Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Spinosad

MRID #: 440451-05

Matrix: Water

Analysis: HPLC/UV

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

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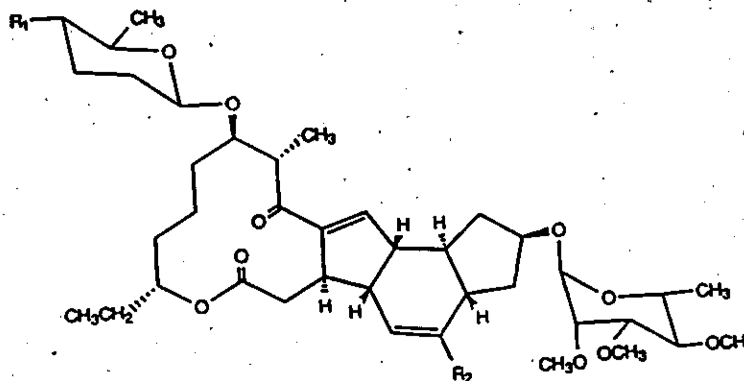
A. Scope

This method is applicable for the quantitative determination of residues of the insecticide XDE-105 and its metabolites in water. 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 0.001-0.1 µg/mL with a validated limit of quantitation of 0.001 µg/mL.



XDE-105
factor A (compound number 232105), $R_1 = N(CH_3)_2$ and $R_2 = H$
factor D (compound number 275043), $R_1 = N(CH_3)_2$ and $R_2 = CH_3$
factor B (compound number 210984), $R_1 = NH(CH_3)$ and $R_2 = H$
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 water by partitioning into methylene chloride. An optional cleanup of the sample extracts may be obtained with 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 sodium hydroxide is corrosive. It is imperative that proper eye and personal protection equipment be worn when handling this reagent.
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. Filtration apparatus for HPLC solvents, catalog number 5-8061M, Supelco, Inc., Bellefonte, PA 16823.
4. High performance liquid chromatograph, Model 1050, with a UV detector, and a recording integrator, Model 33396 Series II, Hewlett-Packard, Wilmington, DE 19808.
5. Rotary vacuum evaporator, Model 1007-4 IN, Rinco Instrument Company, Inc., Greenville, IL 62246.
6. Sep-Pak cartridge rack and reservoirs, part number 22030, Waters, Milford, MA 01757.
7. Water bath, catalog number 15-458-30A, Fisher Scientific, Pittsburgh, PA 15219.
8. 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. Cartridges, Sep-Pak, silica, part number 51900, Waters.
3. Column, ODS-AQ, catalog number AQ-302-5, 5 µm, 150 mm x 4.6 mm i.d., YMC, Inc., Wilmington, NC 28403.
4. Column, C18/ Cation, Mixed Mode, 5 µm, 150 mm x 4.6 mm i.d., catalog number 72575, Alltech/Applied Science, Deerfield, IL 60015.
5. Culture tubes, borosilicate glass, 13 mm x 100 mm, disposable, catalog number 14-961-27, Fisher Scientific.
6. Filters, membrane, Nylon-66, 47-mm i.d., 0.45 µm pore size, catalog number 5-8067M, Supelco, Inc.
7. pH test paper, pHydrion Insta-Chek 0-13, catalog number 14-850-1, Fisher Scientific.
8. Pipets, Pasteur, 9 in, 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. Methanol, ChromAR HPLC grade, catalog number 3041-09, Mallinkrodt Specialty Chemicals Company.
   f. Methylene chloride (dichloromethane), OPTIMA HPLC grade, catalog number DX0831-1, Fisher Scientific.
   g. Sodium hydroxide pellets, certified ACS, catalog number S-318, Fisher Scientific.
   h. Standards.

      Obtain pure active ingredients or reference compounds for factors A, D, B, and "B of D" from Test Substance Coordinator, DowElanco, Indianapolis, IN 46268-1053.

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

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

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

Mix equal volumes of each.

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

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

e. 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. Dilute appropriate aliquots of the above 100.0 μg/mL stock solutions 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/mL
20.0	100	20.0	0.100
15.0	100	15.0	0.075
10.0	100	10.0	0.050
5.0	100	5.0	0.025
2.0	100	2.0	0.010
0.2	100	0.2	0.001

[a] The equivalent sample concentration is based on fortifying a 200-mL sample with 1.0 mL of spiking solution.

2. Preparation of XDE-105 Calibration Standards

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. mL	Final Solution Volume mL	Final Solution Concentration μg/mL
0.0	100	0.000
1.0	100	0.100
2.0	100	0.200
5.0	100	0.500
10.0	100	1.000

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 (Note L.5.)
Detector:	Ultraviolet, 250 nm
Attenuation:	2^3 or 8 (adjust to yield a peak response of approximately 30-60% 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 B is shown in Figure 2. Calibration curves for the other factors are similar to that for factor B.

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 I.3.)
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 3-6. Typical chromatograms for the confirmation of XDE-105 residues under the conditions in Step H.3. are shown in Figure 7. Chromatograms of samples containing trace background interferences (0.00006 µg/mL to 0.00016 µg/mL) are contained in Figures 3 and 4.

I. Determination of Recovery of XDE-105 from Water

1. Preparation of Recovery Samples

a. If the water samples have been frozen or stored under refrigeration, warm the samples to room temperature before beginning the analysis. Using a 250-mL graduated cylinder, measure out a 200-mL aliquot of an untreated control sample. (Notes L.5. and L.6.) Transfer the water sample to a 250-mL separatory funnel.

(If treated water samples have been collected or stored in glass containers, add 20 mL of methanol to the glass containers, cap the containers, and shake. Turn the containers to a horizontal position and slowly rotate to dissolve residues of XDE-105 that have adsorbed to the glass. Transfer the methanol rinse to the separatory funnel.) (Critical step-see Note L.7.)

b. Add 4.0 mL of 1.0 N sodium hydroxide solution and shake for approximately 5 seconds. Check the water sample with pH paper to ensure that the pH is approximately 12 or greater. If necessary, add additional 1.0 N sodium hydroxide to adjust the pH to approximately 12 or greater.

c. For laboratory recovery samples, add 1.0 mL of the 0.2 µg/mL spiking solution (from Step G.1.b.) in 50% methanol/50% acetonitrile to result in recovery samples containing 0.001 µg/mL of all four XDE-105 factors. (If other recovery levels are desired, add 1.0 mL of the appropriate spiking standard solution from Step G.1.b.)

d. Add 50 mL of methylene chloride and vigorously shake the separatory funnel for approximately 30 seconds. (If the water sample has been collected or stored in a glass container, add the methylene chloride to the container instead of the separatory funnel. Cap the container and shake. Turn the container to a horizontal position and slowly rotate to dissolve residues of XDE-105 that have adsorbed onto the glass. Transfer the methylene chloride rinse to the separatory funnel and shake for approximately 30 seconds.) (Critical step-See Note L.7.)

   Wait for the layers to separate for at least 5 minutes. If necessary, use a stirring rod or a wooden applicator stick to break the slight emulsion and to aid in the separation of the layers. Work under low light conditions (e.g., turn off the lights in the hood) during the partitioning procedure in Steps I.1.d. through I.i.f. (Note L.8.)

e. Drain the methylene chloride (lower) layer nearly down to the slight emulsion, collecting the methylene chloride in a 500-mL boiling flask. Retain the slight emulsion with the aqueous layer in the separatory funnel. Do not drain the methylene chloride through sodium sulfate to remove traces of water. (Note L.9.)

f. Repeat Steps d and e with two additional 50-mL aliquots of methylene chloride, combining the three methylene chloride extracts in the same flask.

g. Prior to evaporating the sample, turn on the vacuum and rinse the rotary vacuum evaporator with hexane, followed by methanol, to prevent sample contamination. Evaporate the dichloromethane extract to dryness with the rotary vacuum evaporator and a water bath heated to approximately 35-50 °C. If traces of water remain in the flask upon evaporation of the methylene chloride, add approximately 20 mL of methanol and repeat the evaporation. (Critical step–Note L.10.)

h. If the optional silica SPE cleanup is needed, proceed to Step I.1.i. If a silica SPE cleanup is not needed, proceed to Step I.1.j. (Note L.11.)

i. If desired, purify the sample extracts using the optional silica SPE procedure described below. Refer to Section K.4. for the standardization procedure for silica SPE cartridges.

   (1) Dissolve the residue from Step I.1.g. in 10 mL of hexane.

   (2) Attach a silica SPE cartridge reservoir to a silica SPE cartridge and attach the cartridge to the cartridge rack. Position the rack for discarding the eluate in Steps I.1.i.(3) through I.1.i.(5). Use full vacuum to result in a stream of the eluting solvents for Steps I.1.i.(3) to I.1.i.(5), but reduce the vacuum to result in a dropwise elution in Step I.1.i.(6). Turn off the vacuum between each solvent addition.

   (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. Wait until the previous solution has eluted before adding the next solution.

(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 using dropwise elution to prevent the sample solution from bubbling out of the collection tube.

(7) Transfer the eluate from the culture tube to a 125-mL boiling flask. Rinse the tube with 4 mL of 75% methylene chloride/25% methanol, and add the solvent rinse to the boiling flask. Rinse the neck of the flask with 4 mL of 75% methylene chloride/25% methanol.

(8) Prior to evaporating 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.

j. Dissolve the residue in 2.0 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.12.)

k. 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.c.)

l. 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 0.999 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 3-6 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.

m. 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:

$$C = \frac{(PR - 0.00123)}{12.55215}$$

c. Determine the concentrations (µg/mL) in the final solution of the control and recovery samples. For example, using the peak height responses in Figure 3 for factor B in the control (0.2 cm) and the 0.001 µg/mL recovery sample (1.3 cm):

$$C\ (\text{control}) = \frac{(0.2 - 0.00123)}{12.55215} = 0.01584\ \mu g/mL$$

$$C\ (\text{recovery}) = \frac{(1.3 - 0.00123)}{12.55215} = 0.10347\ \mu g/mL$$

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

$$\mu g/mL = \frac{C \times V_f}{V_i}$$

where V_i is the initial volume of water extracted (normally 200 mL) and V_f is the final volume of the sample extract (normally 2.0 mL unless further diluted).

For the example above, the µg/mL concentration of factor B is calculated as:

$$\mu g/mL\ (\text{control}) = \frac{0.01584\ \mu g/mL \times 2.0\ mL}{200\ mL} = 0.0001584\ \mu g/mL$$

$$\mu g/mL\ (recovery) = \frac{0.10347\ \mu g/mL \times 2.0\ mL}{200\ mL} = 0.0010347\ \mu g/mL$$

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

$$\text{Net concentration} = \mu g/mL\ (recovery) - \mu g/mL\ (control)$$

$$= 0.0010347 - 0.0001584$$

$$= 0.000876\ \mu g/mL$$

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

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

For the above example:

$$R = \frac{0.000876}{0.001} \times 100\%$$

$$R = 87.6\%$$

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.k.) 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%. The sensitivity will be decreased at 235 nm or 275 nm (Figure 1).

d. The optional silica SPE cleanup may also be utilized to afford some additional selectivity to the method that could be useful in removing potential interferences.

J. Determination of XDE-105 and Metabolites in Water

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:

$$\text{Uncorrected Result } (\mu g/mL) = \frac{\mu g/mL \text{ (from std. curve)} \times V_f}{V_i}$$

$$\text{Uncorrected Result } (\mu g/mL) = \frac{0.103\ \mu g/mL \times 2.0\ mL}{200\ mL} = 0.00103\ \mu g/mL$$

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

   a. Determine the XDE-105 concentrations in the water samples as described in Section J.3.

   b. Determine the corrected analyte concentration in the water samples as follows:

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

where 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:

$$\text{Corrected Result } (\mu g/mL) = 0.000876\ \mu g/mL \times \frac{100\%}{87.6\%}$$

$$\text{Corrected Result } (\mu g/mL) = 0.001\ \mu g/mL$$

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 pond water, well water, and tap water. The individual results are summarized in Tables II-VI, with statistical summaries in Tables VI and VII. For pond water, the method was validated with and without the optional silica SPE cleanup. The following recovery values (mean ± one standard deviation) resulted from the method when water samples were fortified over the concentration range of 0.001 to 0.1 µg/mL, and the silica SPE cleanup was not used (Table VII):

Factor A:	93 ± 8%
Factor B:	87 ± 6%
Factor D:	90 ± 8%
Factor B of D:	90 ± 8%

The following recovery values (mean ± one standard deviation) resulted from the method when pond water samples were fortified at 0.001 and 0.01 µg/mL, and the silica SPE cleanup was used (Table VI):

Factor A:	84 ± 10%
Factor B:	87 ± 13%
Factor D:	78 ± 6%
Factor B of D:	81 ± 9%

The relative standard deviation (RSD) for all fortification levels combined ranged from 7% to 16% with the SPE cleanup (Table VI), and from 1% to 12% without the cleanup (Table VII). Typical chromatograms demonstrating the determination of XDE-105 in tap water, well water, and pond water without the optional SPE cleanup are illustrated in Figures 3-5, and typical chromatograms demonstrating the determination of XDE-105 in pond water with the SPE cleanup are contained in Figure 6.

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 0.9999 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 0.001 µg/mL recovery samples in Tables II-VI. 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 22 samples. The results are summarized in Table VIII.

For all four analytes, the calculated statistics support an LOD of 0.0003 µg/mL and an LOQ of 0.001 µg/mL. Results should not be quantified at levels below the validated limit of quantitation, but should instead be reported as less than the validated limit of quantitation (e.g., <0.001 µg/mL).

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 tap water are illustrated in Figure 7. (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 water sample, a minimum of 2 fortified controls for determination of recovery, and 10-12 samples. Without the optional SPE column cleanup, this analytical run can typically be prepared for HPLC analysis in approximately 3-4 hours, with the chromatographic analysis occurring overnight and calculations occurring the following day. The total person-hours required without the SPE cleanup is typically 5-6 hours. With the optional SPE cleanup, the total person-hours is typically 6-8 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.f. The assay may be stopped after the third partitioning with dichloromethane. The samples should be protected from light.
b. Step I.1.i.(1). The analysis may be stopped with the samples dissolved in hexane prior to the optional silica SPE procedure if the boiling flasks are capped and the samples are protected from light.
c. Step I.1.k. 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.i.(2) to I.1.i.(3).
b. Transfer 1.0 mL of the 2.0 µg/mL spiking standard solution (Step G.1.b.) 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 2-mL volumes of 75% methylene chloride/25% methanol to the SPE cartridge and collect each 2-mL fraction in a separate culture tube using dropwise elution.

g. Proceed as described in Steps I.1.i.(7) through I.1.m.

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.i.(5)) or the volume of 75% methylene chloride/25% methanol to be collected (Step I.1.i.(6)).

A typical elution profile is illustrated in Figure 8.

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. 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. Certain parameters in the method may be modified, if desired, to increase the sensitivity of the method or to reduce the sample size requirements. For example, the sensitivity of the method may potentially be increased by increasing the injection volume and/or the volume of water that is extracted.

   Alternatively, it may be possible to maintain the same sensitivity of the method while using smaller sample sizes in conjunction with larger injection volumes (e.g., up to 500 µL). However, any changes in the method must be properly validated in order to statistically calculate the limits of detection and quantitation using the new parameters.

6. If a surfactant is to be added to the water sample to prevent the analytes from adsorbing to the glass container for immunoassay (2), an aliquot of the water sample for the HPLC analysis must be taken before addition of the surfactant. The surfactant can interfere with the HPLC analysis and reduce recovery of the analytes during the SPE column cleanup.

7. XDE-105 readily adsorbs from water samples onto glass containers. Rinsing of the emptied containers with methanol, followed by methylene chloride (Step I.1.d.), is needed to remove residues from the glass.

8. 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 during the partitioning process.

9. Avoid the common technique of drying the organic partitioning solvent by draining through sodium sulfate. Some lots of sodium sulfate adsorb XDE-105 factors and significantly reduce the recovery.

10. The rotary vacuum evaporation usually does not leave traces of water in the boiling flask. However, it is especially important to remove any traces of water if the optional silica SPE procedure is being utilized. Water will alter the elution profile of the XDE-105 factors from the silica.

11. The optional SPE column cleanup has typically not been necessary. The optional cleanup should be used if the methylene chloride extracts of the water samples are highly colored, or if the water samples being analyzed have previously resulted in interference on the HPLC chromatograms.

12. XDE-105 adsorbs tightly 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 Water Using a Magnetic Particle-Based Immunoassay Test Kit", GRM 94.10, unpublished method of DowElanco.

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Table I. Chemical Names for XDE-105 Factors A, D, B, and "B of D"

Factor	Chemical Name
A	2-((6-deoxy-2,3,4-tri-O-methyl-α -L-mannopyranosyl)oxy)-13-((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-14-methyl-1H-as-Indaceno(3,2-d)oxacyclododecin-7,15-dione (CAS Number 131929-60-7).
D	2-((6-deoxy-2,3,4-tri-O-methyl-α -L-mannopyranosyl)oxy)-13-((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-1H-as-Indaceno(3,2-d)oxacyclododecin-7,15-dione (CAS Number 131929-60-0).
B	2-((6-deoxy-2,3,4-tri-O-methyl-α -L-mannopyranosyl)oxy)-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-13-((tetrahydro-6-methyl-5-(methylamino)-2H-pyran-2-yl)oxy)-1H-as-Indaceno(3,2-d)oxacyclododecin-7,15-dione (CAS Number 131929-61-8).
B of D	2-((6-deoxy-2,3,4-tri-O-methyl-α -L-mannopyranosyl)oxy)-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-13-((tetrahydro-6-methyl-5-(methylamino)-2H-pyran-2-yl)oxy)-1H-as-Indaceno(3,2-d)oxacyclododecin-7,15-dione (CAS Number: not yet available).