in each recovery sample by subtracting the concentration found at the retention time of each analyte in the untreated control sample from that of the gross analyte concentration in the recovery sample.

For example, using the data for XDE-175-J from Figure 17:

$$\text{XDE-175-J conc. (net } \mu g/g) = \text{XDE-175-J conc. (gross } \mu g/g) - \text{XDE-175-J conc. (control } \mu g/g)$$

$$\text{XDE-175-J conc. (net } \mu g/g) = 0.0048\ \mu g/g - 0.0000\ \mu g/g$$

$$\text{XDE-175-J conc. (net)} = 0.0048\ \mu g/g$$

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

$$\text{Recovery} = \frac{\text{conc. found}}{\text{conc. added}} \times 100\%$$

$$\text{Recovery} = \frac{0.0048\ \mu g/g}{0.005\ \mu g/g} \times 100\%$$

$$\text{Recovery} = 96\%$$

10.4. Determination of XDE-175 and its Metabolites in Soil and Sediment Samples

10.4.1. Determine the gross concentration of XDE-175 and its metabolites in each soil or sediment sample by substituting the respective peak area into the equation for the calibration curve and calculating the uncorrected residue result as described in Section 10.3.1.

10.4.2. For those samples that require correction for the method procedural recovery, use the average recovery of all the recovery samples at or above the limit of quantitation, as described in Section 9.1, from a given sample set to correct for method efficiency. For example, continuing with the data from Figure 17 and the average recovery from Table 2 for the samples analyzed on 17-Aug-2004:

$$\text{XDE-175-J conc. (corrected } \mu g/g) = \text{XDE-175-J conc. (gross } \mu g/g) \times \left(\frac{100}{\text{Average \% Recovery}}\right)$$

$$\text{XDE-175-J conc. (corrected } \mu g/g) = 0.0048\ \mu g/g \times \frac{100}{92}$$

$$\text{XDE-175-J conc. (corrected)} = 0.0052\ \mu g/g$$

10.5. Determination of Soil Moisture

10.4.1. Accurately weigh a 10-g portion of soil into a tared aluminum weighing dish.

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

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

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

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

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

10.6. Determination of Dry Weight Concentrations of XDE-175 and Metabolites in Soil and Sediment

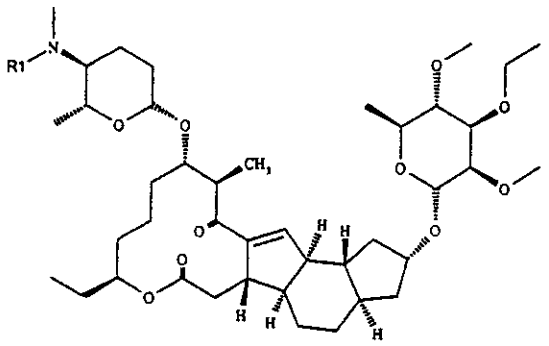
10.6.1. Determine the analyte concentrations in the sample as described in Section 10.4.

10.6.2. Determine the soil moisture as described in Section 10.5.

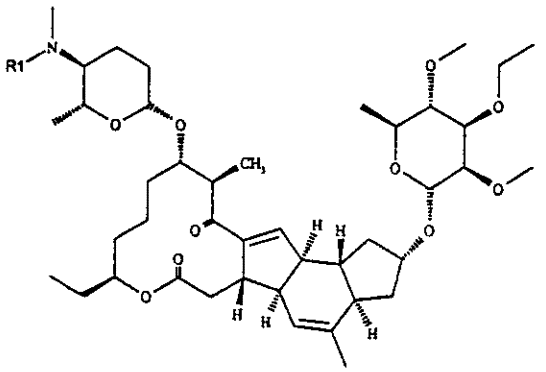
10.6.3. Determine the dry weight analyte concentrations in the samples as follows:

$$\text{XDE-175 conc. (dry weight } \mu g/g) = \text{XDE-175 conc. } (\mu g/g) \times \left(1 + \frac{\%\ \text{Moisture}}{100}\right)$$

Table 1. Identity and Structure of XDE-175, its Metabolites and Stable Isotope Internal Standards



XDE-175-J, R1 = CH_3
XDE-175-*N*-Demethyl-J, R1 = H



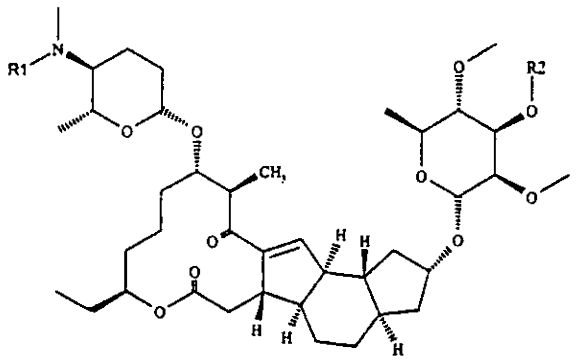
XDE-175-L, R1 = CH_3
XDE-175-*N*-Demethyl-L, R1 = H

Common Name of Compound
XDE-175-J Molecular Formula: $C_{42}H_{69}NO_{10}$ Formula Weight: 748.010 Nominal Mass: 747.5 CAS Registry Number: 187166-40-1 CAS Name: 1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione, 2-[(6-deoxy-3-O-ethyl-2,4-di-O methyl-a-L-mannopyranosyl)oxy]-13-[[(2R,5S,6R)-5-(dimethylamino)tetrahydro-6-methyl 2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,4,5,5a,5b,6,9,10,11,12,13,14,16a,16b-hexadecahydro 14-methyl-, (2R,3aR,5aR,5bS,9S,13S,14R,16aS,16bR)
XDE-175-L Molecular Formula: $C_{43}H_{69}NO_{10}$ Formula Weight: 760.022 Nominal Mass: 759.5 CAS Registry Number: 187166-15-0 CAS Name: 1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione, 2-[(6-deoxy-3-O-ethyl-2,4-di-O-methyl-a-L-mannopyranosyl)oxy]-13-[[(2R,5S,6R)-5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-, (2S,3aR,5aS,5bS,9S,13S,14R,16aS,16bS)

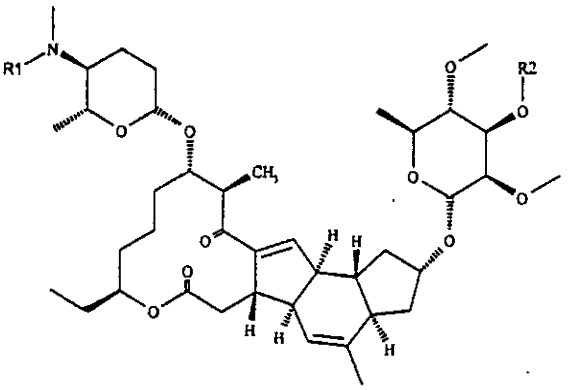
Table 1. (Cont.) Identity and Structure of XDE-175, its Metabolites and Stable Isotope Internal Standards

XDE-175-*N*-Demethyl-J Molecular Formula: $C_{41}H_{67}NO_{10}$ Formula Weight: 733.984 Nominal Mass: 733.5 CAS Registry Number: N/A IUPAC Name: (2R,3aR,5aR,5bS,9S,13S,14R,16aS,16bR)-9-ethyl-14-methyl-13-{[(2S,5S,6R)-6-methyl-5-(methylamino)tetrahydro-2H-pyran-2-yl]oxy}-7,15-dioxo-2,3,3a,4,5,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-octadecahydro-1H-as-indaceno[3,2-d]oxacyclododecin-2-yl 6-deoxy-3-O-ethyl-2,4-di-O-methyl-beta-L-mannopyranoside
XDE-175-*N*-Demethyl-L Molecular Formula: $C_{42}H_{67}NO_{10}$ Formula Weight: 745.995 Nominal Mass: 745.5 CAS Registry Number: N/A IUPAC Name: (2S,3aR,5aS,5bS,9S,13S,14R,16aS,16bS)-9-ethyl-4,14-dimethyl-13-{[(2S,5S,6R)-6-methyl-5-(methylamino)tetrahydro-2H-pyran-2-yl]oxy}-7,15-dioxo-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-1H-as-indaceno[3,2-d]oxacyclododecin-2-yl 6-deoxy-3-O-ethyl-2,4-di-O-methyl-beta-L-mannopyranoside

Table 1. (Cont.) Identity and Structure of XDE-175, its Metabolites and Stable Isotope Internal Standards



XDE-175-J, R1 = $^{13}CD_3$, R2 = C_2D_5
XDE-175-*N*-Demethyl-J, R1 = H, R2 = C_2D_5



XDE-175-L, R1 = $^{13}CD_3$, R2 = C_2D_5
XDE-175-*N*-Demethyl-L, R1 = H, R2 = C_2D_5

Common Name of Internal Standard	
XDE-175-J IS	
Molecular Formula:	$C_{41}{}^{13}CH_{61}D_8NO_{10}$
Formula Weight:	757.010
Nominal Mass:	756.5
CAS Registry Number:	N/A
XDE-175-L IS	
Molecular Formula:	$C_{42}{}^{13}CH_{61}D_8NO_{10}$
Formula Weight:	769.022
Nominal Mass:	768.5
CAS Registry Number:	N/A
XDE-175-N-Demethyl-J IS	
Molecular Formula:	$C_{41}H_{62}D_5NO_{10}$
Formula Weight:	738.984
Nominal Mass:	738.5
CAS Registry Number:	N/A
XDE-175-N-Demethyl-L	
Molecular Formula:	$C_{42}H_{62}D_5NO_{10}$
Formula Weight:	750.995
Nominal Mass:	750.5
CAS Registry Number:	N/A