SPINOSAD
CIPAC Code No. 636
CIPAC Method Extension

STUDY TITLE

Extension of Analytical Method for Determination of Spinosad
in a New Granule Formulation (GR)
by Reversed Phase High Performance Liquid Chromatography

SPONSOR

ESPAC

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I. LIST OF PARTICIPANTS

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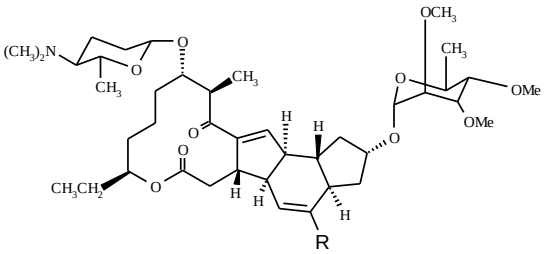
II. INTRODUCTION

Common Name: Spinosad

CIPAC Number: 636



Spinosyn A R = H
Spinosyn D R = CH_3

Chemical name (2*R*,3a*R*,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,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione (Spinosyn A) (CA; 131929-60-7)

mixture with

(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-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-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 (Spinosyn D) (CA; 131929-63-0)

Empirical formula $C_{41}H_{65}NO_{10}$ (Spinosyn A); $C_{42}H_{67}NO_{10}$ (Spinosyn D)

RMM 731.976 (Spinosyn A); 745.988 (Spinosyn D)

m.p. 84-99.5 °C (Spinosyn A); 161.5-170 °C (Spinosyn D)

v.p. 2.4×10^{-10} mm Hg at 25°C (Spinosyn A)
1.6×10^{-10} mm Hg at 25°C (Spinosyn D)

Solubility In water: 89.4 mg/l (Spinosyn A), 0.457 mg/l (Spinosyn D); acetonitrile: 134 g/l (Spinosyn A), 2.55 g/l (Spinosyn D), all at 20 °C

Description White crystalline solid

Stability Hydrolytically stable at 25 °C and pH 5-9
Rapidly photolysed (DT_{50} <1 day)

Formulations Suspension Concentrate (SC), Granule (GR), Tablets for Direct Application (DT); Emulsifiable Concentrate (GR)

An analytical method for quantifying spinosad in technical material and SC and GR formulation was developed and validated by Dow AgroSciences in 2004. Following a pilot trial conducted in 2004 and a full collaborative trial in 2005, the method was accepted as a full CIPAC method during the June 2006 annual CIPAC meeting. Extensions of the method were accepted as full CIPAC method for DT formulation in 2007 and EC formulation in 2010. The full CIPAC method for GR formulation was evaluated for a new GR formulation in 2012. Since the active ingredient content was outside the acceptability range covered by the GR in the full collaborative study, an extension of the method was required. A protocol, data spreadsheet, MSDS, samples and standards were shipped to five laboratories (Section I) to evaluate the method extension. The results from the evaluation are presented in this report.

III. SAMPLES AND STANDARDS

The following samples and standards were sent to the participants in April 2012.

Sample Identification	Batch Identification	Dow AgroSciences Test Substance Number	Lot Number	Concentration/ Purity
Spinosad GR Formulation	Batch 1	TSN300983	100812-11	25 g/kg [1]
Spinosad GR Formulation	Batch 2	TSN300984	100902-04	25 g/kg [1]
Spinosad GR Formulation	Batch 3	TSN300985	100907-07	25 g/kg [1]
Spinosad GR Formulation	Batch 4	TSN300986	100908-05	25 g/kg [1]
Spinosad GR Formulation	Batch 5	TSN300987	100913-11	25 g/kg [1]
Spinosad GR Formulation Blank	—	TSN300981	NB141-01-02	—
Spinosad Analytical Standard	—	TSN105822	F50-F1249-20	82.1% Spinosyn A 13.6% Spinosyn D

[1] Nominal concentration of spinosad

IV. ANALYTICAL METHOD

1. Scope

The method is applicable to the determination of spinosad in GR formulations containing spinosad at nominal concentrations of 25 g/kg. The method was previously demonstrated to be applicable to GR formulations containing spinosad at nominal concentrations of 5 g/kg.

2. Outline of Method

An aliquot of the sample is diluted in a mixture of methanol and water and analyzed by HPLC using a YMC ODS-AQ column and ultraviolet (UV) detection at 250 nm. Quantitation is external standard calibration using peak areas.

3. Procedure

Two calibration standard solutions and one sample solution for each GR sample provided were prepared on each day of analysis. GR samples were injected in duplicate and bracketed by single injections of the calibration solutions. Samples were analyzed on two days. The concentration of spinosyn A and spinosyn D was calculated based on the response factors from the two bracketing calibration standards. Spinosad concentration was the sum of spinosyn A and spinosyn D concentrations. Prior to analyses, the mobile phase and GR formulation blank solutions were analyzed to evaluate interferences with spinosyn A or spinosyn D.

V. REMARKS OF THE PARTICIPANTS

1. Analytical Conditions

Lab.	Column	Column Temp.	Mobile Phase	Flow Rate	Detection	Injection Volume
Protocol	YMC ODS-AQ, S-5 µm, 120Å, 4.6 × 150 mm	35°C	Methanol+acetonitrile+ 2% ammonium acetate in water, pH 5.3 with glacial acetic acid (40+40+20)	1.5 mL/min.	UV λ=250 nm	20 µL
1	Same	Same	Same	Same	Same	Same
2	Same	Same	Same	Same	Same	Same
3	Same	Same	Same	Same	Same	Same
4	Same	Same	Same	Same	Same	Same
5	Same	Same	Same	Same	Same	Same

2. Remarks

Lab 1: Only four batches were received.

Lab 2: The 100ml jars we had available on day 1 were only large enough to add 100ml with no headspace left so I was a bit concerned about the extraction. We had a delivery of new jars on the 2nd day which had a bigger head space so the shaking was more efficient.

Lab 5: In addition to shaking the sample solutions for 1 hour, the day 1 samples were sonicated for approximately 10 minutes after shaking and the day 2 samples were sonicated for approximately 10 minutes prior to shaking. Sonication was a more effective method of removing the outer coating of the granules, as shaking alone was ineffective.

VI. RESULTS AND DISCUSSION

1. Outliers and Stragglers

Five sample sets were distributed, and results from five laboratories were obtained. Laboratory data are presented in Tables I and II and are graphically presented in Figure 1. The statistical evaluations for outlying results were completed according to "CIPAC Guidelines for Collaborative Study Procedures for Assessment of Performance of Analytical Methods" and ISO 5725-2:1994(E). Outliers and stragglers were determined using Cochran's test and Grubb's test. An outlying result was considered a straggler if the test statistic exceeded the critical value at α=5% and was considered an outlier if the test statistic exceeded the critical value at α=1%. The statistical evaluation of the data collected for this trial identified no outliers or stragglers.

2. Repeatability and Reproducibility

Individual data and a graphical presentation of the results are provided in this report (Table I and Figure 1). For the five batches of GR formulation, the repeatability relative standard deviation (RSD_r) ranged from 1.9 to 3.8% (Table II).

The reproducibility relative standard deviation (RSD_R) ranged from 3.1 to 4.8% (Table II). The RSD_R for three of the batches exceeded the Horwitz calculation for acceptable reproducibility (Horwitz RSD_R = 3.5% for all five batches). However, the Horwitz calculation is a prediction of reproducibility in an analytical method rather than a requirement. For granule formulations with active ingredient content of 25 g/kg or less, greater variability in analytical results can be expected due to the difficulty in obtaining a representative sample.

3. Discussion

During the full collaborative study conducted in 2005, data from eleven laboratories were used to evaluate the analytical method for a GR formulation at a nominal spinosad concentration of 5 g/kg. In that study, the RSD_r was 3.3, 3.4, and 4.7%, and the RSD_R was 3.8, 4.5, and 4.9% (Table III and Figure 2). For the study reported here, the RSD_r for three of the five 25 g/kg GR batches was less than the RSD_r for all of the GR batches in the full collaborative study, and the highest pair of RSD_r results were within 0.1%. Similarly, the RSD_R for four of the five 25 g/kg GR batches was less than the RSD_R for all of the GR batches in the full collaborative study, and the all of the RSD_R were less than the highest RSD_R in the full collaborative study. Repeatability and reproducibility are considered to be within the range determined during the full collaborative study.

VII. CONCLUSIONS

Based on the relative standard deviation results, the method extension trial for the 25 g/kg spinosad GR formulation demonstrated repeatability and reproducibility that was comparable to or better than that for the 5 g/kg spinosad GR formulation in the full collaborative study. Therefore, it is recommended that the currently published method for 5 g/kg spinosad GR formulations be adopted for 25 g/kg spinosad GR formulations.

TABLE I. Results Summary by Laboratory

GR Batch	Laboratory	Mean Concentration (g/kg) Day 1	Day 2	Overall	Standard Deviation (s)
1	1	24.4	25.0	24.7	0.45
	2	23.8	24.5	24.1	0.53
	3	22.7	22.5	22.6	0.11
	4	25.0	26.1	25.6	0.79
	5	23.5	24.3	23.9	0.57
	Mean			24.2	
2	1	*	*	*	*
	2	24.4	24.8	24.6	0.26
	3	25.3	24.1	24.7	0.86
	4	24.5	26.1	25.3	1.16
	5	23.9	24.5	24.2	0.45
	Mean			24.7	
3	1	25.5	24.9	25.2	0.39
	2	23.5	24.9	24.2	1.02
	3	24.3	24.5	24.4	0.17
	4	24.6	25.4	25.0	0.54
	5	23.1	23.7	23.4	0.47
	Mean			24.4	
4	1	24.9	24.6	24.7	0.25
	2	23.3	24.2	23.7	0.69
	3	23.4	25.9	24.7	1.77
	4	24.0	25.1	24.5	0.80
	5	25.8	25.5	25.7	0.26
	Mean			24.7	
5	1	24.5	24.4	24.4	0.08
	2	21.9	22.6	22.2	0.49
	3	23.1	24.3	23.7	0.80
	4	24.3	23.7	24.0	0.40
	5	23.4	23.5	23.4	0.06
	Mean			23.6	

* Batch 2 was inadvertently not included in the shipment of test materials to laboratory 1.

TABLE II. Summary of Statistical Evaluation — Method Extension (25 g/kg Spinosad GR Formulation)

		25 g/kg Spinosad GR Formulation				
Batch Number		1	2	3	4	5
Mean (x)		24.2	24.7	24.4	24.7	23.6
Standard Deviation (s_x)		1.10	0.45	0.72	0.68	0.82
Number of Labs (L)		5	4	5	5	5
Number of Analyses per Lab and Batch (n)		2	2	2	2	2
s_r	$s_r = \sqrt{\sum s^2 / L}$	0.54	0.77	0.59	0.94	0.46
s_L	$s_L = \sqrt{(s_x)^2 - (s_r)^2 / n}$	1.03	0.00	0.59	0.15	0.75
s_R	$s_R = \sqrt{(s_r)^2 + (s_L)^2}$	1.16	0.77	0.84	0.95	0.88
r	s_r*2.8	1.50	2.15	1.65	2.63	1.28
R	s_R*2.8	3.25	2.15	2.34	2.66	2.46
RSD_r	s_r/x*100%	2.2%	3.1%	2.4%	3.8%	1.9%
RSD_R	s_R/x*100%	4.8%	3.1%	3.4%	3.9%	3.7%
RSD_R(Horwitz)	$2^{(1-0.5\log(\text{Mean}/100))}$	3.5%	3.5%	3.5%	3.5%	3.5%

TABLE III. Summary of Statistical Evaluation — Full Collaborative Study (5 g/kg Spinosad GR Formulation)

		5 g/kg Spinosad GR Formulation		
Batch Number		1	2	3
Mean (x)		5.7	6.4	6.2
Standard Deviation (s_x)		0.12	0.19	0.26
Number of Labs (L)		11	11	11
Number of Analyses per Lab and Batch (n)		2	2	2
s_r	$s_r = \sqrt{\sum s^2 / L}$	0.27	0.22	0.21
s_L	$s_L = \sqrt{(s_x)^2 - (s_r)^2 / n}$	0.00	0.12	0.21
s_R	$s_R = \sqrt{(s_r)^2 + (s_L)^2}$	0.27	0.25	0.30
r	s_r*2.8	0.76	0.60	0.57
R	s_R*2.8	0.76	0.69	0.83
RSD_r	s_r/x*100%	4.7%	3.4%	3.3%
RSD_R	s_R/x*100%	4.7%	3.8%	4.8%
RSD_R(Horwitz)	$2^{(1-0.5\log(\text{Mean}/100))}$	4.3%	4.3%	4.3%

FIGURE 1. Graphical Presentation of Data — 25 g/kg Spinosad GR Formulation

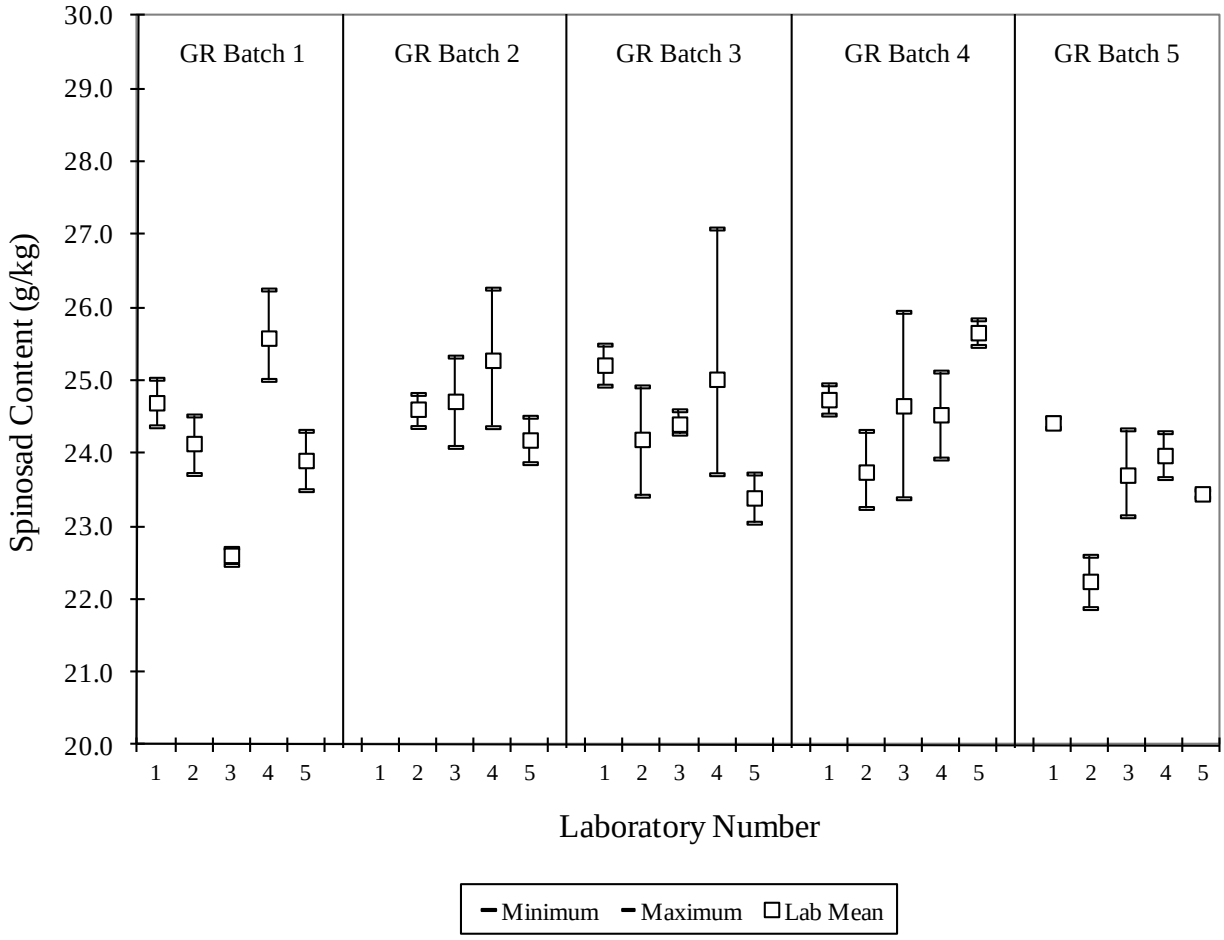


FIGURE 2. Graphical Comparison of Repeatability and Reproducibility Relative Standard Deviations