

**Test Material:** Spinosad (XDE-105)

**MRID:** 44045103

**Title:** Residue Method Validation Report for the Determination of XDE-105 and Metabolites in Soil and Sediment by High Performance Liquid Chromatography with Ultraviolet Detection

**MRID:** 44045104

**Title:** 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

**EPA PC Code:** 110003

**OCSPP Guideline:** 850.6100

**For CDM Smith**

**Primary Reviewer:** Lisa Muto

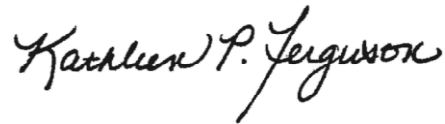
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**Date:** 9/22/15

**Secondary Reviewer:** Kathleen Ferguson

**Signature:**



**Date:** 9/22/15

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**Signature:**



**Date:** 9/22/15

**Analytical method for spinosad [XDE-105 (spinosyns A and D)] and its transformation products, spinosyn B and N-demethyl spinosyn D, in soil and sediment**

- Reports:** ECM: EPA MRID No.: 44045103. West, S.D. 1995. Residue Method Validation Report for the Determination of XDE-105 and Metabolites in Soil and Sediment by High Performance Liquid Chromatography with Ultraviolet Detection. Laboratory Study ID: RES94124. Report prepared, sponsored and submitted by North American Environmental Chemistry Laboratory, DowElanco, Indianapolis, Indiana; 79 pages. Final report dated March 27, 1995; Method dated January 27, 1995 (pp. 1, 16).  
ILV: EPA MRID No. 44045104. Lochhaas, C. 1995. 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. ABC Laboratories Final Report No.: 42463. Dow AgroSciences PTR No.: 10000943-5008-1 and Study No.: 050036. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, and sponsored and submitted by DowElanco, Indianapolis, Indiana; 41 pages. Final report issued April 12, 1995.
- Document No.:** MRIDs 44045103 & 44045104
- Guideline:** 850.6100
- Statements:** ECM: The study was conducted in accordance with USEPA FIFRA and OECD Good Laboratory Practice (GLP) standards (1982), with the exception of the radiolabeled extraction study, ultraviolet spectra, and untreated control samples, as well as minor GLP deviations regarding soil characterization, silica SPE elution profiles and purity determination for N-demethyl spinosyn D (p. 3 of MRID 44045103). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).  
ILV: The study was conducted in accordance with USEPA GLP standards, with the exception of the collection of the control soil, as well as minor GLP deviations regarding purity determination for some of the reference compounds (p. 3 of MRID 44045104). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A statement of authenticity was not included.
- Classification:** This analytical method is classified as supplemental. In the ILV, the number of samples was insufficient for all analyses, and no samples were prepared at 10×LOQ. In the ECM, the number of samples was insufficient for all analyses, except for sediment at the LOQ, and only sediment samples were prepared at 10×LOQ. The soil matrices were insufficiently characterized in the ILV; it could not be determined if the ILV was provided with the most difficult soil type with which to validate the method. One of the individual recoveries in the ILV was <70%. The ECM confirmation method was not validated by the ILV, only primary HPLC/UV. ECM and ILV representative chromatograms did not adequately support the method due to baseline interference and/or scale. Sample recoveries were corrected in the ILV.

**PC Code:** 110003

**Reviewer:** Larry Liu



Date: 5/3/17

The page numbers of MRID 44045103 refer to those listed in the upper right-hand corner of the MRID. The page numbers of MRID 44045104 refer to those listed in the lower right-hand corner of the MRID, without the ABC Labs # included.

### **Executive Summary**

The analytical method, DowElanco Method GRM 94.20, is designed for the quantitative determination of XDE-105 (spinosyns A and D) and its transformation products, spinosyn B and N-demethyl spinosyn D, in soil and sediment at the LOQ of 0.01 µg/g using HPLC/UV. The LOQ is less than the lowest toxicological level of concern in soil/sediment for all analytes. The ECM utilized two soils (sandy loam and clay loam) and one pond sediment (clay loam); a confirmatory HPLC/UV was performed, but these results were not quantified. Method GRM 94.20 was validated by the ILV in the second trial using clay loam soil after optimization of the HPLC conditions and laboratory equipment; however, the confirmatory HPLC/UV was not performed. The soil matrix was insufficiently characterized in the ILV; it could not be determined if the ILV was provided with the most difficult soil type with which to validate the method. In the ILV and soil portion of the ECM, the number of samples was insufficient for all analyses, and no samples were prepared at 10×LOQ. Only pond sediment samples in the ECM were prepared at 10×LOQ (n = 3). ECM and ILV representative chromatograms did not adequately support the method due to baseline interference and/or scale.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
XDE-105 (spinosyn A)	44045103 (GRM 94.20)	44045104		Soil <sup>1,2</sup>	27/03/1995 (Report date)  27/01/1995 (Method date)	DowElanco	HPLC/UV <sup>3</sup>	0.01 µg/g
XDE-105 (spinosyn D)								
Spinosyn B								
N- demethyl spinosyn D								

1 For ECM Method GRM 94.20, sandy loam soil (76.0% sand, 13.6% silt, 10.4% clay; 0.88% organic matter), clay loam soil (34.8% sand, 34.0% silt, 31.2% clay; 1.26% organic matter) and clay loam pond sediment (24.0% sand, 42.2% silt, 33.8% clay; 3.94% organic matter) were used (pp. 8, 10; Appendix A, Table VIII, p. 44 of MRID 44045103).

2 The matrix of the ILV was uncharacterized clay loam soil obtained from the sponsor was used for validation (p. 11). It could not be determined if the characterization data applied to the soil matrices of the ILV validation.

3 Method GRM 94.20 described a primary and a confirmatory HPLC/UV analysis. In the ECM, performance data (recovery results) were only provided for quantitative HPLC/UV analysis; recovery results from the confirmatory HPLC/UV analysis were not reported. Confirmation of residue identity was performed by analyzing the samples under different HPLC/UV conditions, possibly using different wavelengths; the method noted that the confirmatory HPLC analysis could be performed, if necessary (Appendix A, pp. 26-27 of MRID 44045103). No confirmatory HPLC/UV was performed by the ILV.

## I. Principle of the Method

Samples (20 g) of soil or sediment in 8-oz bottles were fortified, as necessary, then extracted twice with methanol:5% sodium chloride/1N sodium hydroxide (65:27:8, v:v:v; 2 x 60 mL) via sonication (ultrasonic cleaner for *ca.* 5 minutes) and shaking on a gyrator shaker for *ca.* 30 minutes at *ca.* 275 rpm (pp. 9-10; Appendix A, pp. 22-24 of MRID 44045103). Solids and solvent were separated using centrifugation (*ca.* 10 minutes at *ca.* 2250 rpm). The method noted that samples should be protected from light either with paper towels or aluminum foil-wrapped vials. The volume of the combined extracts was adjusted to 130 mL using methanol:5% sodium chloride/1N sodium hydroxide (65:27:8, v:v:v). After mixing, an aliquot (65 mL) of the extraction solvent was diluted with 65 mL of 0.16 N hydrochloric acid in 5% sodium chloride. The pH of aqueous phase should be *ca.* 2; additional acidic salt solution can be added to achieve the proper pH. The extract was partitioned with 50 mL of hexane (shaking in separatory funnel for *ca.* 20-25 seconds). The hexane layer was discarded, and the aqueous layer was returned to the separatory funnel. The pH of the aqueous layer was adjusted to *ca.* 10-12 using 10.0 mL of 1.0 N sodium hydroxide solution (more can be used to ensure that the pH = *ca.* 10-12). The aqueous layer was extracted three times with 50 mL of hexane (shaking in separatory funnel for *ca.* 20-25 seconds). After separation, the hexane layers were drained into a beaker through a small plug of hexane-washed glass wool containing approximately 10 mL of hexane-washed sodium sulfate. After the final extraction, the glass wool/sodium sulfate was rinsed with 15 mL of hexane. The combined extracts were evaporated to dryness using a rotary vacuum evaporator and water bath set at 35-50°C (the method noted that care should be taken to clear contamination from the rotary evaporator with solvents before the sample extracts were concentrated). The samples were purified by silica solid phase extraction (SPE). The SPE cartridge was pre-conditioned with methylene chloride:methanol (75:25, v:v; 10 mL), acetonitrile (10 mL), methylene chloride (10 mL) and hexane (20 mL). The concentrated extract was applied to the column with 10 mL of hexane. The evaporating flask was rinsed with 10 mL x 2 and 40 mL of hexane (each passed through the SPE separately). All hexane eluate solutions were discarded. The evaporation flask was rinsed with methylene chloride (2 x 5 mL) and acetonitrile (2 x 4 mL; each passed through the SPE separately). All eluate solutions were discarded. For sample collection, the evaporation flask was rinsed with 3 x 8 mL of methylene chloride:methanol (75:25, v:v; each passed through the SPE separately). The analytes were collected into culture tubes. The combined eluate solutions from the culture tubes were concentrated to dryness via rotary vacuum evaporation (water bath set at 35-50°C). When combining the eluate solutions, 4 mL of methylene chloride:methanol (75:25, v:v) was used to rinse all flasks and the neck of the boiling flask. The residue was reconstituted in 1.0 mL of methanol:acetonitrile:2% ammonium acetate (1:1:1, v:v:v). The solution was transferred to an HPLC sample vial, and XDE-105 and its metabolites were analyzed by liquid chromatography with UV detection.

Samples were analyzed for XDE-105 (spinosyns A and D), spinosyn B and N-demethyl spinosyn D using a Hewlett-Packard Model 1050 with a UV detector (p. 10; Appendix A, p. 21 of MRID 44045103). The instrumental conditions consisted of a YMC ODS-AQ column (4.6 x 150 mm, 5- $\mu$ m; column temperature 30°C), a mobile phase of methanol/acetonitrile/2% ammonium acetate:acetonitrile (67:33, v:v) [44:44:12, v:v:v], UV detection (250 nm), and injection volume 175  $\mu$ L. Retention times for spinosyn A, spinosyn D, spinosyn B and N-demethyl spinosyn D were *ca.* 11, 12.5, 8 and 9 minutes, respectively (based on provided clay loam and pond sediment

chromatograms; retention times were not noted in the sandy loam chromatograms; Appendix A, Figures 4-5, pp. 48-50).

Confirmation of the identities of the analytes was performed with the same instrument with the following instrumental condition changes: C18/Cation Mixed Mode column (4.6 x 150 mm, 5- $\mu$ m; column temperature 30°C), a mobile phase of methanol/acetonitrile/2% ammonium acetate:acetonitrile (67:33, v:v) [40:40:20, v:v:v] and UV detection (250 nm, 235 nm or 275 nm). (Appendix A, pp. 21-22, 26-27 of MRID 44045103). Retention times could not be determined from the provided chromatogram; however, the compounds eluted in the order: A, D, B and B of D (slightly reversed of the primary HPLC/UV method; UV 250 nm; Appendix A, Figure 6, p. 51).

The method suggested the use of “an alternative detection system such as HPLC-mass spectrometry or immunoassay” if additional confirmation of analyte identity was required; however, no instrumental parameters were suggested or reported for these additional detection methods (Appendix A, p. 29 of MRID 44045103).

The method contained several notes regarding important information for the success of the method, including the attention and instruction to reduce interferences in the samples due to the nonselective UV wavelength (250 nm), the use of sodium sulfate to eliminate water in the samples prior to SPE elution, the desorption of the XDE-105 from glass with ammonium acetate and the use of low laboratory lighting to reduce the occurrence of aqueous photolysis of XDE-105 during partitioning (Appendix A, pp. 31-32 of MRID 44045103). The ECM study author noted that GRM 94.20 was performed exactly as written, except for a few minor deviations which had no effect on the outcome of the method validation study (p. 10).

### ILV

In the ILV, Method GRM 94.20 was performed as written, with minor modifications which were allowed by the method: the altering of the HPLC mobile phase and the use of silica gel packed in glass columns instead of silica gel SPE columns (pp. 10, 12, 39-41 of MRID 44045104). The samples were analyzed using a Shimadzu 6A HPLC with a Shimadzu SPD-6A UV detector (250 nm). The LC column, flow rate and injection volume were the same as those in the ECM. The mobile phase was optimized to a mobile phase of methanol/acetonitrile/2% ammonium acetate [44:44:12, v:v:v] to improve peak separation. Retention times for spinosyn A, spinosyn D, spinosyn B and N-demethyl spinosyn D were 13.4, 16.0, 7.2 and 8.4 minutes, respectively (p. 40; Figures 13-15, pp. 35-37). No confirmation method was performed.

### LOQ/LOD

The LOQ for all four analytes was the same in the ECM and ILV at 0.010  $\mu$ g/g (pp. 11-12, 14; Appendix A, p. 29; Appendix A, Table VII, p. 43; Appendix B, p. 55 of MRID 44045103; pp. 10-11 of MRID 44045104). The LOD for all analytes was 0.003  $\mu$ g/g in the ECM. The LOD was not reported in the ILV.

## II. Recovery Findings

ECM (MRID 44045103; Method GRM 94.20): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean recovery 70-120%; RSD  $\leq$ 20%) for analysis of XDE-105 (spinosyns A and D), spinosyn B and N-demethyl spinosyn D (B of D) at fortification levels of 0.010  $\mu\text{g/g}$  (LOQ) and 1.00  $\mu\text{g/g}$  (100 $\times$ LOQ) in two soils (sandy loam and clay loam) and of 0.010  $\mu\text{g/g}$  (LOQ), 0.025  $\mu\text{g/g}$  (2.5 $\times$ LOQ), 0.050  $\mu\text{g/g}$  (5 $\times$ LOQ), 0.075  $\mu\text{g/g}$  (7.5 $\times$ LOQ), 0.100  $\mu\text{g/g}$  (10 $\times$ LOQ) and 1.00  $\mu\text{g/g}$  (100 $\times$ LOQ) in one sediment (clay loam), except for one recovery of N-demethyl spinosyn D in clay loam pond sediment (69%; Appendix A, Tables II-VI, pp. 34-42 of MRID 44045103 and DER Attachment 2). The number of samples ( $n = 3$ ) was insufficient for all analyses, except for those at the LOQ in clay loam pond sediment ( $n = 8$ ). No 10 $\times$ LOQ samples were prepared in the two soils. Performance data (recovery results) were only provided for quantitative HPLC/UV analysis; recovery results from the confirmatory HPLC/UV analysis were not reported. Confirmation of residue identity was performed by analyzing the samples under different HPLC/UV conditions, possibly using different wavelengths; the method noted that the confirmatory HPLC analysis could be performed, if necessary (Appendix A, pp. 26-27). Method GRM 94.20 allowed for recovery data to be corrected for residues found in the control samples; however, residues were not quantified in any of the control samples (Appendix A, pp. 25-26; Appendix A, Tables II-VI, pp. 34-42). Recoveries from samples fortified at 0.003  $\mu\text{g/g}$  (LOD) were reported as  $<$ LOQ. The soil/sediment matrices were fully characterized (pp. 8, 10; Appendix A, Table VIII, p. 44). Classifications were not specified as USDA. Sandy loam soil (76.0% sand, 13.6% silt, 10.4% clay; 0.88% organic matter) was collected from Oconee, Georgia. Clay loam soil (34.8% sand, 34.0% silt, 31.2% clay; 1.26% organic matter) was collected from Tunica, Mississippi. Clay loam pond sediment (24.0% sand, 42.2% silt, 33.8% clay; 3.94% organic matter) was collected from a pond near Greenfield, Indiana.

ILV (MRID 44045104): Individual recoveries were within guidelines for analysis of XDE-105 (spinosyns A and D), spinosyn B and N-demethyl spinosyn D (B of D) in clay loam soil at fortification levels of 0.01  $\mu\text{g/g}$  (LOQ) and 0.05  $\mu\text{g/g}$  (5 $\times$ LOQ) using the quantitative HPLC/UV analysis, except for one recovery of N-demethyl spinosyn D (66%; Tables I-VII, pp. 13-19). The number of samples ( $n = 2$ ) was insufficient for all analyses. Mean recoveries and relative standard deviations (RSDs) of statistical significance could not be calculated. No samples were prepared at 10 $\times$ LOQ. Confirmatory HPLC/UV analysis was not performed. Calculations allowed for recovery data to be corrected for residues found in the control samples; however, only residues of Spinosyn A were quantified in the control samples (p. 21; Tables I-VII, pp. 13-19; Figures 10-12, pp. 32-34). Uncharacterized clay loam soil obtained from the sponsor was used for validation (p. 11). Method GRM 94.20 was validated in the second trial after optimization of the HPLC conditions and laboratory equipment (pp. 40-41; Tables I-VIII, pp. 13-20).

**Table 2. Initial Validation Method Recoveries for XDE-105 and Its Transformation Products in Sandy Loam Soil, Clay Loam Soil and Clay Loam Pond Sediment<sup>1,2</sup>**

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>GRM 94.20</b>						
Sandy Loam Soil						
XDE-105 (spinosyn A)	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	<b>3</b>	76	76	0	0
	1.000	<b>3</b>	85-88	86	2	2
XDE-105 (spinosyn D)	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	<b>3</b>	75-90	80	9	11
	1.000	<b>3</b>	86-87	86	1	1
Spinosyn B	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	<b>3</b>	70-80	72	6	8
	1.000	<b>3</b>	80-83	81	2	2
N-demethyl spinosyn D (B of D)	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	<b>3</b>	68-73	70	3	4
	1.000	<b>3</b>	79-83	81	2	2
Clay Loam Soil						
XDE-105 (spinosyn A)	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	<b>3</b>	83-89	87	3	4
	1.000	<b>3</b>	85-86	86	1	1
XDE-105 (spinosyn D)	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	<b>3</b>	88-95	93	4	4
	1.000	<b>3</b>	84	84	0	0
Spinosyn B	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	<b>3</b>	74	74	0	0
	1.000	<b>3</b>	82-83	83	1	1
N-demethyl spinosyn D (B of D)	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	<b>3</b>	75-81	79	3	4
	1.000	<b>3</b>	81-84	82	2	2
Clay Loam Pond Sediment						
XDE-105 (spinosyn A)	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	8	76-89	80	5	6
	0.025	<b>3</b>	73-88	81	8	9
	0.050	<b>3</b>	71-79	76	5	6
	0.075	<b>3</b>	76-89	82	7	8
	0.100	<b>3</b>	79-87	83	4	5
	1.000	<b>3</b>	81-86	84	3	3
XDE-105 (spinosyn D)	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	8	74-89	80	8	10
	0.025	<b>3</b>	76-88	84	7	8
	0.050	<b>3</b>	71-82	77	6	8
	0.075	<b>3</b>	77-90	82	7	9
	0.100	<b>3</b>	80-87	83	4	5
	1.000	<b>3</b>	80-85	82	3	3
Spinosyn B	0.003 (LOD)	<b>1</b>	<LOQ	--	--	--
	0.010 (LOQ)	8	69-85	78	6	8



Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.025	3	75-87	82	6	8
	0.050	3	64-76	72	7	10
	0.075	3	75-87	80	6	8
	0.100	3	80-87	82	3	5
	1.000	3	77-80	78	2	2
N-demethyl spinosyn D (B of D)	0.003 (LOD)	1	<LOQ	--	--	--
	0.010 (LOQ)	8	63-75	73	4	6
	0.025	3	67-81	76	8	10
	0.050	3	61-73	69	7	10
	0.075	3	73-85	78	6	8
	0.100	3	76-85	79	5	7
	1.000	3	74-78	76	2	3

Data (uncorrected recovery results; Appendix A, pp. 25-26; Appendix A, Tables II-VI, pp. 34-42) were obtained from Appendix A, Tables II-VI, pp. 34-42 of MRID 44045103 and DER Attachment 2 (means, s.d. and RSDs for LOQ and 100×LOQ).

- The soil/sediment matrices were fully characterized (pp. 8, 10; Appendix A, Table VIII, p. 44). Classifications were not specified as USDA. Sandy loam soil (76.0% sand, 13.6% silt, 10.4% clay; 0.88% organic matter) was collected from Oconee, Georgia. Clay loam soil (34.8% sand, 34.0% silt, 31.2% clay; 1.26% organic matter) was collected from Tunica, Mississippi. Clay loam pond sediment (24.0% sand, 42.2% silt, 33.8% clay; 3.94% organic matter) was collected from a pond near Greenfield, Indiana.
- HPLC/UV analysis was employed for analyte identification (Appendix A, pp. 21-22). A second different HPLC/UV analysis was used as a confirmation method; however, recovery results were not reported.

**Table 3. Independent Validation Method Recoveries for XDE-105 and Its Transformation Products in Clay Loam Soil<sup>1,2</sup>**

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>GRM 94.20</b>						
Clay Loam Soil						
XDE-105 (spinosyn A)	0.01 (LOQ)	2	72, 89	--	--	--
	0.05	2	80, 87	--	--	--
XDE-105 (spinosyn D)	0.01 (LOQ)	2	82	--	--	--
	0.05	2	86, 93	--	--	--
Spinosyn B	0.01 (LOQ)	2	72, 77	--	--	--
	0.05	2	80, 84	--	--	--
N-demethyl spinosyn D (B of D)	0.01 (LOQ)	2	66, 77	--	--	--
	0.05	2	79, 84	--	--	--

Data (corrected results; p. 21; Tables I-VII, pp. 13-19) were obtained from Tables I-VII, pp. 13-19 of MRID 44045104.

- Uncharacterized clay loam soil obtained from the sponsor was used for validation (p. 11).
- HPLC/UV analysis was employed for analyte identification (p. 39). No confirmation HPLC/UV analysis was used.

**III. Method Characteristics**

The LOQ for XDE-105 (spinosyns A and D), spinosyn B and N-demethyl spinosyn D was the same in the ECM and ILV at 0.010 µg/g (pp. 11-12, 14; Appendix A, p. 29; Appendix A, Table

VII, p. 43; Appendix B, p. 55 of MRID 44045103; pp. 10-11 of MRID 44045104). The LOD for all analytes was 0.003 µg/g in the ECM. The LOD was not reported in the ILV. Following the method of Keith, L. H., *et al.* (see section V. **References** below), the LOD and LOQ for determination of XDE-105 and its transformation products in soil/sediment were calculated in the ECM Method using the standard deviation from the 0.01 µg/g recovery results. 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 recovery results. The calculated values support the LOQ and LOD established for the study and are presented in **Table 4** below.

**Table 4. Method Characteristics (GRM 94.20)<sup>1</sup>**

		XDE-105 (spinosyn A)	XDE-105 (spinosyn D)	Spinosyn B	N-demethyl spinosyn D (B of D)
Limit of Quantitation (LOQ)	Established	0.010 µg/g			
	Calculated (ECM)	0.005 µg/g	0.009 µg/g	0.005 µg/g	0.005 µg/g
Limit of Detection (LOD)	Established	0.003 µg/g			
	Calculated (ECM)	0.002 µg/g	0.003 µg/g	0.002 µg/g	0.001 µg/g
Linearity (Least squares calibration curve r and concentration range)	ECM <sup>2</sup>	r <sup>2</sup> = 0.9998- 0.99995	r <sup>2</sup> = 0.9997	r <sup>2</sup> = 0.9997- 1.0000	r <sup>2</sup> = 0.9997- 0.9998
	ILV	r <sup>2</sup> = 0.99997	r <sup>2</sup> = 0.99998	r <sup>2</sup> = 0.99997	r <sup>2</sup> = 1.00000
	Concentration range	0.0-1.5 µg/mL			
Repeatable	ECM <sup>3</sup>	Soils	Yes at LOQ and 100×LOQ, but n = <b>3</b> . <b>No</b> samples were prepared at 10×LOQ.		
		Sediment	Yes at LOQ (n = 8). Yes at 2.5×LOQ, 5×LOQ, 7.5×LOQ, 10×LOQ, and 100×LOQ, but n = <b>3</b> .	Yes at LOQ (n = 8). Yes at 2.5×LOQ, 7.5×LOQ and 10×LOQ, and 100×LOQ, but n = <b>3</b> . <b>No</b> at 5×LOQ (mean, 69%, n = 3).	
	ILV <sup>4</sup>	Yes at LOQ and 5×LOQ, but n = <b>2</b> . <b>No</b> samples were prepared at 10×LOQ.			
Reproducible	Yes at the LOQ, but n = <b>2</b> . <b>No</b> samples were prepared at 10×LOQ.				
Specific	ECM/ILV	The chromatographic profile (primary and confirmatory) of the soils and sediment showed a large peak (>1000×LOQ size) which eluted before the analytes and sloped the baseline with a large tailing portion. This large tailing portion interfered with the peak integrations of first three analytes which eluted from the column.			
	ECM	No additional interferences were observed in the matrix controls; however, analyte peaks were small in the LOQ chromatograms.			

		<b>XDE-105 (spinosyn A)</b>	<b>XDE-105 (spinosyn D)</b>	<b>Spinosyn B</b>	<b>N-demethyl spinosyn D (B of D)</b>
	ILV	No confirmation of analyte identification was performed; confirmatory HPLC/UV analysis was not validated.			
		Matrix interferences were <i>ca.</i> 50% of the LOQ based on peak quantification.	No additional interferences were observed in the matrix controls.		A peak in the baseline interred with peak integration ( <i>ca.</i> 30% of the LOQ based on peak estimation).

Data were obtained from pp. 8, 10-12, 14; Appendix A, p. 29; Appendix A, Tables II-VIII, pp. 34-44; Appendix A, Figures 2-6, pp. 46-51; Appendix B, pp. 55-75 of MRID 44045103; pp. 10-11; Tables I-VII, pp. 13-19; Figures 1-16, pp. 23-38 of MRID 44045104.

- 1 Methods GRM 94.20, HPLC/UV analysis was employed for analyte identification (Appendix A, pp. 21-22 of MRID 44045103). A second different HPLC/UV analysis was used as a confirmation method; however, recovery results were not reported. In the ILV, the HPLC/UV method of the ECM was employed with minor allowed modifications of the mobile phase for optimization; however, no confirmation HPLC/UV method was employed (pp. 10, 12, 39-41 of MRID 44045104).
- 2 Only one calibration curve and correlation coefficient was provided in the ECM: XDE-105 (spinosyn A) in pond sediment, LOQ set (Appendix A, Figure 2, p. 46 of MRID 44045103). The reported ranges for  $r^2$  were reviewer-calculated based on the data in the study report (Appendix B, pp. 56-75; DER Attachment 2), as well as including the one  $r^2$  value provided in the study report. The reviewer determined that the compound codes AA-060, AA-069, AA-059 and AA-061 corresponded to Spinosyn B, N-demethyl spinosyn D (B of D), Spinosyn A and Spinosyn D, respectively, based on comparison of recovery results (Appendix A, Tables II-VI, pp. 34-42; Appendix B, pp. 56-75). For pond sediment, three sets of calibration curve data were provided: LOQ analysis, 2.5×LOQ, 5×LOQ, 7.5×LOQ and 10×LOQ analyses, and 100×LOQ analysis. The reviewer only calculated those for the LOQ data set; the other data sets looked similar.
- 3 Only quantitative HPLC/UV results were provided in the ECM. For ECM Method GRM 94.20, soil/sediment matrices were fully characterized (pp. 8, 10; Appendix A, Table VIII, p. 44 of MRID 44045103). Classifications were not specified as USDA. Sandy loam soil (76.0% sand, 13.6% silt, 10.4% clay; 0.88% organic matter) was collected from Oconee, Georgia. Clay loam soil (34.8% sand, 34.0% silt, 31.2% clay; 1.26% organic matter) was collected from Tunica, Mississippi. Clay loam pond sediment (24.0% sand, 42.2% silt, 33.8% clay; 3.94% organic matter) was collected from a pond near Greenfield, Indiana.
- 4 Only quantitative HPLC/UV results were provided in the ILV. Uncharacterized clay loam soil obtained from the sponsor was used for validation (p. 11 of MRID 44045104).

#### IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, the number of samples ( $n = 2$ ) was insufficient for all analyses (Tables I-VII, pp. 13-19 of MRID 44045104). Mean recoveries and relative standard deviations (RSDs) of statistical significance could not be calculated.

In the ECM, the number of samples ( $n = 3$ ) was insufficient for all analyses, except for those at the LOQ in clay loam pond sediment ( $n = 8$ ; Appendix A, Tables II-VI, pp. 34-42 of MRID 44045103).

OSCPP guidelines recommend a minimum of five samples spiked at each fortification level.

2. In the ILV, no samples were prepared at  $10\times\text{LOQ}$  (Tables I-VII, pp. 13-19 of MRID 44046104). Samples were only prepared at LOQ and  $5\times\text{LOQ}$ .

In the ECM, no  $10\times\text{LOQ}$  samples were prepared in the two soils, only in the one pond sediment (Appendix A, Tables II-VI, pp. 34-42 of MRID 44045103). In the soils, samples were only prepared at LOQ and  $100\times\text{LOQ}$ .

OSCPP guidelines recommend the minimal concentrations of the LOQ and  $10\times\text{LOQ}$  for each analyte/matrix.

3. One of the individual recoveries in the ILV was  $<70\%$ : N-demethyl spinosyn D (66%; Tables I-VII, pp. 13-19 of MRID 44045104). OSCPP guidelines recommend that mean recoveries are within the range 70-120% and RSDs are  $\leq 20\%$ .
4. The clay loam soil matrix was not characterized in the ILV (p. 11 of MRID 44046104). Due to the lack of characterization data, it could not be determined if the ILV was provided with the most difficult soil type with which to validate the method.
5. In GRM 94.20, HPLC/UV analysis was employed for analyte identification (Appendix A, pp. 21-22 of MRID 44045103). A second different HPLC/UV analysis was used as a confirmation method; however, recovery results were not reported. In the ILV, the HPLC/UV method of the ECM was employed with minor allowed modifications of the mobile phase for optimization; however, no confirmation HPLC/UV method was employed (pp. 10, 12, 39-41 of MRID 44045104). The confirmation method which was specified by the ECM should have been validated by the ILV, especially since HPLC/UV was employed as the primary quantification method.
6. In the ECM and ILV, the chromatographic profile (primary and confirmatory) of the soils and sediment showed a large peak ( $>1000\times\text{LOQ}$  size) which eluted before the analytes and sloped the baseline with a large tailing portion (Appendix A, Figures 3-6, pp. 47-51 of MRID 44045103; Figures 5-16, pp. 27-38 of MRID 44045104). This large tailing portion interfered with the peak integrations of first three analytes which eluted from the column.

In the ILV, matrix interferences were observed for spinosyn A and N-demethyl spinosyn D (Figures 10-16, pp. 32-38 of MRID 44045104). For spinosyn A, a matrix contaminant at the same retention time was measured at *ca.* 50% of the LOQ. For N-demethyl spinosyn D, baseline contaminants which measured *ca.* 30% of the analyte peak interfered with peak integration.

In the ECM, the scale of the representative chromatograms was not ideal for specificity assessment, i.e. the analyte peak at the LOQ was small (Appendix A, Figures 3-6, pp. 47-51 of MRID 44045103). Also, a reagent blank was not included.

7. Samples recoveries were corrected in the ILV. In the ILV, calculations allowed for recovery data to be corrected for residues found in the control samples; however, only residues of Spinosyn A were quantified in the control samples (p. 21; Tables I-VII, pp. 13-19; Figures 10-12, pp. 32-34 of MRID 44045104). In the ECM, Method GRM 94.20 allowed for recovery data to be corrected for residues found in the control samples; however, residues were not quantified in any of the control samples (Appendix A, pp. 25-26; Appendix A, Tables II-VI, pp. 34-42 of MRID 44045103).
8. Method GRM 94.20 was validated by the ILV in the second trial after optimization of the HPLC conditions and laboratory equipment (pp. 10, 12, 39-41; Tables I-VIII, pp. 13-20 of MRID 44045104). The mobile phase was optimized to a mobile phase of methanol/acetonitrile/2% ammonium acetate (44:44:12, v:v:v) to improve peak separation; the mobile phase of the ECM was methanol/acetonitrile/2% ammonium acetate:acetonitrile (67:33, v:v) [44:44:12, v:v:v]. Also, silica gel packed in glass columns was used in instead of silica gel SPE columns. The ILV study author noted that these modifications were allowed by the method as chromatographic-condition optimization and use of equivalent laboratory equipment. The reviewer considered these modifications acceptable since the most significant one, the mobile phase constitution change, involved the change of 4% of the mobile phase constitution.

The ILV study author noted that the failure of the first trial was likely due to the degradation of the extracts (p. 41 of MRID 44045104).

9. In the ECM, representative primary HPLC/UV chromatograms were provided for matrix blanks, one calibration standard (175 ng), spiked soil samples at the LOD, LOQ and 100×LOQ, and spiked sediment samples at the LOD, LOQ, 2.5×LOQ, 5×LOQ, 7.5×LOQ, 10×LOQ and 100×LOQ (Appendix A, Figures 3-5, pp. 47-50 of MRID 44045103). Representative confirmatory HPLC/UV chromatograms were provided for the pond sediment only (175 ng calibration standard, control, LOD and LOQ; Appendix A, Figure 6, p. 51). The confirmatory HPLC/UV chromatograms should have been provided for all matrices.

In the ILV, representative chromatograms were provided for all samples/analytes (Figures 5-16, pp. 27-38 of MRID 44045104).

10. The toxicological level of concern was not reported for the analytes in soil.

LOD was not reported in the ILV.

11. In the ILV, the communications between the ILV and the sponsor were documented in summary form in the discussion of the problems encountered during validation (pp. 40-41 of MRID 44045104).
12. It was reported for the ILV that the analytical procedure for one set of 6 samples required approximately eight person hours (1.5 calendar days) for preparation, analysis and result interpretation (p. 41 of MRID 44045104).

## V. References

Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, 55, 2210-2218 (p. 14 of MRID 44045103).

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

## Attachment 1: Chemical Names and Structures

**Spinosyn A [Spinosad, XDE-105 Factor A, Spin A, spinosyn factor A, DE-105 Factor A]**

**IUPAC Name:** (2*R*,3*aR*,5*aR*,5*bS*,9*S*,13*S*,14*R*,16*aS*,16*bR*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-alpha-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetra-deoxy-beta-D-erythro-pyranosyloxy)-9-ethyl-2,3,3*a*,5*a*,6,7,9,10,11,12,13,14,15,16*a*,16*b*-hexadeca-hydro-14-methyl-1*H*-8-oxacyclododeca[*b*]as-indacene-7,15-dione.

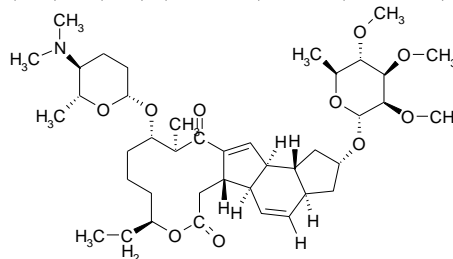
(2*R*,3*aS*,5*aR*,5*bS*,9*S*,13*S*,14*R*,16*aS*,16*bR*)-2-(6-deoxy-2,3,4-tri-*O*-methyl- $\alpha$ -L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetra-deoxy- $\beta$ -D-erythro-pyranosyloxy)-9-ethyl-2,3,3*a*,5*a*,5*b*,6,7,9,10,11,12,13,14,15,16*a*,16*b*-hexadeca-hydro-14-methyl-1*H*-8-oxacyclododeca[*b*]as-indacene-7,15-dione.

**CAS Name:** 2-[(6-deoxy-2,3,4-tri-*O*-methyl-alpha-L-mannopyranosyl)oxy]-13-[[[(2*R*,5*S*,6*R*)-5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl]oxy]-9-ethyl-2,3,3*a*,5*a*,5*b*,6,9,10,11,12,13,14,16*a*,16*b*-tetradeca-hydro-14-methyl-,(2*R*,3*aS*,5*aR*,5*bS*,9*S*,13*S*,14*R*,16*aS*,16*bR*)-1*H*-as-Indaceno[3,2-*d*]oxacyclododecin-7,15-dione.

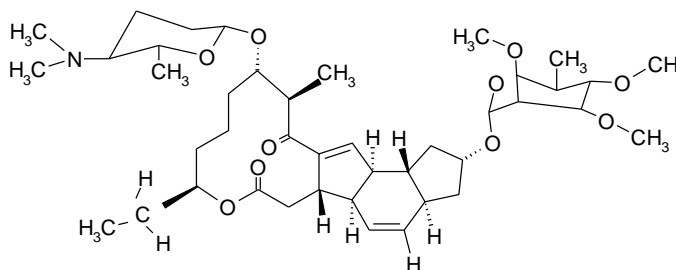
(2*R*,3*aS*,5*aR*,5*bS*,9*S*,13*S*,14*R*,16*aS*,16*bR*)-2-[(6-deoxy-2,3,4-tri-*O*-methyl- $\alpha$ -L-mannopyranosyl)oxy]-13-[[[(2*R*,5*S*,6*R*)-5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl]oxy]-9-ethyl-2,3,3*a*,5*a*,5*b*,6,9,10,11,12,13,14,16*a*,16*b*-tetradeca-hydro-14-methyl-1*H*-as-indaceno[3,2-*d*]oxacyclododecin-7,15-dione.

**CAS Number:** 131929-60-7.

**SMILES String:** CCC6CCCC(OC1CCC(N(C)C)C(C)O1)C(C)C(=O)C5=CC4C(C=CC3CC(OC(O)C2CC(OC)C(OC)C(C)C2OC)CC34)C5CC(=O)O6



Or



**Spinosyn D [Spinosad, XDE-105 Factor D, Spin D, spinosyn factor D]**

**IUPAC Name:** (2*R*,3*aR*,5*aS*,5*bS*,9*S*,13*S*,14*R*,16*aS*,16*bS*)-2-(6-deoxy-2,3,4-tri-*O*-methyl- $\alpha$ -L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetra-deoxy- $\beta$ -D-erythro-pyranosyloxy)-9-ethyl-2,3,3*a*,5*a*,6,7,9,10,11,12,13,14,15,16*a*,16*b*-hexadeca-hydro-4,14-dimethyl-1*H*-8-oxacyclododeca[*b*]as-indacene-7,15-dione.

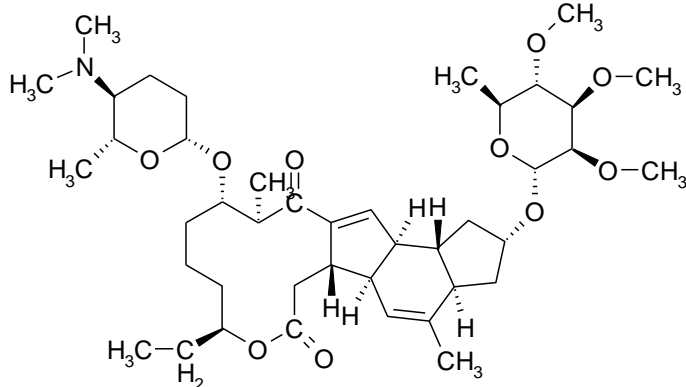
(2*S*,3*aR*,5*aS*,5*bS*,9*S*,13*S*,14*R*,16*aS*,16*bR*)-2-(6-deoxy-2,3,4-tri-*O*-methyl- $\alpha$ -L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetra-deoxy- $\beta$ -D-erythro-pyranosyloxy)-9-ethyl-2,3,3*a*,5*a*,5*b*,6,7,9,10,11,12,13,14,15,16*a*,16*b*-hexadeca-hydro-4,14-dimethyl-1*H*-8-oxacyclododeca[*b*]as-indacene-7,15-dione.

**CAS Name:** 2-[(6-deoxy-2,3,4-tri-*O*-methyl-( $\alpha$ )-L-mannopyranosyl)oxy]-13-[[2*R*,5*S*,6*R*)-5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl]oxy]-9-ethyl-2,3,3*a*,5*a*,5*b*,6,9,10,11,12,13,14,16*a*,16*b*-tetradeca-hydro-4,14-dimethyl-(2*S*,3*aR*,5*aS*,5*bS*,9*S*,13*S*,14*R*,16*aS*,16*bS*)-1*H*-as-Indaceno[3,2-*d*]oxacyclododecin-7,15-dione.

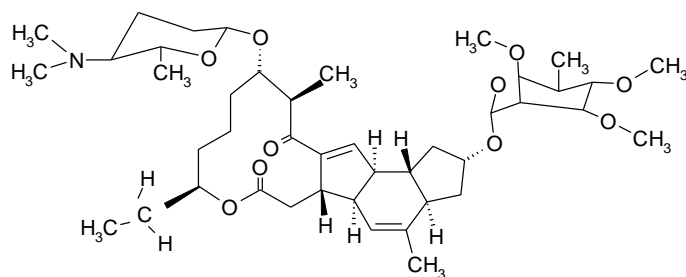
(2*S*,3*aR*,5*aS*,5*bS*,9*S*,13*S*,14*R*,16*aS*,16*bS*)-2-[(6-deoxy-2,3,4-tri-*O*-methyl- $\alpha$ -L-mannopyranosyl)oxy]-13-[[2*R*,5*S*,6*R*)-5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl]oxy]-9-ethyl-2,3,3*a*,5*a*,5*b*,6,9,10,11,12,13,14,16*a*,16*b*-tetradeca-hydro-4,14-dimethyl-1*H*-as-indaceno[3,2-*d*]oxacyclododecin-7,15-dione.

**CAS Number:** 131929-63-0.

**SMILES String:** CCC6CCCC(OC1CCC(N(C)C)C(C)O1)C(C)C(=O)C5=CC3C(C=C(C)C4CC(OC(O)C2CC(OC)C(OC)C(C)C2OC)CC34)C5CC(=O)O6



Or





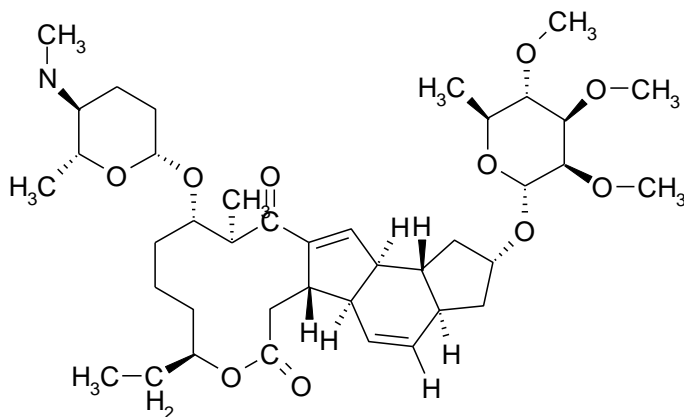
**Spinosyn B [Spin B, spinosyn factor B, DE-105 factor B, N-demethyl spinosyn A]**

**IUPAC Name:** 2-[(6-Deoxy-2,3,4-tri-O-methyl- $\alpha$ -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.

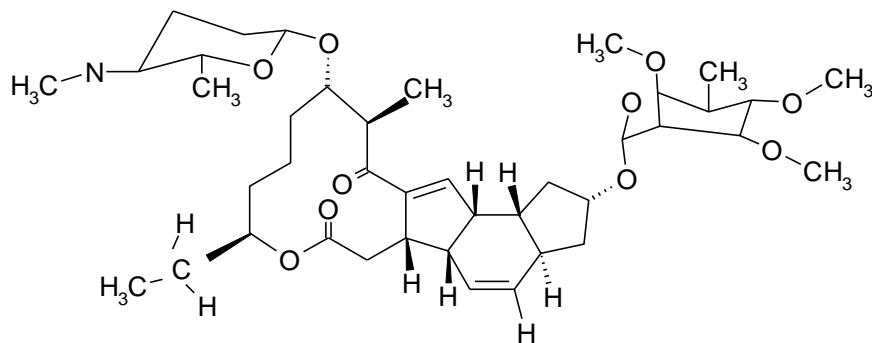
**CAS Name:** 2-[(6-Deoxy-2,3,4-tri-O-methyl- $\alpha$ -L-mannopyranosyl)oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-13-[(tetrahydro-6-methyl-5-(methylamino)-2H-pyran-2-yl)oxy]-1H-as-indaceno[3,2-d]oxacyclododecin-7,15-dione.

**CAS Number:** 131929-61-8.

**SMILES String:** Not found



Or



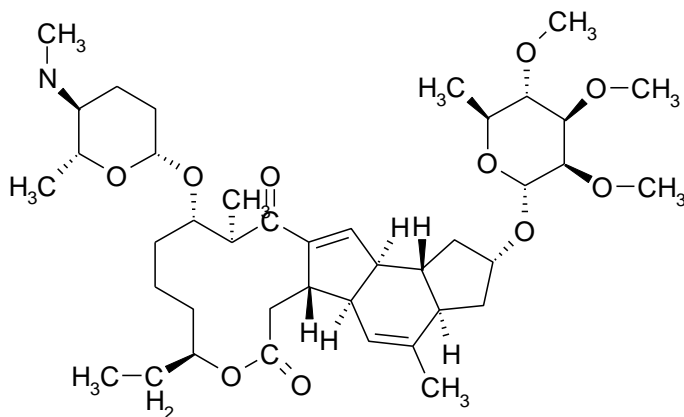
**N-Demethyl spinosyn D [Spinosyn B of D, N-demethyl D, Ndem D]**

**IUPAC Name:** 2-[(6-Deoxy-2,3,4-tri-O-methyl-alpha-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 Name:** 2-[(6-Deoxy-2,3,4-tri-O-methyl-alpha-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:** 149439-70-3.

**SMILES String:** Not found



Or

