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Dow AgroSciences

Determination of Residues of XDE-175 and its Metabolites in Soil and Sediment by Liquid Chromatography with Tandem Mass Spectrometry

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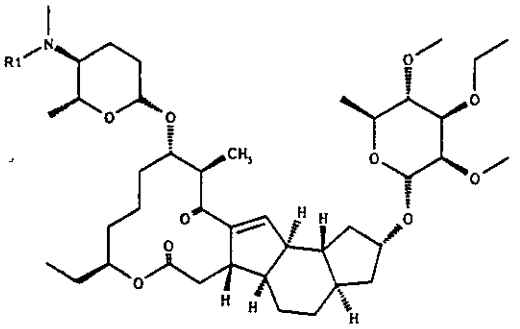
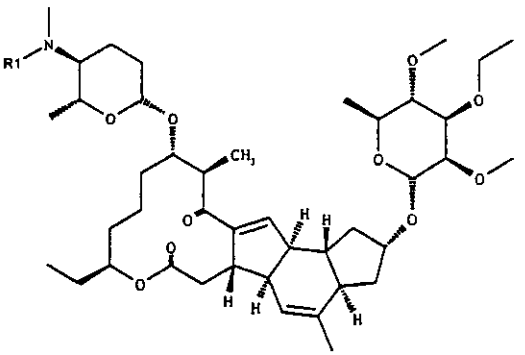
1. SCOPE

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/0.1N sodium hydroxide solution (90:10). An aliquot of the extraction solvent is diluted with 10% sodium chloride and XDE-175 and its metabolites are partitioned into methyl *tert*-butyl ether (MTBE). The MTBE is evaporated and the residues are reconstituted in an acetonitrile/methanol/water (35:35:30) solution. The final solution is 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, methanol, and methyl *tert*-butyl ether 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 is 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 22-mL and 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. Evaporating unit, Reacti-Vap, Model 18780, Pierce Chemical Company, Rockford, IL 61105.

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

4.1.7. Heating module, Reacti-Therm, Model 18870, Pierce Chemical Company.

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

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

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

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

4.1.12. 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. 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, 2-oz (60-mL) with PTFE cap, catalog number 03-326-3C, Fisher Scientific.

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

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

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

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

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

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

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

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

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

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

5.13. Pipet, disposable seriological, 2-mL, catalog number 13-666-7C, Fisher Scientific.

5.14. Pipet, disposable seriological, 10-mL, catalog number 13-666-7E, Fisher Scientific.

5.15. Pipet, polyethylene disposable transfer, 3-mL, 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. Pipetter tips, Gilson Microman CP250, catalog number F148114, Gilson Inc.

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

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

5.25. Vial, 12-mL, with PTFE-lined screw cap, catalog number B7800-12, National Scientific Company.

5.26. Vial, 22-mL, with PTFE-lined screw cap, catalog number B7800-5, National Scientific Company.

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

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

6.1.3. Glycerin, certified ACS grade, catalog number G33-500, 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. Methyl *tert*-butyl ether (MTBE), OmniSolv grade, catalog number MX0826-1, EM Science, Gibbstown, NJ 08027.

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

6.1.8. Sodium chloride, USP/FCC grade, catalog number S640-500, Fisher Scientific.

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

6.1.10. Water, HPLC grade, catalog number WX0004-1, EM Science.

6.2. Standards

6.2.1. XDE-175-J, XDE-175-L, XDE-175-*N*-Demethyl-J, XDE-175-*N*-Demethyl-L. Standard information 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.

6.3. Prepared Solutions

6.3.1. 10% aqueous sodium chloride (w/v)

Weigh 100 g of sodium chloride into a 1-L bottle. Measure 1 L of HPLC water using a 1-L graduated cylinder and transfer to the bottle containing the salt. Cap the bottle and shake to dissolve.

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/methanol/water (35:35:30)

Measure 350 mL of acetonitrile using a 500-mL graduated cylinder and then transfer into a 1.0-L bottle. Measure 350 mL of methanol using a 500-mL graduated cylinder and then transfer into the 1.0-L bottle. Measure 300 mL of water 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/glycerine (8:2 w/w)

Weigh 2.0 g of glycerine into a 60-mL bottle. Weigh 8.0 g of methanol and transfer to the 50-mL bottle containing the glycerine. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.5. 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.

6.3.6. 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.

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 Mixed XDE-175 Calibration Solutions

7.2.1. Prepare dilutions of the 1.0, 0.1 and 0.01-µg/mL mixed XDE-175 spiking solutions (Section 7.1.2.) in acetonitrile/methanol/water (35:35:30) 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	0.40
1.0	3.5	100	35	0.28
1.0	2.0	100	20	0.16
1.0	1.0	100	10	0.08
0.1	5.0	100	5.0	0.04
0.1	1.0	100	1.0	0.008
0.01	5.0	100	0.5	0.004
0.01	1.0	100	0.1	0.0008

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

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

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

Run Time: 7 minutes

Mobile Phase: A –acetonitrile/methanol (1:1) containing 10 mM ammonium acetate
B –water containing 10 mM ammonium acetate

Flow Rate: 1.0 mL/min

Gradient:

Time, (min:secs)	A, %	B, %
00:01	70	30
05:00	100	0
05:30	100	0
05:45	70	30
07:00	70	30

Flow Diverter Program: 1) 0.0→2.5 min: flow to waste
2) 2.5→6.0 min: flow to source
3) 6.0→7.0 min: flow to waste

8.2. 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 psi
Ion Source Gas 2 (GS2):	N/A
Period 1	
Acquisition Time Delay:	2.5 mins
Period Duration:	3.5 mins
Nebulizer Current (NC):	5 µA

Compound:	Ion, *m/z*		Time, ms	Collision Energy, V
	Q1	Q3		
XDE-175-J	748.6	142.2	150	37
XDE-175-L	760.9	142.2	150	37
XDE-175-*N*-Demethyl-J	734.9	128.2	150	31
XDE-175-*N*-Demethyl-L	746.7	128.2	150	33

8.3. Typical Mass Spectra

Typical mass spectra and product ion spectra of XDE-175 and its metabolites are presented in Figures 1-8.

8.4. Typical Calibration Curve

Typical calibration curves for the determination of XDE-175 and its metabolites in soil and sediment are shown in Figures 9-12.

8.5. 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 13-16.

Typical chromatograms generated using the confirmatory HPLC column are presented in Figures 17-20.

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 1.0 mL of the extraction solution into a 22-mL vial.

9.3.10. Add 10 mL of a 10% aqueous sodium chloride solution and 5.0 mL of MTBE.

9.3.11. Shake on a flat bed shaker at approximately 180 excursions/minute for 5 minutes. Centrifuge the sample for 5 minutes at 2000 rpm.

9.3.12. Transfer the top MTBE layer to a clean 12-mL vial using a polyethylene disposable transfer pipette. **Do not transfer any of the aqueous layer to the 12-mL vial.**

9.3.13. Add 100 µL of a methanol/glycrine (80:20) solution to the sample vial.

9.3.14 Evaporate the MTBE using a dry block heater set at 40 °C and a gentle stream of nitrogen until approximately 20 µL of glycerine remains. **Do not use a Turbovap evaporator. Do not attempt to evaporate to dryness**. Check the evaporation progress regularly and remove the vials when no further volume change is noticed.

9.3.15. Reconstitute the sample in 980 µL of an acetonitrile/methanol/water (35:35:30) solution and vortex mix.

9.3.16. Transfer the samples and standards to 2-mL vials (or a 2-mL 96-well plate), and chromatograph the samples using the conditions given in Section 8, injecting the calibration standards interspersed throughout the run. For sample extracts which contain XDE-175 concentrations > 50 ng/mL (equivalent to >0.4 µg/g), dilute with acetonitrile:methanol:water (35:35:30). 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 13-16 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for the 0.5-ng/mL calibration standard (equivalent to 0.004 µg/g of XDE-175 and or metabolites in a soil or sediment sample).

10. CALCULATIONS

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

10.1.1. Inject a series of calibration standards (Section 7.2.) using the conditions described in Section 8 and determine the peak areas for XDE-175 and its metabolites 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

10.1.2. Prepare a standard curve by plotting the concentration of the analytes on the abscissa (x-axis) and the respective peak areas on the ordinate (y-axis), as shown in Figures 9-12. Using linear regression analysis (13.1.) with a 1/x weighting (13.2.), determine the equation for the curve with respect to the abscissa.

For example, using the XDE-175-J data from Figure 9:

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

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

$$\text{XDE-175-J conc. (ng/mL)} = \left(\frac{\text{XDE-175-J peak area} - (-132.4)}{17077}\right)$$

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

10.2.1. 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. 17, Figure 9:

$$\text{XDE-175-J conc. (gross ng/mL)} = \left(\frac{\text{XDE-175-J peak area} - (-132.4)}{17077}\right)$$

$$\text{XDE-175-J conc. (gross ng/mL)} = \left(\frac{9716-(-132.4)}{17077}\right)$$

$$\text{XDE-175-J conc. (gross)} = 0.577 \text{ ng/mL}$$

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:

$$\text{XDE-175-J conc. (gross ng/g)} = 0.577 \text{ ng/mL} \times \frac{(40 \text{ mL}/1.0 \text{ mL}) \times 1.0 \text{ mL}}{5 \text{ g}} \times \text{DF}$$

$$\text{XDE-175-J conc. (gross)} = 4.616 \text{ ng/g or } 0.00462 \text{ µg/g}$$

Where DF = final dilution factor for samples diluted at Step 9.3.16.

10.2.2. 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 9:

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

$$\text{XDE-175-J conc. (net µg/g)} = 0.00462 \text{ µg/g} - 0.0000 \text{ µg/g}$$

$$\text{XDE-175-J conc. (net)} = 0.00462 \text{ µg/g}$$

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

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

$$\text{Recovery} = \frac{0.00462 \text{ µg/g}}{0.005 \text{ µg/g}} \times 100\%$$

$$\text{Recovery} = 92\%$$

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

10.3.1. 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 10.2.1.

10.3.2. 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 9 and the average recovery from Table 2 for the samples analyzed on 17-Aug-2004:

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

$$\text{XDE-175-J conc. (corrected µg/g)} = 0.00462\ \mu g/g \times \frac{100}{95}$$

$$\text{XDE-175-J conc. (corrected)} = 0.00486\ \mu g/g$$

10.4. 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:

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

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

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

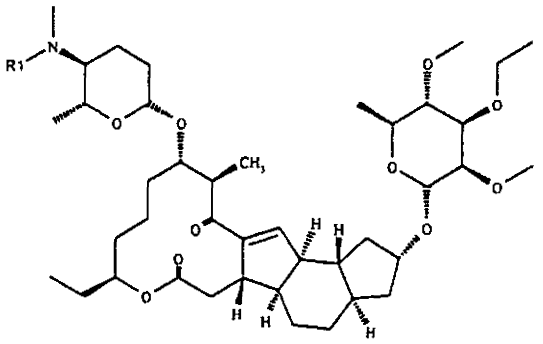
10.5.1. Determine the analyte concentrations in the sample as described in Section 10.3.

10.5.2. Determine the soil moisture as described in Section 10.4.

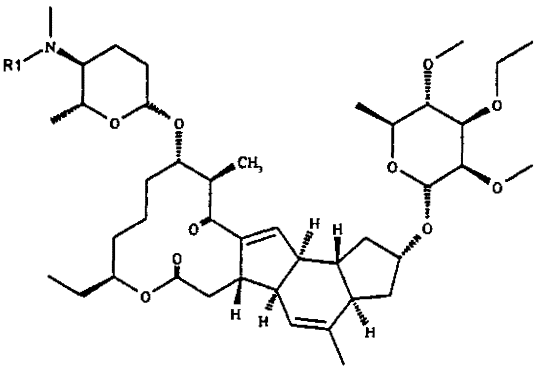
10.5.3. Determine the dry weight analyte concentrations in the samples as follows:

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

Table 1. Identity and Structure of XDE-175 and its Metabolites



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 and its Metabolites

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

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

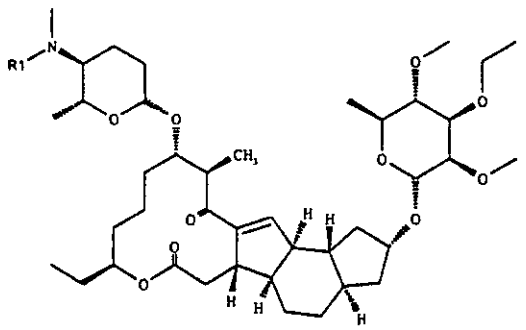
Dow AgroSciences

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

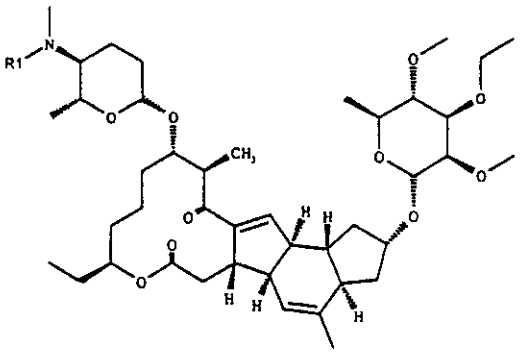
M. J. Hastings

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:

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

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

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

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

10.2.3. 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:

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

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

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

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:

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

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

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

10.5. Determination of Soil Moisture

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

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

10.5.3. 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.

10.5.4. 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

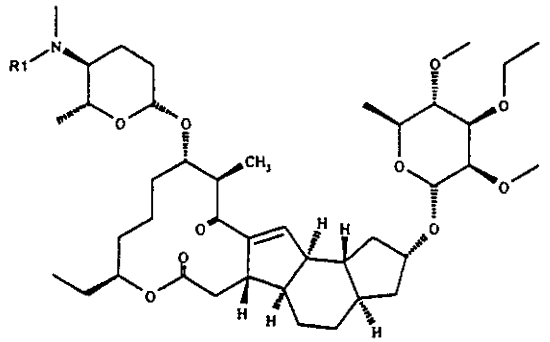
10.6.1. Determine the analyte concentrations in the sample as described in Section 10.4.

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

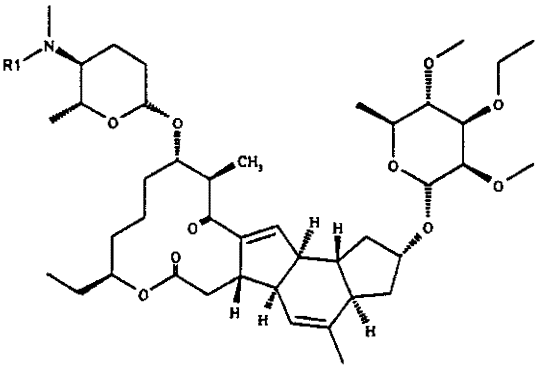
10.6.3. Determine the dry weight analyte concentrations in the samples as follows:

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

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



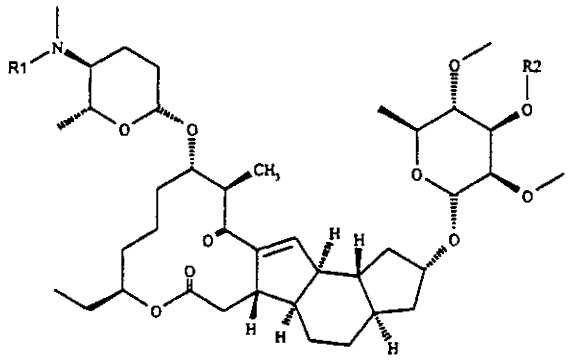
XDE-175-L, R1 = CH_3
XDE-175-*N*-Demethyl-L, R1 = H

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)

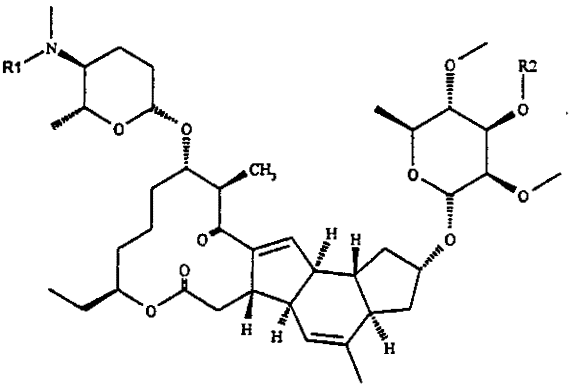
Table 1. (Cont.) Identity and Structure of XDE-175, its Metabolites and Stable Isotope Internal Standards

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