Test Material:	Spinosad (XDE-105)
MRID:	44045105
Title:	Residue Method Validation Report for the Determination of XDE-105 and Metabolites in Water by High Performance Liquid Chromatography with Ultraviolet Detection
MRID:	44045106
Title:	INDEPENDENT LABORATORY VALIDATION OF METHOD GRM 94.12 - DETERMINATION OF XDE-105 AND METABOLITES IN WATER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION
EPA PC Code:	110003
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 9/22/15

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Date: 9/22/15

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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 water

Reports: ECM: EPA MRID No.: 44045105. West, S.D. 1995. Residue Method Validation Report for the Determination of XDE-105 and Metabolites in Water by High Performance Liquid Chromatography with Ultraviolet Detection. Laboratory Study ID: RES94092. Report prepared, sponsored and submitted by North American Environmental Chemistry Laboratory, DowElanco, Indianapolis, Indiana; 78 pages. Final report dated March 27, 1995; Method dated January 17, 1995 (pp. 1, 15).
ILV: EPA MRID No. 44045106. Stenzel, J., and B.J. Markley. 1995. INDEPENDENT LABORATORY VALIDATION OF METHOD GRM 94.12 - DETERMINATION OF XDE-105 AND METABOLITES IN WATER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION. Wildlife International Ltd. Project No.: 379C-115. DowElanco Protocol No.: RES95037. Report prepared by Wildlife International Ltd., Easton, Maryland, and sponsored and submitted by DowElanco, Indianapolis, Indiana; 26 pages. Final report issued March 30, 1995.

Document No.: MRIDs 44045105 & 44045106

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 water pH determination, silica SPE elution profiles and purity determination for N-demethyl spinosyn D (p. 3 of MRID 44045105). 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 water, as well as minor GLP deviations regarding purity determination for some of the reference compounds (p. 3 of MRID 44045106).). 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).

Classification: This analytical method is classified as supplemental. An ILV for Method GRM 94.12 without SPE clean-up was not provided. 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 pond water at the LOQ, and only pond water samples were prepared at 10×LOQ. The water matrices were insufficiently characterized in the ECM and ILV; it could not be determined if the ILV was provided with the most difficult water type with which to validate the method. The ECM confirmation method was not validated by the ILV, only

primary HPLC/UV. No linearity data was provided in the ILV. ECM and ILV representative chromatograms did not adequately support the method due to baseline interference. Sample recoveries were corrected in the ECM and ILV.

PC Code: 110003

Reviewer:

Larry Liu

Date: 5/3/17

The page numbers refer to those listed in the upper right-hand corner of the MRID.

Executive Summary

The analytical method, DowElanco Method GRM 94.12, 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 water at the LOQ of 0.001 µg/mL using HPLC/UV. The LOQ is less than or equal to the lowest toxicological level of concern in water for all analytes. The ECM utilized tap, well and pond water matrices; a confirmatory HPLC/UV was performed, but these results were not quantified. Method GRM 94.12 with SPE clean-up was validated by the ILV in the first trial using pond water provided by the sponsor with minor modifications; however, the confirmatory HPLC/UV was not performed. Method GRM 94.12 without SPE clean-up was not performed by the ILV. The water matrices were insufficiently characterized in the ECM and ILV; it could not be determined if the ILV was provided with the most difficult water type with which to validate the method. In the ILV and tap and well water portions of the ECM, the number of samples was insufficient for all analyses. Only pond water samples in the ECM were prepared at 10×LOQ (n = 3). ECM and ILV representative chromatograms did not adequately support the methods due to baseline interference. Samples recoveries were corrected in the ECM and ILV.

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)	44045105 (GRM 94.12)	44045106		Water[1,2]	27/03/1995 (Report date) 17/01/1995 (Method date)	DowElanco	HPLC/UV[3]	0.001 µg/mL
XDE-105 (spinosyn D)								
Spinosyn B								
N-demethyl spinosyn D								

1 For ECM Method GRM 94.12, water matrices were minimally characterized (pp. 8, 10 of MRID 44045105). Tap water (SN 14884901; pH 5.4) was collected from Indianapolis. Well water (SN 14883001; pH 6.8) was collected from Indianapolis, Indiana. Pond water (SN 14882201; pH 7.2) was collected from a pond near Greenfield, Indiana.

2 Uncharacterized pond water obtained from the sponsor (SN 14882201) was used for validation; this was the same pond water which was used in the ECM (p. 9 of MRID 44045106).

3 Method GRM 94.12 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 44045105). No confirmatory HPLC/UV was performed by the ILV.

I. Principle of the Method

Samples (200 mL) of water in 250-mL separatory funnels were mixed with 4.0 mL of 1.0 N sodium hydroxide solution (more can be used to ensure that the pH = *ca*. 12+; p. 9; Appendix A, pp. 20-22 of MRID 44045105). The method noted that the original sample container should be rinsed with methanol if it was glass. After fortification, as necessary, the samples were extracted three times with 50 mL portions of methylene chloride (shaking in separatory funnel for *ca*. 30 seconds under low lighting conditions). After separation, the methylene chloride layers were combined into a boiling flask. The method noted that the sodium sulfate should not be used to remove water from the methylene chloride. 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). As an optional step, 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 1 x 8 mL of methylene chloride:methanol (75:25, v:v). The analytes were collected into culture tubes then transferred to a boiling flask prior to concentration to dryness via rotary vacuum evaporation (water bath set at 35-50°C). When combining the eluate solutions, 2 x 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 2.0 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. 9; Appendix A, p. 19 of MRID 44045105). The instrumental conditions consisted of a YMC ODS-AQ column (4.6 x 150 mm, 5-µm; 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 µL. Retention times for spinosyn A, spinosyn D, spinosyn B and N-demethyl spinosyn D were *ca*. 11, 12.5, 7.8 and 8.8 minutes, respectively, without SPE clean-up and *ca*. 9, 10.8, 5 and 5.8 minutes, respectively, with SPE clean-up (Appendix A, Figures 3-6, pp. 45-49).

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-µm; 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. 20, 26-27 of MRID 44045105). Retention times for spinosyn A, spinosyn D, spinosyn B and N-demethyl spinosyn D were *ca*. 6.9, 7.9, 10 and 12 minutes, respectively (slightly reversed of the primary HPLC/UV method; UV 250 nm; Appendix A, Figure 7, p. 50).

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, pp. 26-27 of MRID 44045105).

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 instruction to not use sodium sulfate for the removal of water, the desorption of the XDE-105 from glass with methanol followed by methylene chloride or just ammonium acetate, the use of SPE was not typically necessary and only used for highly colored samples, and the use of low laboratory lighting to reduce the occurrence of aqueous photolysis of XDE-105 during partitioning (Appendix A, pp. 28-30 of MRID 44045105). The ECM study author noted that GRM 94.12 was performed exactly as written, except for two minor deviations which had no effect on the outcome of the method validation study (p. 9).

ILV

In the ILV, Method GRM 94.12 was performed as written with SPE clean-up, with minor modifications which were allowed by the method: the altering of the volumes of some of the SPE elution solvents (pp. 10-12, 23-24 of MRID 44045106). The HPLC/UV instrument and conditions were the same as those in the ECM. Retention times for spinosyn A, spinosyn D, spinosyn B and N-demethyl spinosyn D were *ca*. 10, 11.5, 6 and 7 minutes, respectively (based on reviewer estimations from representative chromatograms; Figures 1-4, pp. 19-22). No confirmation method was performed.

LOQ/LOD

The LOQ for all four analytes was the same in the ECM and ILV at 0.001 µg/mL (pp. 7, 11; Appendix A, p. 26; Appendix A, Table VIII, p. 42; Appendix B, p. 54 of MRID 44045105; p. 8; Figure 3, p. 21 of MRID 44045106). The LOD for all analytes was 0.0003 µg/mL in the ECM. The LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 44045105: Method GRM 94.12): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean recovery 70-120%; RSD ≤20%) for analysis of XDE-105 (spinosyns A and D), spinosyn B and N-demethyl spinosyn D (B of D) at fortification levels of 0.001 µg/mL (LOQ) and 0.100 µg/mL (100×LOQ) in tap and well water matrices and of 0.001 µg/mL (LOQ), 0.010 g/mL (10×LOQ), 0.025 µg/mL (25×LOQ), 0.050 µg/mL (50×LOQ), 0.075 µg/mL (75×LOQ) and 0.100 µg/mL (100×LOQ) in pond water matrix (Appendix A, Tables II-VII, pp. 32-41 and DER Attachment 2). The number of samples (n = 3) was insufficient for all analyses, except for those at the LOQ in pond water (n = 8). No 10×LOQ samples were prepared in the tap and well waters. The LOQ and 10×LOQ samples in pond water were analyzed with and without SPE clean-up; all other samples/matrices were analyzed only without SPE clean-up. 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 94.12 allowed for recovery data to be corrected for residues found in the control samples; residues were quantified in the control samples, but not reported in the tables (Appendix A, pp. 23-24; Appendix A, Tables II-VII, pp. 32-41). The amounts of Spinosyn B in the control residues were found in the report as 0.0001584 µg/mL (Appendix A, p. 24) and 0.00006-0.00016 µg/mL (tap and well water; Figures 3-4, pp. 45-46). Recoveries from samples fortified at 0.0003 µg/mL (LOD) were reported as <LOQ. The water matrices were minimally characterized (pp. 8, 10). Tap water (SN 14884901; pH 5.4) was collected from Indianapolis. Well water (SN 14883001; pH 6.8) was collected from Indianapolis, Indiana. Pond water (SN 14882201; pH 7.2) was collected from a pond near Greenfield, Indiana.

ILV (MRID 44045106): 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 pond water at fortification levels of 0.001 µg/mL (LOQ) and 0.005 µg/mL (5×LOQ) using the quantitative HPLC/UV analysis (Tables 1-7, pp. 13-16). 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×LOQ. Confirmatory HPLC/UV analysis was not performed. Recovery results of Spinosyn B were corrected for an interference in the control samples; no other recovery corrections were made (pp. 17, 25). Uncharacterized pond water obtained from the sponsor (SN 14882201) was used for validation; this was the same pond water which was used in the ECM (p. 9). Method GRM 94.12 was validated in the first trial with insignificant modifications; a second trial was performed to see if chromatogram contamination of Spinosyn B could be eliminated, but the eluate of one sample at 5×LOQ was spilled prior to analysis and the background contamination of Spinosyn B was still present in the controls (pp. 24-25; Tables 1-7, pp. 13-16).

Table 2. Initial Validation Method Recoveries for XDE-105 and Its Transformation Products in Pond, Tap and Well Water[1,2]

Analyte	Fortification Level (µg/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
GRM 94.12 (with SPE)						
	Pond Water					
XDE-105 (spinosyn A)	0.001 (LOQ)	8	71-105	85	11	13
	0.010	3	74-90	81	8	10
XDE-105 (spinosyn D)	0.001 (LOQ)	8	71-87	77	6	8
	0.010	3	74-85	81	6	7
Spinosyn B	0.001 (LOQ)	8	73-117	90	14	16
	0.010	3	74-91	82	9	11
N-demethyl spinosyn D (B of D)	0.001 (LOQ)	8	72-97	82	10	12
	0.010	3	72-82	78	5	7
GRM 94.12 (no SPE)						
	Tap Water					
XDE-105 (spinosyn A)	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	3	94-104	97	6	6
	0.100	3	88-94	91	3	3
XDE-105 (spinosyn D)	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	3	87-99	95	7	7
	0.100	3	88-94	91	3	3
Spinosyn B	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	3	72-87	79	8	9
	0.100	3	88-93	90	3	3
N-demethyl spinosyn D (B of D)	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	3	89-98	92	5	6
	0.100	3	87-91	89	2	2
	Well Water					
XDE-105 (spinosyn A)	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	3	93-103	100	6	6
	0.100	3	82-93	86	6	7
XDE-105 (spinosyn D)	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	3	87-100	96	8	8
	0.100	3	82-93	86	6	7
Spinosyn B	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	3	80-88	85	5	5
	0.100	3	83-91	86	4	5
N-demethyl spinosyn D (B of D)	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	3	90-99	96	5	5

Analyte	Fortification Level (µg/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.100	3	80-90	84	6	7
	Pond Water					
XDE-105 (spinosyn A)	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	8	85-106	99	10	10
	0.010	3	82-96	90	7	8
	0.025	3	81-95	90	8	9
	0.050	3	87-91	88	5	3
	0.075	3	89-94	91	3	3
	0.100	3	77-92	86	8	9
XDE-105 (spinosyn D)	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	8	75-100	94	10	10
	0.010	3	78-94	87	8	9
	0.025	3	78-93	88	9	9
	0.050	3	86-104	92	10	12
	0.075	3	84-92	88	4	5
	0.100	3	73-89	83	9	10
Spinosyn B	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	8	71-96	88	8	9
	0.010	3	86-96	91	5	5
	0.025	3	76-92	86	9	10
	0.050	3	86-90	88	2	2
	0.075	3	90-92	91	1	1
	0.100	3	86-91	89	3	3
N-demethyl spinosyn D (B of D)	0.0003 (LOD)	1	<LOQ	--	--	--
	0.001 (LOQ)	8	93-111	99	7	7
	0.010	3	82-94	90	7	7
	0.025	3	71-88	82	10	12
	0.050	3	80-84	82	2	2
	0.075	3	85-87	86	1	1
	0.100	3	80-89	86	5	6

Data (corrected recovery results; Appendix A, pp. 23-24; Appendix A, Tables II-VII, pp. 32-41) were obtained from Appendix A, Tables II-VII, pp. 32-41 of MRID 44045105 and DER Attachment 2 (means, s.d. and RSDs for LOQ and 100×LOQ for GRM 94.12 no SPE only).

1 The water matrices were minimally characterized (pp. 8, 10). Tap water (SN 14884901; pH 5.4) was collected from Indianapolis. Well water (SN 14883001; pH 6.8) was collected from Indianapolis, Indiana. Pond water (SN 14882201; pH 7.2) was collected from a pond near Greenfield, Indiana.

2 HPLC/UV analysis was employed for analyte identification (Appendix A, pp. 19-20). 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 Pond Water[1,2]

Analyte	Fortification Level (µg/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
GRM 94.12 (with SPE) – Trial 1 Results						
	Pond Water					
XDE-105 (spinosyn A)	0.001 (LOQ)	2	96, 102	--	--	--
	0.005	2	98, 100	--	--	--
XDE-105 (spinosyn D)	0.001 (LOQ)	2	87, 95	--	--	--
	0.005	2	95, 96	--	--	--
Spinosyn B	0.001 (LOQ)	2	80, 93	--	--	--
	0.005	2	97, 99	--	--	--
N-demethyl spinosyn D (B of D)	0.001 (LOQ)	2	82, 89	--	--	--
	0.005	2	93, 94	--	--	--

Data (uncorrected results for all analytes, except Spinosyn B; pp. 17, 25) were obtained from Tables 1-7, pp. 13-16 of MRID 44045106.

1 Uncharacterized pond water obtained from the sponsor (SN 14882201) was used for validation; this was the same pond water which was used in the ECM (p. 9).

2 HPLC/UV analysis was employed for analyte identification (p. 23). 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 0.001 µg/mL (pp. 7, 11; Appendix A, p. 26; Appendix A, Table VIII, p. 42; Appendix B, p. 54 of MRID 44045105; p. 8; Figure 3, p. 21 of MRID 44045106). The LOD for all analytes was 0.0003 µg/mL 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 water/sediment were calculated in the ECM Method using the standard deviation from the 0.001 µg/mL recovery results. The LOD was calculated as three times the standard deviation (3*s*), and the LOQ was calculated as ten times the standard deviation (10*s*) 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 94.12)[1]

			XDE-105 (spinosyn A)	XDE-105 (spinosyn D)	Spinosyn B	N-demethyl spinosyn D (B of D)
Limit of Quantitation (LOQ)	Established		0.001 µg/mL			
	Calculated (ECM)		0.001 µg/mL			
Limit of Detection (LOD)	Established		0.0003 µg/mL			
	Calculated (ECM)		0.0003 µg/mL			
Linearity (Least squares calibration curve r and concentration range)	ECM[2]	No SPE	r^2 = 0.9995-0.9999	r^2 = 0.9999-1.0000	r^2 = 0.9998-1.0000	r^2 = 0.9998-1.0000
		SPE[3]	r^2 = 0.9998	r^2 = 0.9988	r^2 = 0.9997	r^2 = 0.9977
	ILV		No data or curves reported; reported $r^2 \geq 0.999$.			
	Concentration range		0.0-1.0 µg/mL			
Repeatable	ECM[4]	No SPE	Yes at LOQ (n = 8). Yes at 10×LOQ, 25×LOQ, 50×LOQ, 75×LOQ and 100×LOQ, but n = **3**. (pond water)			
			Yes at LOQ and 100×LOQ, but n = **3**. **No** samples were prepared at 10×LOQ. (tap and well water)			
		SPE	Yes at LOQ (n = 8). Yes at 10×LOQ, but n = **3**. (pond water)			
	ILV (SPE)[5]		Yes at LOQ and 5×LOQ, but n = **2**. **No** samples were prepared at 10×LOQ.			
Reproducible			Yes at the LOQ, but n = **2**. **No** samples were prepared at 10×LOQ.			
Specific	ECM	No SPE	The chromatographic profile (primary and confirmatory) of the waters showed a large peak (100-1000×LOQ size) which eluted before the analytes and sloped the baseline with a tailing portion. This tailing portion interfered with the peak integrations of first two analytes which eluted from the column in the tap water. Some minor (≤50% LOD) residues of Spinosyn B found in controls of tap and well waters.			
		SPE	The chromatographic profile (primary) of the pond water showed a large peak (100-1000×LOQ size) which eluted before the analytes and sloped the baseline with a tailing portion. At the LOQ, significant baseline interference was noted with the first two analytes which eluted from the column.			
	ILV (SPE)		No confirmation of analyte identification was performed; confirmatory HPLC/UV analysis was not validated.			
			No interferences were observed in the matrix controls.		Matrix interferences were *ca*. 70% of the LOQ based on peak estimation.	Matrix interferences were *ca*. 5% of the LOQ based on peak estimation.

Data were obtained from pp. 7-8, 10-11; Appendix A, p. 26; Appendix A, Tables II-VIII, pp. 32-42; Appendix A, Figures 2-7, pp. 44-50; Appendix B, p. 54 of MRID 44045105; pp. 8-9, 17; Tables 1-7, pp. 13-16; Figures 1-4, pp. 19-22 of MRID 44045106.

1 Methods GRM 94.12, HPLC/UV analysis was employed for analyte identification (Appendix A, pp. 21-22 of MRID 44045105). 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 44045106).

2 Only one calibration curve and correlation coefficient was provided in the ECM: Spinosyn B in tap water (Appendix A, Figure 2, p. 44 of MRID 44045105). The reported ranges for r^2 were reviewer-calculated based on the data in the study report (Appendix B, pp. 55-62, 67-74; 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-VII, pp. 32-41; Appendix B, pp. 55-62, 67-74). For pond water with no SPE, two sets of calibration curve data were provided: LOQ and 10×LOQ analysis, and 25×LOQ, 50×LOQ, 75×LOQ and 100×LOQ analyses. The reviewer only calculated those for the LOQ data set; the other data sets looked similar.

3 GRM 94.12 with SPE was only performed for the LOQ and 10×LOQ samples in pond water (Table VI, p. 40 of MRID 44045105).

4 Only quantitative HPLC/UV results were provided in the ECM. For ECM Method GRM 94.12, water matrices were minimally characterized (pp. 8, 10 of MRID 44045105). Tap water (SN 14884901; pH 5.4) was collected from Indianapolis. Well water (SN 14883001; pH 6.8) was collected from Indianapolis, Indiana. Pond water (SN 14882201; pH 7.2) was collected from a pond near Greenfield, Indiana.

5 Only quantitative HPLC/UV results were provided in the ILV. Uncharacterized pond water obtained from the sponsor (SN 14882201) was used for validation; this was the same pond water which was used in the ECM (p. 9 of MRID 44045106).

IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, Method GRM 94.12 was performed as written with SPE clean-up, with minor modifications which were allowed by the method (pp. 10-12, 23-24 of MRID 44045106). In the ECM, Method GRM 94.12 was performed without SPE clean-up using pond, tap and well water and with SPE clean-up using pond water (pp. 21-22; Tables II-VIII, pp. 32-41 of MRID 44045105). Although more matrices and samples were analyzed without SPE clean-up, samples were prepared at the LOQ and 10×LOQ in pond water for ECM validation of GRM 94.12 with SPE clean-up. In the ILV, samples were prepared at the LOQ and 5×LOQ in pond water for validation of GRM 94.12 with SPE clean-up. ILV validation was not performed for GRM 94.12 without SPE clean-up.

2. In the ILV, the number of samples (n = 2) was insufficient for all analyses (Tables 1-7, pp. 13-16 of MRID 44045106). 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 (with and without SPE clean-up), except for those at the LOQ in pond water (n = 8; Appendix A, Tables II-VIII, pp. 32-42 of MRID 44045105).

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

3. In the ILV, no samples were prepared at 10×LOQ (Tables 1-7, pp. 13-16 of MRID 44046104). Samples were only prepared at LOQ and 5×LOQ.

   In the ECM, no 10×LOQ samples were prepared in the tap and well water matrices, only in the pond water matrix (with and without SPE clean-up; Appendix A, Tables II-VIII, pp. 32-42 of MRID 44045105). In the tap and well water matrices, samples were only prepared at LOQ and 100×LOQ.

   OSCPP guidelines recommend the minimal concentrations of the LOQ and 10× LOQ for each analyte/matrix.

4. The water matrices were minimally characterized in the ECM and not characterized in the ILV (pp. 8, 10 of MRID 44045105; p. 9 of MRID 44045106). The pond water matrix of the ILV was the supplied by the sponsor and was same as that of the ECM (SN 14882201). Due to the lack of characterization data, it could not be determined if the ILV was provided with the most difficult water type with which to validate the method.

5. In GRM 94.12, HPLC/UV analysis was employed for analyte identification (Appendix A, pp. 19-20 of MRID 44045105). 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 without modification; however, no confirmation HPLC/UV method was employed (pp. 10-12, 23-24 of MRID 44045106). 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 ILV, no linearity data or curves reported; therefore, individual correlation coefficients could not be reported or determined by the reviewer. The correlation coefficients were reported as greater than or equal to 0.999 (p. 17 of MRID 44045106). Calibration curve slopes and y-intercepts were reported in the tables (Tables 1-8, pp. 13-16).

7. In the ECM, the chromatographic profile (primary and confirmatory) of the waters showed a large peak (100-1000×LOQ size) which eluted before the analytes and sloped the baseline with a large tailing portion (Appendix A, Figures 3-7, pp. 45-50 of MRID 44045105). In the no SPE analysis of tap water, this large tailing portion interfered with the peak integrations of first two analytes which eluted from the column. In the SPE analysis, the large tailing portion also sloped the baseline slightly, but interference with the first two analytes which eluted from the column was caused by contaminants. Some minor (≤50% LOD) residues of Spinosyn B found in controls of tap and well waters.

   In the ILV, matrix interferences were observed for Spinosyn B and N-demethyl spinosyn D (Figures 1-4, pp. 19-22 of MRID 44045106). For Spinosyn B, a matrix contaminant at the same retention time was reviewer-estimated at *ca*. 70% of the LOQ. For N-demethyl

spinosyn D, a matrix contaminant at the same retention time was reviewer-estimated at *ca*. 5% of the LOQ.

8. Samples recoveries were corrected in the ECM and ILV. In the ILV, calculations allowed for recovery data to be corrected for residues found in the control samples; however, only recovery results of Spinosyn B were corrected for an interference in the control samples; no other recovery corrections were made (pp. 17, 25 of MRID 44045106). In the ECM, Method GRM 94.12 allowed for recovery data to be corrected for residues found in the control samples; residues were quantified in the control samples, but not reported in the tables (Appendix A, pp. 23-24; Appendix A, Tables II-VII, pp. 32-41 of MRID 44045105). The amounts of Spinosyn B in the control residues were found in the report as 0.0001584 µg/mL (Appendix A, p. 24) and 0.00006-0.00016 µg/mL (tap and well water; Figures 3-4, pp. 45-46).

9. Method GRM 94.12 was validated by the ILV in the first trial; however, a second trial was performed in order to see if the matrix interference which eluted at the same retention time as Spinosyn B could be eliminated (pp. 24-25; Tables 1-8, pp. 13-16 of MRID 44045106). In the second trial, the interference could not be eliminated, and the recovery results were undesirable because part of the eluate of one of the 5×LOQ samples was spilled. Therefore, the reviewer only reported results from trial 1. The chromatograms were not specified as trial 1 or 2, and only one set of chromatograms was presented in the report (Figures 1-4, pp. 19-22).

10. In the ECM, representative primary HPLC/UV chromatograms were provided for matrix blanks, one calibration standard (50 or 87.5 ng), spiked tap water samples at the LOD and LOQ, spiked well water samples at the LOQ and 100×LOQ, spiked pond water samples without SPE at the LOQ, 25×LOQ, 50×LOQ, 75×LOQ and 10×LOQ, and spiked pond water samples with SPE at the LOQ and 10×LOQ (Appendix A, Figures 3-6, pp. 45-49 of MRID 44045105). Representative confirmatory HPLC/UV chromatograms were provided for the tap water matrix only (87.5 ng calibration standard, control, LOQ and 100×LOQ; Appendix A, Figure 7, p. 50). The confirmatory HPLC/UV chromatograms should have been provided for all matrices.

    In the ILV, representative chromatograms were provided for all samples/analytes (Figures 1-4, pp. 19-22 of MRID 44045106).

    A reagent blank was not included in the ECM or ILV.

11. The toxicological level of concern was not reported for the analytes in water.

    The LOD was not reported in the ILV.

12. In the ILV, the communications between the ILV and the sponsor were documented and contained the discussion of the problems encountered during validation (pp. 24-25 of MRID 44045106).

13. It was reported for the ILV that the analytical procedure for one set of 6 samples required approximately ten person hours (1 calendar day; p. 24 of MRID 44045106).

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. 30 of MRID 44045105).

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

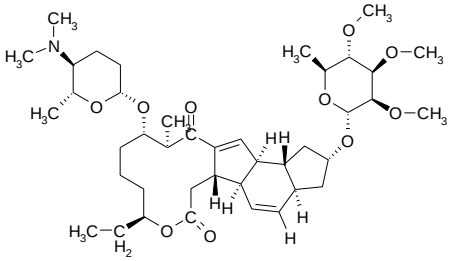
Spinosyn A [Spinosad, XDE-105 Factor A, Spin A, spinosyn factor A, DE-105 Factor A]

IUPAC Name: (2*R*,3aR,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-alpha-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-beta-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione.
(2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-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-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-,(2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-1*H*-*as*-Indaceno[3,2-*d*]oxacyclododecin-7,15-dione.
(2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-[(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyl)oxy]-13-[[(2*R*,5*S*,6*R*)-5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-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



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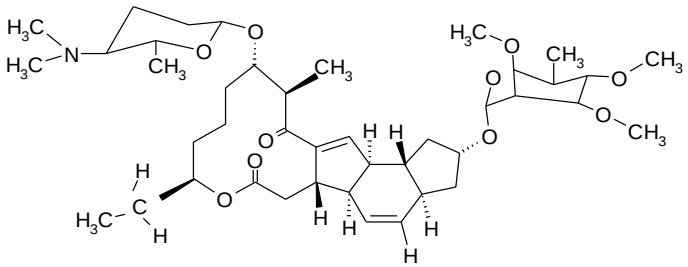


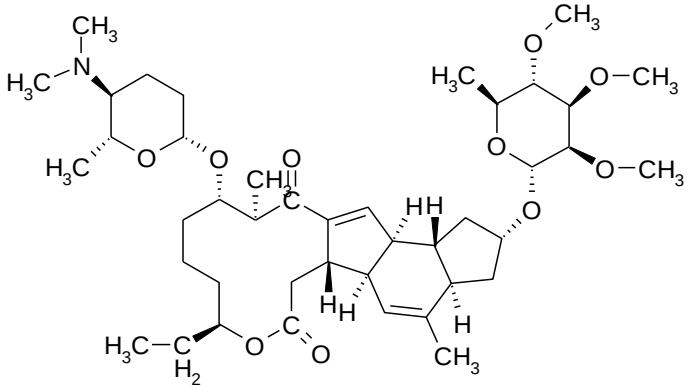
Spinosyn D [Spinosad, XDE-105 Factor D, Spin D, spinosyn factor D]

IUPAC Name: (2R,3a*R*,5a*S*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*S*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-alpha-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-beta-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-4,14-dimethyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione.
(2*S*,3a*R*,5a*S*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-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-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-(2*S*,3a*R*,5a*S*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*S*)-1*H*-*as*-Indaceno[3,2-*d*]oxacyclododecin-7,15-dione.
(2*S*,3a*R*,5a*S*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*S*)-2-[(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyl)oxy]-13-[[(2*R*,5*S*,6*R*)-5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-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



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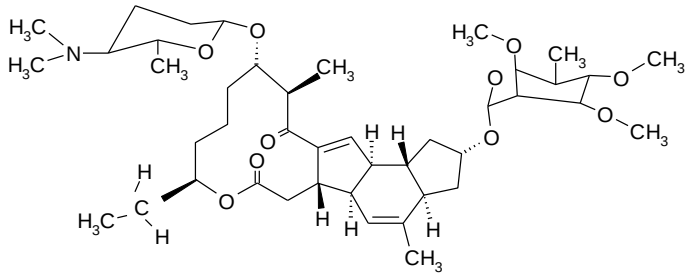

Spinosyn B [Spin B, spinosyn factor B, DE-105 factor B, N-demethyl spinosyn A]

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.

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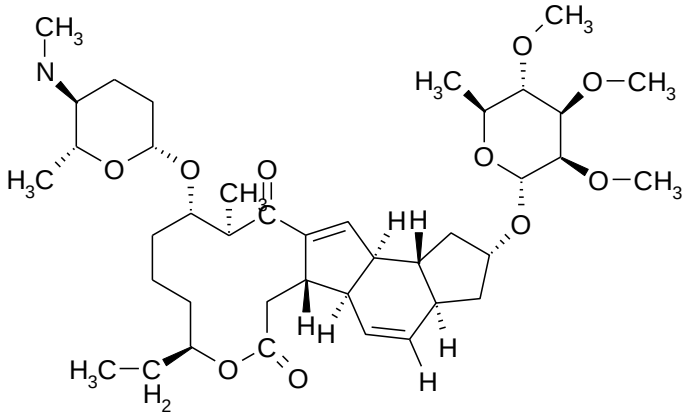

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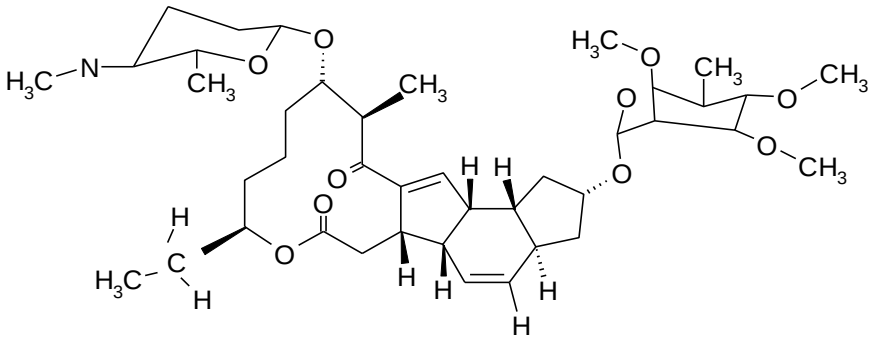

N-Demethyl spinosyn D [Spinosyn B of D, N-demethyl D, Ndem D]

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.

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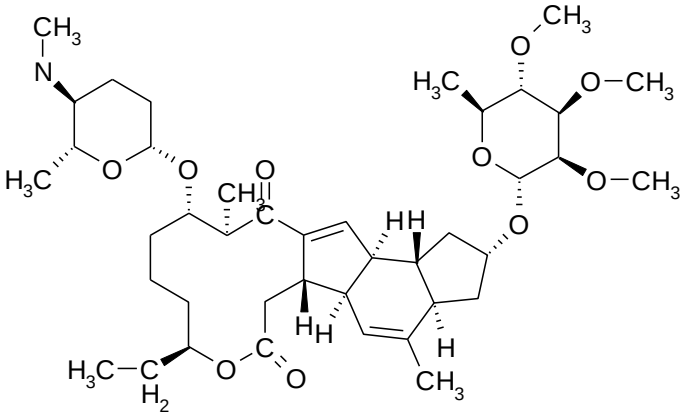

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