DESCRIPTION OF ANALYTICAL METHOD

Method Identification Number: Residue Determination Method GRM 94.20

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

Scope of Method: 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.

Test substance identification: XDE-105

Name:	Factor A	Factor B	Factor D	Factor B of D
TSN Number:	100221	100111	100222	100259
% Purity:	97	96	98	94.5
Analytical Report:	FA&PC 950019	FA&PC 940114	FA&PC 950123	FA&PC 940172
Date:	01-Mar-1995	10-Mar-1994	05-Oct-1993	27-Sept-1994

Certificates of Analysis for all the compounds are on file at DowElanco.

Identification of test systems used:

Type:	Clay loam soil
Source:	DowElanco
Sample Identification:	Each sample assigned a unique identifier

Method Outline

RESIDUE DETERMINATION METHOD GRM 94.20

INDEPENDENT LABORATORY VALIDATION OF METHOD GRM 94.20 - DETERMINATION OF XDE-105 AND METABOLITES IN SOIL AND SEDIMENT BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

Weigh 20 grams of soil into a 150-mL glass centrifuge bottle. Fortify as necessary.

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Extract soil by first sonicating and then shaking with 60 mL of methanol:5% sodium chloride:1 N sodium hydroxide (65:27:8). Centrifuge and decant extraction solution into a 250-mL mixing cylinder. Add 60 more milliliters of methanol:5% sodium chloride:1 N sodium hydroxide (65:27:8), sonicate, shake, centrifuge and decant into the same 250-mL mixing cylinder. Bring the combined extracts to a final volume of 130 mL with methanol:5% sodium chloride:1 N sodium hydroxide (65:27:8) and mix.

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Transfer 65 mL of sample extract to a 250-mL separatory funnel. Adjust pH to approximately 2 with 0.16 N hydrochloric acid in 5% sodium chloride. Partition with 50 mL hexane. Allow layers to separate. Drain the aqueous portion into a beaker. Discard the hexane.

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Return the aqueous portion to the separatory funnel. Adjust the pH to approximately 10-12 with 1 N sodium hydroxide solution. Partition 3 X 50 mL of hexane. Allow phases to separate. Drain hexane from each partition through a funnel containing sodium sulfate. Collect all hexane in the same 500-mL boiling flask. Rinse sodium sulfate bed with 15 mL of hexane.

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Evaporate hexane to dryness using a rotary evaporator (water bath temperature approximately 35-50 °C). Reconstitute extract with 10 mL of hexane.

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Plug a 25 cm x 10.5 mm glass column with glass wool. Add a slurry of 0.7 grams silica gel in methylene chloride:methanol (75:25). Allow silica gel to settle. Top with sodium sulfate. Rinse column with acetonitrile, then methylene chloride, then hexane. Add extract in hexane. Wash column with 60 mL of hexane, then 10 mL of methylene chloride, then 4 mL of acetonitrile. Elute the analyte from the column with 24 mL methylene chloride:methanol (75:25).

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Evaporate the column eluate to dryness using a rotary evaporator (water bath temperature approximately 35-50 °C). Dissolve the residue in 1.0 mL of methanol:acetonitrile:2% ammonium acetate (1:1:1).

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Analyze by HPLC

ANALYTICAL RESULTS (Continued)

Acquisition: Data acquisition and analysis, results reporting, and information management was accomplished using a computer program called MULTICHROM® purchased from VG Data Systems. This program was run on a Digital Equipment Corporation MicroVax 3800 computer. The chromatographic signals were digitized and stored in VG Chromatography Servers. The digitized chromatograms acquired were then downloaded to the computer for storage and data processing.

Calculations: The MULTICHROM® computer program measured peak height for standards and samples and then used the standard concentrations versus peak height to form a linear regression curve. The analyte concentration in the sample extracts was interpolated from the regression curve. These concentrations were then converted to ppm in the sample by entering the final volume and milligrams injected into the MULTICHROM® computer program.

$$found\ ppm\ in\ sample = \frac{\mu gs\ detected\ in\ extract \times dilution\ volume\ (mL)}{sample\ weight\ (grams)}$$

Recovery of the fortified samples was calculated as follows:

$$\%\ recovery = \frac{(ppm\ found - ppm\ in\ control)}{theoretical\ ppm\ calculated} \times 100$$

Statistical Methods: The mean was calculated for each compound in each sample set by dividing the sum of the percent recoveries of the sample set by the number of samples in the set.

The standard deviation (s) was calculated for each compound in each sample set by summing the squares of the individual deviations from the mean, dividing by the number of degrees of freedom, and extracting the square root of the quotient.

FULL DESCRIPTION OF ANALYTICAL INSTRUMENTATION USED

Instrumentation:	Shimadzu 6A High Performance Liquid Chromatography System
Detector:	Shimadzu SPD-6A Ultraviolet Detector
Wavelength:	250 nm
Column:	YMC ODS-AQ, 4.6 mm x 150 mm, 5μm
Mobile Phase:	44% methanol, 44% acetonitrile, 12% of 2% ammonium acetate
Flow Rate:	0.8 mL/minute
Injection Volume:	175 μL

DESCRIPTION OF PROBLEMS ENCOUNTERED IN VALIDATING THE METHOD

Chromatography of initial extracts showed interferences, particularly for Factor B and Factor B of D. These interferences were of sufficient magnitude to prevent quantitation of these two components. It was noted in the chromatography that the elution time of the four components was somewhat faster than that demonstrated in the chromatography presented in the method. Examples of this were elution times of 6.0, 6.8, 10.1, and 11.6 minutes for Factor B, Factor B of D, Factor A, and Factor D, respectively, versus elution times of 8.0, 9.1, 11.0, and 12.6 minutes shown in the method. The mobile phase and column used were identical to that described in the method. To obtain better separation, the mobile phase was altered slightly to increase the elution times of all components. This was done by changing the 12% fraction containing 2% ammonium acetate:acetonitrile (67:33) to just 2% ammonium acetate. The resulting mobile phase was then 44% methanol:44% acetonitrile:12% of 2%ammonium acetate. Elution times obtained were 7.2, 8.4, 13.4, and 16.0 minutes for the four respective compounds. An improvement in separation from interferences was obtained; however, it was insufficient to obtain quantitation for the first two components. (Altering the chromatographic conditions to optimize performance was permitted by note L.4. in method GRM 94.20.)

Notations in the methodology indicated interferences could occur due to contaminated glassware. Method steps were provided regarding cleaning of glassware to prevent these problems. A reagent blank was run through the method to determine if interferences existed or if the interferences were due to the sample matrix. Results obtained with this reagent blank showed significant interferences were present. A second reagent blank was run through the method up to the SPE column cleanup. Although some peaks were observed, they did not correspond with the elution times of any of the analytes. Two additional reagent blanks were run, one obtained from an SPE column prepared and eluted as per the method, and the second prepared by concentrating the volumes of solvent used to prepare and elute the SPE column. Significant interferences were observed in the column eluate but not in the solvents used to prepare and elute the column. A second lot of silica SPE columns from Waters and a lot from Baker were tested and the same interferences were observed. A lot of silica gel obtained from EM Science packed into a glass bell column (25 cm x 10.5 mm i.d.) was tested and the interferences were significantly reduced. A set of sample extracts (1 week old) were analyzed using the silica gel packed in the glass columns. This set is noted in the report as the failed set. Discussions with the sponsor concerning the problems indicated the results obtained from the week old extracts were probably low due to instability of the components in the solutions for an extended period of time. The problems of interferences were discussed at length. The sponsor felt the use of silica gel in a glass column was acceptable but asked that a sample of SPE columns from a lot they had used for the same analysis be tried.

In the methodology, half of the sample extract is taken through the sample cleanup and analyzed. For the analysis set which resulted in good data, half of the sample extract was taken through the method and cleaned up using the glass column with silica gel. The other half of the extract was taken through the method and cleaned up using either the silica gel SPE columns obtained from DowElanco or a silica gel SPE column obtained directly from Waters. In both instances, significant interferences were observed in the SPE columns, preventing quantitation of Factor B and Factor B of D at the 0.01 ppm fortification level. Acceptable recoveries were obtained from the extracts using silica gel packed in a glass column. Data for solvent and column test runs

may be found in the "Raw Data Package" of this report. (The use of alternative reagents and equipment to avoid interferences was permitted by Notes L.1. and L.2. of method GRM 94.20.)