Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Spinosad (XDE-105)

MRID #: 440451-03

Matrix: Soil

Analysis: HPLC/UV

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Determination of XDE-105 and Metabolites in Soil and Sediment by High Performance Liquid Chromatography with Ultraviolet Detection

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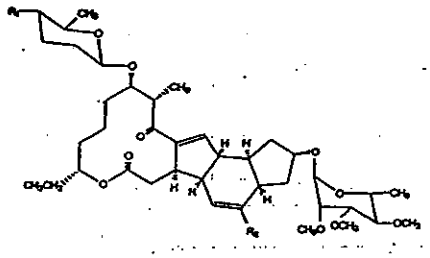
A. Scope

This method is applicable for the quantitative determination of residues of the XDE-105 and its metabolites in soil and sediment. The method determines the active ingredients in XDE-105 (factors A and D) and two degradation products (factors B and "B of D"). The method has been validated over the concentration range of 0.01-1.0 µg/g with a validated limit of quantitation of 0.01 µg/g.



XDE-105 factor A (compound number 232105), $R_1 = N(CH_3)_2$ and $R_2 = H$
XDE-105 factor D (compound number 275043), $R_1 = N(CH_3)_2$ and $R_2 = CH_3$
XDE-105 factor B (compound number 210984), $R_1 = NH(CH_3)$ and $R_2 = H$
XDE-105 factor B of D (compound number 202149), $R_1 = NH(CH_3)$ and $R_2 = CH_3$

The chemical names for these four factors are presented in Table I.

B. Principle

Residues of XDE-105 factors A, D, B, and "B of D" are extracted from soil or sediment with methanol/5% sodium chloride/1 N sodium hydroxide (65:27:8). An aliquot of the extract is purified by aqueous-organic partitioning and silica solid phase extraction (SPE). All four analytes are separated and determined simultaneously by reversed-phase high performance liquid chromatography with ultraviolet (UV) detection at 250 nm.

C. Safety Precautions

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-DowElanco products should be obtained from the container label or from the supplier. Disposal of reagents and solvents must be in compliance with local, state, and federal laws and regulations.
2. Flammable and/or volatile solvents such as acetone, acetonitrile, hexane, methanol, and methylene chloride should be used in well-ventilated areas away from ignition sources.
3. Concentrated hydrochloric acid is corrosive, and sodium hydroxide is caustic. When working with these reagents, proper eye protection, long laboratory coats, and rubber gloves must be worn.
4. Erlenmeyer flasks under vacuum are susceptible to implosion. Use polypropylene flasks or glass flasks covered with electrical tape. Evaporations under vacuum must be conducted behind appropriate shields while wearing eye protection.

D. Equipment (Note L.1.)

1. Balance, analytical, Model AE-160, Mettler Instrument Corporation, Hightstown, NJ 08520.
2. Balance, toploading, Model P-1200 or BB2240, Mettler Instrument Corporation.
3. Centrifuge, Model CU-5000, International Equipment Company, Needham Heights, Massachusetts 02194.
4. Cleaner, ultrasonic, Bransonic Model 8210, Branson Ultrasonics Corporation, Danbury, CT 06810.
5. Filtration apparatus for HPLC solvents, catalog number 5-8061M, Supelco, Inc., Bellefonte, PA 16823.
6. High performance liquid chromatograph, Model 1050, with a UV detector, and a recording integrator, Model 3396 Series II, Hewlett-Packard, Wilmington, DE 19808.
7. Rotary vacuum evaporator, Model 1007-4 IN, Rinco Instrument Company, Inc., Greenville, IL 62246.
8. Sep-Pak cartridge rack and reservoirs, part number 22030, Waters, Milford, MA 01757.
9. Shaker, gyratory, New Brunswick Model G-33, Fisher Scientific, Pittsburgh, Pennsylvania 15219.

10. Water bath, catalog number 15-458-30A, Fisher Scientific.
11. Water purifier, Milli-Q UV Plus, Millipore Corporation, Milford, MA 01730.

E. Glassware and Materials (Notes L.1. and L.2.b.)

1. Applicators, wooden, 12 in x 1/12 in, catalog number 805, Hardwood Products Company, Guilford, ME 04443.
2. Bottles, glass, 8-ounce (237-mL), catalog number 03-320-10D, Fisher Scientific.
3. Caps, TFE-lined, 58-mm, catalog number 03-321-11B, Fisher Scientific.
4. Cartridges, Sep-Pak, silica, part number 51900, Waters.
5. Column, C18/ Cation, Mixed Mode, 5 µm, 150 mm x 4.6 mm i.d., catalog number 72575, Alltech/Applied Science, Deerfield, IL 60015.
6. Column, ODS-AQ, catalog number AQ-302-5, 5 µm, 150 mm x 4.6 mm i.d., YMC, Inc., Wilmington, NC 28403.
7. Culture tubes, borosilicate glass, 13 mm x 100 mm, disposable, catalog number 14-961-27, Fisher Scientific.
8. Filters, membrane, Nylon-66, 47-mm i.d., 0.45 µm pore size, catalog number 5-8067M, Supelco, Inc.
9. Glass wool, catalog number 11-390, Fisher Scientific:

   Purify by submerging approximately 100 g of glass wool in 400 mL of methanol for at least 5 minutes. Vacuum filter to remove the methanol. Repeat the procedure with 400 mL of methylene chloride. Dry in a hood for approximately 2 hours (Note L.2.c.).
10. pH test paper, pHydrion Insta-Chek 0-13, catalog number 14-850-1, Fisher Scientific.
11. Pipets, Pasteur, 9-inch, catalog number 13-678-7C, Fisher Scientific.

F. Reagents and Chemicals (Note L.1 and L.2.)

1. Reagents

   a. Acetone, OmniSolv, catalog number AX0116-1, EM Science, Gibbstown, NJ 08207.
   b. Acetonitrile, ChromAR HPLC grade, catalog number 2856-09, Mallinkrodt Specialty Chemicals Company, Paris, KY 40361.
   c. Ammonium acetate, HPLC grade, catalog number A639-500, Fisher Scientific.
   d. Hexane, OmniSolv, catalog number HX0296-1, EM Science.
   e. Hydrochloric acid, concentrated (approximately 12 N), Reagent ACS, catalog number A144S-500, Fisher Scientific.
   f. Hydrochloric acid solution, 1.0 N, Certified, catalog number SA48-500, Fisher Scientific.
   g. Methanol, ChromAR HPLC grade, catalog number 3041-09, Mallinkrodt Specialty Chemicals Company.

h. Methylene chloride (dichloromethane), Optima grade, catalog number DX0831-1, Fisher Scientific.

i. Sodium chloride crystals, catalog number SX0420-5, EM Science, Cherry Hills, NJ 08034.

j. Sodium hydroxide pellets, certified ACS, catalog number S-318, Fisher Scientific.

k. Sodium sulfate, anhydrous granular, catalog number S421-3, Fisher Scientific:

Purify by rinsing approximately 800 g of sodium sulfate with approximately 1000 mL of hexane. Vacuum filter to remove hexane, and then dry the sodium sulfate in a hood for approximately 15 minutes with occasional stirring. (Note L.2.c.)

l. Standards

Obtain pure active ingredients or reference compounds for factors A, D, B, and "B of D" from Test Substance Coordinator, DowElanco, 9330 Zionsville Road, Building 306/A1, Indianapolis, IN 46268-1053.

m. Water, ultra-pure, purified using Milli-Q UV Plus.

2. Prepared Solutions

a. 2% ammonium acetate/acetonitrile (67:33) (v/v).

Dissolve 20.0 g of ammonium acetate per liter of ultra-pure water. For each liter of the 67:33 solution, mix 670 mL of 2% ammonium acetate and 330 mL of acetonitrile. Filter through a 0.45-µm membrane.

b. 0.16 N hydrochloric acid/5% sodium chloride (v/v):

Dissolve 50 g of sodium chloride per liter of ultra-pure water. Carefully add 13.4 mL of concentrated hydrochloric acid per liter of 5% sodium chloride solution. (See Safety Precaution C.3.)

c. 50% methanol/50% acetonitrile (v/v).

Prepare by mixing 500 mL of methanol and 500 mL of acetonitrile for each liter of solution.

d. Methanol/acetonitrile/2% ammonium acetate (1:1:1) (v/v/v).

Mix equal volumes of each.

e. Methanol/5% sodium chloride/1 N sodium hydroxide (65:27:8) (v/v/v):

Dissolve 50 g of sodium chloride per liter of ultra-pure water. Dissolve 40 g of sodium hydroxide pellets per liter of ultra-pure water. (See Safety Precaution C.3.) For each liter of the final solution, add 650 mL of methanol, 270 mL of 5% sodium chloride, and 80 mL of 1 N sodium hydroxide.

f. 75% methylene chloride/25% methanol (v/v).

Mix 750 mL of methylene chloride and 250 mL of methanol for each liter of solution.

g. Sodium hydroxide, 1.0 N.

For each liter of solution, dissolve 40 g of sodium hydroxide pellets in ultra-pure water and then dilute to volume with ultra-pure water.

G. Preparation of Standards (Note L.3.)

1. Preparation of XDE-105 Spiking Solutions

a. For each analyte (factors A, D, B, and B of D), weigh 10.0 mg of the pure active ingredient or reference compound (corrected for purity). Quantitatively transfer each one to separate 100-mL volumetric flasks and dissolve in 50% methanol/50% acetonitrile. Mix or shake until the solids completely dissolve. Dilute to volume to obtain stock solutions containing 100.0 µg/mL.

b. Prepare a mixture containing 20.0 µg/mL of the four analytes by diluting 20-mL aliquots of each of the 100.0-µg/mL stock solutions to volume with 50% methanol/50% acetonitrile in a 100-mL volumetric flask.

c. Dilute appropriate aliquots of the above 20.0-µg/mL solution to volume with 50% methanol/50% acetonitrile in 100-mL volumetric flasks to obtain the desired concentrations for the fortification of recovery samples as shown in the table below.

Aliquot of Stock Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]
mL	mL	µg/mL	µg/g
1.0	100	0.2	0.010
2.5	100	0.5	0.025
5.0	100	1.0	0.050
7.5	100	1.5	0.075
10.0	100	2.0	0.100

[a] The equivalent sample concentration is based on fortifying a 20-g sample with 1.0 mL of spiking solution.

2. Preparation of XDE-105 Calibration Solutions

a. Combine 10.0-mL aliquots of each 100.0-µg/mL stock solution from Step G.1.a. in a 100-mL volumetric flask and dilute to volume with methanol/acetonitrile/2% ammonium acetate (1:1:1) to obtain a solution containing 10.0 µg/mL of each factor.

b. Dilute aliquots of the above 10.0-µg/mL solution to volume with methanol/acetonitrile/2% ammonium acetate (1:1:1) in 100-mL volumetric flasks to obtain the following calibration standards:

Aliquot of Stock Soln.	Final Solution Volume	Final Solution Concentration
mL	mL	µg/mL
0.0	100	0.000
1.0	100	0.100
5.0	100	0.500
10.0	100	1.000
15.0	100	1.500

H. High Performance Liquid Chromatography

1. Typical Operating Conditions (Note L.4.)

Instrumentation:	Hewlett-Packard Model 1050 with a UV detector, an autosampler capable of injecting at least 175 µL, and a recording integrator
Column:	YMC ODS-AQ 5 µm 150 mm x 4.6 mm i.d.
Column (Oven) Temp.:	30 °C
Mobile Phase:	44% reservoir A/44% reservoir B/12% reservoir C reservoir A = methanol reservoir B = acetonitrile reservoir C = 2% ammonium acetate/acetonitrile (67:33)
Flow Rate:	0.8 mL/min
Injection Volume:	175 µL
Detector:	Ultraviolet, 250 nm
Attenuation:	2^3 or 8 (adjust to yield a peak response of approximately 25%-35% of full-scale deflection for the 0.5 µg/mL standard)
Chart Speed:	0.2 cm/min
Run Time:	20 minutes per sample (Longer time may be used if late-eluting peaks occur)

UV spectra for factors A, B, and D are shown in Figure 1.

2. Calibration Curves

A typical calibration curve for the determination of XDE-105 factor A is shown in Figure 2. Calibration curves for the other factors are similar to that for factor A.

3. Column and Typical Conditions for Confirmation of XDE-105 Residue (Note L.4.)

Column:	C18/Cation Mixed Mode 5 µm 150 mm x 4.6 mm i.d.
Mobile Phase:	40% reservoir A/40% reservoir B/20% reservoir C Reservoir A = methanol Reservoir B = acetonitrile Reservoir C = 2% ammonium acetate/acetonitrile (67:33)
Flow Rate:	1.0 mL/min
UV Wavelength:	250 nm, 235 nm, or 275 nm (Step L3.)
Other Parameters:	Same as in Step H.1.

4. Typical Chromatograms

Typical chromatograms obtained under the conditions in Step H.1. are illustrated in Figures 3-5. Typical chromatograms for the confirmation of XDE-105 residues under the conditions in Step H.3. are shown in Figure 6. These chromatograms are typical in that none of the control samples in the method validation study resulted in background interference peaks in the chromatograms at the retention times of the analytes.

I. Determination of Recovery of XDE-105 from Soil or Sediment

1. Preparation of Recovery Samples

a. Weigh out a 20-g sample of previously homogenized control soil or sediment into an 8-oz. bottle. For laboratory recovery samples, add 1.0 mL of the appropriate fortification solution(s) from Step G.1.

b. Add 60 mL of methanol/5% sodium chloride/1 N sodium hydroxide (65:27:8). If necessary, use a spatula to thoroughly break up clumps of sediment or soil so that the particles will readily suspend in the extracting solvent. Seal the jar with a TFE-lined cap.

c. Sonicate the sample in an ultrasonic cleaner for approximately 5 minutes. Shake the sample for approximately 30 minutes on a gyratory shaker at approximately 275 rpm.

d. Centrifuge the sample at approximately 2250 rpm for approximately 10 minutes.

e. Decant the supernatant liquid into a 250-mL graduated cylinder. Using aluminum foil or paper towels, protect the solution in the graduated cylinder from light during Step I.1.f. (Note L.5.)

f. Repeat Steps I.1.b. to I.1.e. one additional time.

g. Dilute the sample extract in the graduated cylinder to 130 mL with methanol/5% sodium chloride/1 N sodium hydroxide (65:27:8). Stir or shake briefly to mix the solution. Transfer one-half of the sample extract (65 mL) to a 250-mL separatory funnel. Add 65 mL of an acidic salt solution containing

0.16 N hydrochloric acid in 5% sodium chloride. Check with pH paper to ensure that the pH of the aqueous phase is approximately 2. If necessary, add a sufficient volume of 1.0 N hydrochloric acid to adjust the pH to approximately 2.

h. Add 50 mL of hexane to the separatory funnel. (Note L.5.) Shake the separatory funnel vigorously for approximately 20-25 seconds. Wait for approximately 2 minutes for the layers to separate. If needed, use a glass stirring rod or a wooden applicator stick to aid the separation of the layers. Using a 250-mL beaker, drain the aqueous (lower) layer nearly down to the slight emulsion, leaving the emulsion and approximately 1 mL of the aqueous phase in the separatory funnel. Wait for approximately 2 minutes for the layers to further separate, then drain the remaining aqueous layer into the beaker, leaving the emulsion in the separatory funnel. If necessary, use an applicator stick or reshake the separatory funnel briefly to dislodge the emulsion from the glass, then drain out and discard the hexane (upper) layer, including the slight emulsion.

i. Return the aqueous phase to the separatory funnel. Add 10.0 mL of 1.0 N sodium hydroxide solution. Shake the separatory funnel briefly, and check with pH paper to ensure that the pH is approximately 10-12. If necessary, add additional 1.0 N sodium hydroxide to adjust the pH to approximately 10-12.

j. Add 50 mL of hexane, and shake the separatory funnel vigorously for approximately 20-25 seconds. Wait for approximately 2 minutes for the layers to separate. If needed, use a glass stirring rod or a wooden applicator stick to aid the separation of the layers. Using a 250-mL beaker, drain the aqueous (lower) layer nearly down to the slight emulsion, leaving the emulsion and approximately 1 mL of the aqueous phase in the separatory funnel. Wait for approximately 2 minutes for the layers to further separate, then drain the remaining aqueous layer, the slight emulsion, and approximately 1 mL of the hexane layer into the beaker. Drain the remaining hexane through a funnel containing a small plug of hexane-washed glass wool and approximately 10 mL (approximately 16.5 g) of hexane-washed sodium sulfate into a 500-mL boiling flask. (Note L.6.)

k. Return the aqueous phase to the separatory funnel, and repeat Step L.1.j. two more times (for a total of three times), combining the three hexane extracts in the same 500-mL boiling flask. Rinse the sodium sulfate with 15 mL of hexane.

l. Prior to concentrating the extract, turn on the vacuum and rinse the rotary vacuum evaporator with hexane, followed by methanol, to prevent sample contamination. Evaporate the hexane extract to dryness with the rotary vacuum evaporator and a water bath heated to approximately 35-50 °C.

m. Before using a new lot of silica SPE cartridges, determine the elution profile as described in Section K.4. If the elution profile differs from that described below, modify the volumes of acetonitrile to be discarded in Step L.1.m.(5) or the volume of 75% methylene chloride/25% methanol to be collected in Steps L.1.m.(6) and L.1.m.(7).

(1) Dissolve the residue from Step L.1.l. in 10 mL of hexane.

(2) Position the SPE rack for discarding the eluate in Steps L.1.i.(3) through L.1.i.(5). Use full vacuum to result in a stream of the eluting solvents for Steps L.1.i.(3) to L.1.i.(5), but reduce the vacuum for a dropwise elution in Steps L.1.i.(6) and L.1.i. (7). In all steps, turn off the vacuum between each solvent addition, and wait until the previous solution has eluted before adding the next solution. Although it is not necessary to dry the cartridges between

solvent additions, the cartridges may be allowed to dry without affecting the results.

(3) Prior to adding the sample, condition the cartridge under vacuum using the following sequence of eluants: 10 mL of 75% methylene chloride/ 25% methanol, then 10 mL of acetonitrile, followed by 10 mL of methylene chloride, and 20 mL of hexane.

(4) Add the sample from Step I.1.i.(1) in 10 mL of hexane. After the hexane has eluted, rinse the evaporating flask with 10 mL of hexane, add the hexane to the cartridge, and elute the solvent. Repeat with an additional 10 mL of hexane. Rinse the evaporating flask with an additional 40 mL of hexane, add the solvent to the cartridge, and elute.

(5) Rinse the evaporating flask with two 5-mL aliquots of methylene chloride, add both rinses separately to the SPE cartridge, and elute. Rinse the flask with two 4-mL aliquots of acetonitrile, add both rinses separately to the cartridge, and elute.

(6) After the second 4-mL acetonitrile rinse has eluted, position the cartridge rack for solvent collection. Rinse the evaporating flask with 8 mL of 75% methylene chloride/25% methanol and add the solvent to the cartridge. Collect the eluate in a clean culture tube (100 mm x 13 mm) using dropwise elution to prevent the sample solution from bubbling out of the collection tube.

(7) Rinse the evaporating flask with two additional 8-mL volumes of 75% methylene chloride/25% methanol, add them separately to the SPE cartridge, and collect them in separate culture tubes.

(8) Combine the eluate from the three culture tubes in a 125-mL boiling flask. Rinse the tubes with 4 mL of 75% methylene chloride/25% methanol, and add the solvent rinses to the boiling flask. Rinse the neck of the flask with 4 mL of 75% methylene chloride/25% methanol.

(9) Prior to concentrating the samples, turn on the vacuum and rinse the rotary vacuum evaporators with hexane and then methanol. Evaporate the eluate in the boiling flask to dryness using rotary vacuum evaporation and a water bath heated to approximately 35-50 °C.

n. Dissolve the residue in 1.0 mL of methanol/acetonitrile/2% ammonium acetate (1:1:1). Cap the flask. Swirl to thoroughly dissolve the residue on the bottom of the flask, then tilt the flask to a horizontal position and slowly rotate so that the solvent dissolves the residue on the sides of the flask. (Note L.7.)

o. Using a disposable Pasteur pipet, transfer the solution to an HPLC sample vial and cap the vial with a crimper. Do not filter the final solution. (Note L.2.d.)

p. Analyze the sample and standard solutions by HPLC as described in Section H. Determine the suitability of the chromatographic system using the following performance criteria:

(1) Standard curve linearity: Determine that the correlation coefficient (r^2) equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration.

(2) Peak resolution: Determine that baseline resolution has been achieved for all four XDE-105 factors.

(3) Appearance of chromatograms: Determine that the chromatograms resemble those shown in Figures 3-5 in terms of peak response, baseline noise, and background interference. A signal-to-noise ratio of approximately 5:1 to 10:1 should be attainable for the 0.1-µg/mL standard calibration solution.

q. If the peak response for any of the samples exceeds the range of the standard calibration curve, dilute the samples with methanol/acetonitrile/2% ammonium acetate (1:1:1) to yield a response within the range of the calibration curve.

2. Calculation of Percent Recovery

a. Inject the series of calibration standards described in Section G.2.b. and determine the peak responses for all four XDE-105 factors.

b. Prepare separate standard curves for all four factors by plotting the concentrations on the abscissa (x-axis) and the resulting peak responses on the ordinate (y-axis) as shown in Figure 2. Using regression analysis, determine the equation for the curve with respect to the abscissa for each analyte.

For example, the general equation for calculating the least squares line for the standard calibration curve is as follows:

$$PR = mC + b$$

where C is the concentration (µg/mL) of the analyte in the final solution, PR is the peak response, m is the slope of the line, and b is the y-axis intercept. Rearranging the above equation, the concentration (C) of the analyte in the final solution can be calculated from the standard curve as:

$$C = \frac{(PR - b)}{m}$$

For example, the following equation results from a least squares regression analysis with the data in Figure 2:

$$C = \frac{[PR - (-0.01499)]}{8.04030}$$

c. Determine the concentration (µg/mL) in the final solution of the recovery samples. For example, using the peak height responses in Figure 3(D) for factor A in the 0.01-µg/g recovery sample (0.6 cm):

$$C\,(\text{recovery}) = \frac{[0.6 - (-0.01499)]}{8.04030} = 0.076\ \mu g/mL$$

d. Determine the residue concentration (µg/g) of the analyte in the fortified recovery sample as follows:

$$\mu g/g = \frac{C \times AF \times V}{W}$$

where AF is the aliquot factor (normally 2.0 because one-half of the sample extract is purified for analysis), V is the final volume of the sample extract (normally 1.0 mL unless further diluted), and W is the weight of the sample (normally 20 g).

For the example above, the µg/g concentration of factor A is calculated as:

$$\mu g/g \text{ (recovery)} = \frac{0.076\ \mu g/mL \times 2.0 \times 1.0\ mL}{20\ g} = 0.0076\ \mu g/g$$

e. Calculate the net concentration (µg/g) in the recovery sample as follows:

Net concentration = µg/g (recovery) - µg/g (control)

Because the corresponding control in Figure 3(B) contained no detectable residue, the net concentration of factor A in the recovery is the same as the gross concentration (i.e., 0.0076 µg/g).

f. Determine the net percent recovery (R) by dividing the net concentration (µg/g) by the theoretical concentration (µg/g) that was added:

$$R = \frac{\text{Concentration Found}}{\text{Concentration Added}} \times 100\%$$

For the above example:

$$R = \frac{0.0076\ \mu g/g}{0.01\ \mu g/g} \times 100\%$$

$$R = 76\%$$

If desired, the average percentage recovery for all of the recovery samples may be used to correct for method efficiency.

3. Confirmation of Residue Identity

a. If necessary, confirm the identity of XDE-105 by analyzing the same final solution (from Step I.1.o.) under the different chromatographic conditions specified in Step H.3.

b. For maximum sensitivity, maintain the UV wavelength at 250 nm and utilize the alternative column and mobile phase conditions listed in Step H.3. Compare the resulting retention times of the analytes in the samples with those of the standards. Also, compare the resulting concentrations of the analytes in the samples with those obtained using the conditions in Step H.1. to determine if they are similar (i.e., within approximately 20 percent.)

c. To utilize different wavelengths, inject the standard and sample solutions with the UV wavelength set at 235 nm. Repeat at 275 nm. Compare the resulting concentrations with those obtained at 250 nm (Step H.1.) to determine if they agree within approximately ± 20%. However, the absorbance of the analytes will be decreased at 235 nm or 275 nm (Figure 1).

J. Determination of XDE-105 and Metabolites in Soil or Sediment

1. Prepare reagent blank, control, recovery, and treated samples as described in Section I.1.

2. Prepare separate standard calibration curves for all four analytes and determine the percentage recovery for each analyte as described in Section I.2.

3. Determine the concentration (µg/mL) for each analyte from the appropriate calibration curve, and calculate the uncorrected residue result. For example, using the same data from the same sample in section I.2., the uncorrected residue is calculated as:

$$\text{Uncorrected Result (µg/g)} = \frac{\text{µg/mL (from std. curve)} \times AF \times V}{W}$$

$$\text{Uncorrected Result (µg/g)} = \frac{0.076 \text{ µg/mL} \times 2.0 \times 1.0 \text{ mL}}{20 \text{ g}} = 0.0076 \text{ µg/g}$$

4. For those analyses that require a correction for moisture content of the sample, weigh 10.0 g of the soil or sediment sample into an aluminum or glass container. Place the sample in an oven at approximately 130 °C and allow it to dry for a minimum of 16 hours. Remove the sample from the oven, place it in a desiccator until the sample has cooled to ambient temperature, and then re-weigh. Calculate the percent moisture on a dry-weight basis as follows:

$$\text{Percent Moisture} = \frac{\text{soil moisture weight (g)}}{\text{dehydrated soil weight (g)}} \times 100$$

$$= \frac{\left(\begin{matrix}\text{soil weight} \\ \text{before drying}\end{matrix} - \begin{matrix}\text{soil weight} \\ \text{after drying}\end{matrix}\right)}{\begin{matrix}\text{soil weight} \\ \text{after drying}\end{matrix}} \times 100$$

Then determine the dry-weight concentration of the analytes in soil or sediment samples as follows:

$$\text{Dry-Wt. Conc. (µg/g)} = \text{Uncorr. Result (µg/g)} \times [1 + (\% \text{ Moisture}/100)]$$

5. For those analyses that require correction for method recovery, use the following procedure:

   a. Determine the XDE-105 concentrations in the soil or sediment samples as described in Section J.3. (and J.4., if desired).

   b. Determine the corrected analyte concentration in the soil or sediment samples as follows:

$$\text{Corrected Result } (\mu g/g) = \text{Uncorrected Result } (\mu g/g) \times \frac{100\%}{R_a}$$

   where the uncorrected result can be on either a wet-weight or dry-weight basis, and R_a is the average net % recovery from fortified samples (Section I.2.)

   For the example given in Step I.2., the residue corrected for recovery is calculated as:

$$\text{Corrected Result } (\mu g/g) = 0.0076\ \mu g/g \times \frac{100\%}{76\%}$$

$$\text{Corrected Result } (\mu g/g) = 0.01\ \mu g/g$$

K. Results and Discussion

1. Method Validation

   a. Recovery Levels and Precision

   A method validation study was conducted to determine the recovery levels and the precision of the method for the four analytes in two types of soil (sandy loam and clay loam) and in pond sediment. The individual results are summarized in Tables II-V, with a statistical summary in Table VI. The following recovery values (mean ± one standard deviation) resulted from the method when soil and sediment samples were fortified over the concentration range of 0.01 to 1.0 µg/g (Table VI):

Factor A:	82 ± 5%
Factor D:	83 ± 6%
Factor B:	78 ± 6%
Factor B of D:	76 ± 6%

   The relative standard deviation (RSD) ranged from 2% to 11% for all four analytes at all fortification levels (Table VI). Typical chromatograms demonstrating the determination of XDE-105 in sandy loam soil, clay loam soil, and pond sediment are illustrated in Figures 3-5.

   b. Standard Curve Linearity

   The average correlation coefficient (r^2) for the least squares regression equations describing the detector response as a function of the standard calibration curve concentration was 0.9998-0.9999 for all four analytes.

c. Calculated Limits of Quantitation and Detection

Following established guidelines (1), the limits of detection (LOD) and quantitation (LOQ) were calculated using the standard deviation of the net results from the 0.01-µg/g recovery samples in Tables II-V. The LOD was calculated as three times the standard deviation (3s), and the LOQ was calculated as ten times the standard deviation (10s) of the net results from the analysis of 14 samples. The results are summarized in Table VII.

For all four analytes, the calculated LOD (0.001-0.003 µg/g) and the calculated LOQ (0.005-0.009 µg/g) supported the targeted limits of detection (0.003 µg/g) and quantitation (0.010 µg/g). Results should not be quantified at levels below the validated limit of quantitation, but should instead be reported as less than the validated LOQ (e.g., <0.01 µg/g).

d. Extraction Efficiency

To determine the extraction efficiency of the method for aged residues of the analytes in pond sediment, an untreated control sample was fortified with 0.2 µg/g each of ^{14}C XDE-105 factors A, B, and D. (Radiolabeled factor "B of D" was not available.) The sample was aged for 34 days in a freezer and then analyzed by both HPLC-UV and radiochemical analysis. The two analytical techniques gave nearly identical results, which ranged from 90%-100% of theory for all three analytes. For factor A, the results were 0.18 µg/g for both the HPLC-UV and radiochemical analyses. For factor B, the results were 0.20 µg/g with both techniques; and for factor D, the results were 0.19 µg/g by HPLC and 0.18 µg/g by radiochemical analysis.

e. Chemical and Physical Properties of Soils

The method validation was conducted using two different types of terrestrial soil (sandy loam and clay loam) and a pond sediment. The chemical and physical properties of these soils are summarized in Table VIII.

2. Confirmation of XDE-105 Residues

Confirmation of XDE-105 residues is described in Section I.3. If the retention times of the analytes in the samples do not match those of the standard when using the different column and mobile phase, consider the residue to be due to compounds other than XDE-105. If the retention times match, but significantly different concentrations are obtained using the primary and confirmatory conditions, consider the detected residue to be due at least in part to interfering compounds and not to XDE-105.

Typical chromatograms demonstrating the confirmation of XDE-105 in pond sediment are illustrated in Figure 6. (Note that the alternative conditions utilized for confirmation result in a different order of elution for the four analytes compared to the primary conditions.) If additional confirmation is required beyond that discussed in this method, an alternative detection system such as HPLC-mass spectrometry or immunoassay (2) might be required.

3. Assay Time

A typical analytical run consists of a reagent blank, an untreated control soil or sediment sample, a minimum of 2 fortified controls for determination of recovery, and 10-12 samples. This analytical run can typically be prepared for HPLC analysis in an 8-hour working day, with the chromatographic analysis occurring overnight and calculations occurring the following day. The total person-hours required is typically 8-10 hours.

There are three acceptable stopping points in the method, where sample preparation (Section I) may be suspended without deleterious effects on the sample analysis. These are indicated below:

a. Step I.1.k. The assay may be stopped after the third partitioning with hexane. The samples should be protected from light.

b. Step I.1.m.(1). The analysis may be stopped with the samples dissolved in hexane prior to the silica SPE procedure if the boiling flasks are capped and the samples are protected from light.

c. Step I.1.n. The samples are stable in final solution for several days if protected from light. For extended storage, the samples should be refrigerated or frozen.

4. Standardization of SPE Elution Profile

Variation in the silica SPE cartridges can influence the elution profile of XDE-105. It is necessary to obtain an elution profile for each lot of SPE cartridges used to ensure optimum recovery and clean-up efficiency. The following procedures can be used:

a. Prepare a silica SPE cartridge as described in Steps I.1.m.(2) to I.1.m.(3).

b. Transfer 1.0 mL of the 2.0-µg/mL spiking standard solution (Step G.1.c.) to a 125-mL boiling flask and evaporate to dryness using a rotary vacuum evaporator. Dissolve the sample in 10 mL of hexane.

c. Add the sample to the SPE cartridge. Rinse the evaporating flask with two separate 10-mL portions of hexane and add both hexane rinses separately to the SPE cartridge.

Rinse the boiling flask with an additional 40 mL of hexane, add the solvent to the cartridge, and discard all of the solvent that has eluted thus far.

d. Rinse the evaporating flask with two separate 5-mL aliquots of methylene chloride, add them separately to the cartridge, and discard the eluate.

e. Rinse the evaporating flask with two separate 4-mL aliquots of acetonitrile, add them separately to the cartridge, and collect both of the eluates in a culture tube. Transfer the acetonitrile to a 125-mL boiling flask. Rinse the tube with 4 mL of acetonitrile, and add the rinse to the boiling flask.

f. Add at least eight 4-mL volumes of 75% methylene chloride/25% methanol to the SPE cartridge and collect each 4-mL fraction in a separate culture tube using dropwise elution.

g. Proceed as described in Steps I.1.m.(8) through I.1.q.

h. Calculate separate percentage recoveries for all four analytes as described in Step I.2.

i. If the elution pattern for XDE-105 differs from that described in Section I.1., adjust the volume of acetonitrile rinse to be discarded (Step I.1.m.(5)) or the volume of 75% methylene chloride/25% methanol to be collected (Step I.1.m.(6) and (7)).

A typical elution profile is illustrated in Figure 7.

L. Notes

1. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are, therefore, not listed.

2. Because it is necessary to use a nonselective UV wavelength (250 nm) to obtain adequate sensitivity, certain precautions must be taken to avoid interferences that can result from the reagents or equipment. When following the procedures as described, interferences greater than the LOD have not occurred in the chromatograms of reagent blank samples. However, if interferences occur, individual reagents and chemicals must be tested for purity by treating them as they are used in the procedure and then analyzing the resulting solutions by HPLC to isolate the source(s) of interferences. Those reagents or equipment found to be a source of interference must be suitably purified or replaced.

   Some sources of potential interferences have been previously identified, and the following recommendations should be implemented:

   a. Thoroughly rinse the rotary vacuum evaporators as described to prevent contamination of the samples.

   b. Shortly after using, rinse glassware with water before machine washing. Thoroughly rinse detergent residues from the glassware with water and acetone before drying. Rinse the glassware with acetone again prior to use.

   c. To prevent interferences, prepare the glass wool and sodium sulfate as described.

   d. Do not filter the final solution through 0.45-µm filters prior to injection into the HPLC unless it has been demonstrated that the filters do not produce interferences under the HPLC conditions specified. (Failure to filter the final solutions has not produced a noticeable chromatographic problem after several months of daily operation.)

3. If desired, prepare standard solutions at other concentrations by making appropriate dilutions.

4. The typical HPLC conditions may be modified as needed to obtain optimum performance.

5. XDE-105 is subject to aqueous photolysis in the presence of some photosensitizers. As a precaution, it is recommended that low laboratory lighting be used before and during the partitioning process (e.g., turn off the lights in the hood).

6. The use of sodium sulfate is necessary to remove traces of water that will alter the elution profile on the silica SPE cartridge. However, use only a minimal amount of sodium sulfate (i.e., 10 mL), because some lots of sodium sulfate have been observed to strongly adsorb the analytes and significantly reduce recovery.

7. XDE-105 adsorbs strongly to glass. Redissolving the residue from the glass is aided by the ammonium acetate and by the technique described.

M. References

1. Keith, L. H.; Crummett, W. B.; Deegan, J.; Libby, R. A.; Taylor, J. T.; Wentler, G., "Principles of Environmental Analysis", *Anal. Chem.*, 1983, 55, 2210-2218.

2. Mihaliak, C. A.; Young, D. L., "Determination of Residues of XDE-105 in Sediment Using a Magnetic Particle-Based Immunoassay Test Kit", GRM 94.21, unpublished method of DowElanco.