2. DESCRIPTION OF ANALYTICAL METHOD

Method Identification Number: Residue Determination Method GRM 94.12

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

Scope of Method: 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 a validated limit of quantitation of 0.001 µg/mL.

Identification of test substance used:

Name:	Spinosyn A
TSN Number:	100221
% Purity:	97%
Reference:	FA & PC 950019
Date:	3/01/95

Identification of test substance used:

Name:	Spinosyn D
TSN Number:	100222
% Purity:	98%
Reference:	FA & PC 950123
Date:	10/5/93

Identification of test substance used:

Name:	Spinosyn B
TSN Number:	100111
% Purity:	96%
Reference:	FA & PC 940114
Date:	3/10/94

2. DESCRIPTION OF ANALYTICAL METHOD (Continued)

Identification of test substance used:	Name:	N-Dimethyl Spinosyn D
	TSN Number:	100259
	% Purity:	94.5%
	Reference:	FA & PC 940172
	Date:	9/27/94

Identification of test systems used:	Type:	Pond water
	Source:	DowElanco
	Sample identification:	14882201

METHOD OUTLINE

RESIDUE DETERMINATION METHOD: GRM 94.12

INDEPENDENT LABORATORY VALIDATION OF METHOD GRM 94.12 - DETERMINATION OF XDE-105 AND METABOLITES IN WATER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

Allow the pond water sample to warm to room temperature.
↓

Measure a 200 mL aliquot into an 8 oz. French square bottle.
↓

Add 4 mL of 1.0 N sodium hydroxide.
↓

Shake for approximately 5 seconds and check pH.
(if pH < 12, add more NaOH).
↓

Fortify the sample, as required, with 1.0 mL of the appropriate fortification standard solution.
↓

Transfer the sample to a 250 mL separatory funnel.
↓

Rinse the sample bottle with 20 mL of methanol and add this rinse to the separatory funnel.
↓

Rinse the sample bottle with 50 mL of methylene chloride, and add this rinse to the separatory funnel.
↓

Shake vigorously for 30 seconds (work under dimmed lights during partitioning).
↓

Allow the layers to separate for at least 5 minutes, then drain the methylene chloride layer into a 500 mL round-bottom flask (do not drain the slight emulsion or use sodium sulfate to dry).
↓

METHOD OUTLINE (Continued)

RESIDUE DETERMINATION METHOD: GRM 94.12

INDEPENDENT LABORATORY VALIDATION OF METHOD GRM 94.12 - DETERMINATION OF XDE-105 AND METABOLITES IN WATER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

Repeat the methylene chloride extraction two more times, and combine all three extracts into the 500 mL round-bottom flask.

↓

Pre-rinse the rotovap under vacuum using hexane followed by methanol.

↓

Evaporate the methylene chloride extract to dryness on the rotovap with the water bath at approximately 35-50°C.

↓

Dissolve the residue in 10 mL of hexane.

↓

Condition a silica SPE cartridge with the following solvents:

10 mL 75% methylene chloride/25% methanol
10 mL acetonitrile
10 mL methylene chloride
20 mL hexane

Allow the solvents to pass in a stream, but do not allow the column to go dry.

↓

Load the sample residue, dissolved in 10 mL of hexane, onto the silica SPE cartridge.

↓

Rinse the flask with 10 mL of hexane and elute through the cartridge.

↓

Rinse the flask with 10 mL of hexane and elute through the cartridge.

↓

Rinse the flask with 40 mL of hexane and elute through the cartridge.

↓

Rinse the flask with 5 mL of methylene chloride and elute through the cartridge.

↓

METHOD OUTLINE (Continued)

RESIDUE DETERMINATION METHOD: GRM 94.12

INDEPENDENT LABORATORY VALIDATION OF METHOD GRM 94.12 - DETERMINATION OF XDE-105 AND METABOLITES IN WATER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

Rinse the flask with 5 mL of methylene chloride and elute through the cartridge.

↓

Rinse the flask with 5 mL of acetonitrile and elute through the cartridge.

↓

Rinse the flask with 10 mL of 75% methylene chloride/25% methanol, elute dropwise through the column, and collect the eluate in a 125 mL round bottom flask.

↓

Pre-rinse the rotovap under vacuum using hexane followed by methanol.

↓

Evaporate the eluate to dryness on the rotovap with the water bath at approximately 35-50°C.

↓

Dissolve the residue in 2.0 mL of methanol/acetonitrile/2% ammonium acetate (1:1:1).

↓

Transfer the sample to a vial using a disposable pipet, and analyze by HPLC.

3. ANALYTICAL RESULTS (Continued)

Calculations: Calibration standards were analyzed with each sample set. Linear regression equations were generated for each analyte using the concentrations of the calibration standards versus the respective peak area responses. The correlation coefficient (R^2 value) of each linear regression equation was 0.999 or greater. Concentrations of the analytes in the final solutions were determined by substituting the peak area responses into the applicable linear regression equation as shown below:

$$\mu g/mL \text{ at instrument} = \frac{y - b}{m}$$

where, m is the slope, y is the *peak area response* of the analyte and b is the y-intercept generated from the linear regression equation.

The analyte concentration (µg/mL) in the original sample was calculated using the equation shown below:

$$\mu g/mL \text{ Found} = \frac{\mu g/mL \text{ at instrument} \times \text{Final volume}}{\text{Initial volume}}$$

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

The standard deviation (s) was calculated for each analyte 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.

5. FULL DESCRIPTION OF ANALYTICAL INSTRUMENTATION USED

Instrument:	Hewlett-Packard Model 1090 Series II High Performance Liquid Chromatograph (LC) and a Model G1030A Chemstation
Detector:	Hewlett-Packard Diode Array Detector at 250 mm
Analytical Column:	YMC ODS-AQ; 5 µm; 4.6 mm i.d. x 150 mm
Column Temperature:	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)
Injection Volume:	175 µL
Flow Rate:	0.8 mL/min.
Retention Time:	Approximately 6.0 minutes for XDE-105 Factor B Approximately 6.9 minutes for XDE-105 Factor B of D Approximately 10.0 minutes for XDE-105 Factor A Approximately 11.6 minutes for XDE-105 Factor D