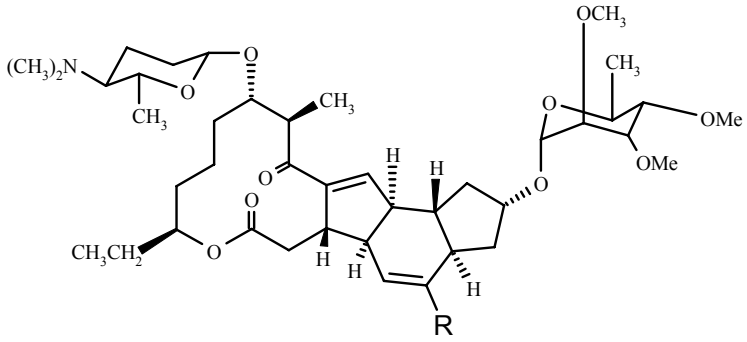
SPINOSAD
636



spinosyn A	R = H
spinosyn D	R = CH_3

ISO common name Spinosad, a mixture of spinosyn A and spinosyn D in the proportion 50-95% to 50-5%

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-erythopyranosyloxy)-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 (spinosyn A) (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-erythopyranosyloxy)-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 (spinosyn D) (IUPAC)

(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*R*,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	732.0 (spinosyn A); 746.0 (spinosyn D)
m.p.	84-99.5 °C (spinosyn A); 161.5-170 °C (spinosyn D)
v.p.	3.2 × 10-8 Pa at 25°C (spinosyn A) 2.1 × 10-8 Pa 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 (DT50 <1 day)
Formulations	Suspension concentrates and granules

SPINOSAD TECHNICAL
*636/TC/(M)/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1HPLC. Use the HPLC method below. The relative retention time of spinosyn A and spinosyn D for the sample solution should not deviate by more than 2% from that of the calibration solution.

2.2 LC-MS. Use an HPLC coupled to a mass spectrometer operating in the positive electrospray ionisation mode. Use LC conditions and sample preparation outlined below. The spectra obtained from the sample should be consistent with the spectra obtained from the standard. The quasi molecular ions, $[M-H]^-$ at *m/z* 747 and *m/z* 732 should be observed in both spectra.

3 Spinosad

OUTLINE OF METHOD Spinosad is dissolved in methanol and determined by high-pressure liquid chromatography using an octadecylsilyl column, ultraviolet detection at 250 nm and external standardisation.

REAGENTS

Spinosad, of known purity, purity higher than 90%

Methanol

Acetonitrile

Acetic acid glacial

Ammonium acetate

Ammonium acetate solution. Dissolve 20 g of ammonium acetate in water (1l). Adjust to pH = 5.3 with acetic acid.

Mobile phase methanol - acetonitrile - ammonium acetate solution, 40+40+20 (v/v)

Calibration solution. Weigh (to the nearest 0.1 mg) about 25 mg (*s* mg) of spinosad into a flask (150 ml). Add by pipette methanol (100 ml) and mix thoroughly.

* Provisional CIPAC method 2005. Prepared by a committee chaired by D Heim. Based on a method supplied by Dow Agroscience–LLC, USA.

APPARATUS

Liquid chromatograph equipped with a spectrometric detector capable of measuring at 250 nm

Liquid chromatographic column, 150 × 4.6 (i.d.) mm, packed YMC ODS-AQ with spherical 5 µm particles and 12 nm pore size (YMC Co. Ltd., No AQ12S051546WT, or equivalent).

Integrator or *data system*

PROCEDURE

(a) Operating conditions (typical):

Column	150 × 4.6 (i.d.) mm packed with YMC ODS-AQ, with spherical 5 µm particles and 12 nm pore size
Mobile phase	water - acetonitrile - acetic acid, 40 + 40 + 20 (v/v)
Flow rate	1.5 ml/min
Temperature	35 °C
Detector wavelength	250 nm
Injection volume	20 µl
Run duration	20 min
Retention times	spinosyn A: about 9.0 min spinosyn D: about 12.0 min

(b) Sample preparation. Weigh (to the nearest 0.1 mg) into a flask (150 ml) sufficient sample to contain about 25 mg of spinosad (*w* mg). Add by pipette methanol (100 ml) and mix thoroughly.

(c) System equilibration. Pump sufficient eluant through the column to equilibrate the system. Inject 20 µl calibration solution into the column and adjust the operating conditions to give retention times about 9.0 and 12.0 min for spinosyn A and spinosyn D respectively. Resolution between spinosyn A and D should be ≥ 1.5. Repeat the injections until the peak areas are within ±1% of the previous injection.

(e) Determination. Inject in duplicate 20 µl portions of the sample solution bracketing them by injections of the calibration solution. Determine the relevant peak areas and calculate the sum of the spinosyn A plus spinosyn D peak areas.

(f) Calculation

$$\text{Content of spinosad} = \frac{H_w \times s \times P}{H_s \times w} \text{ g/kg}$$

where:

H_w = average of the sum of the spinosyn A plus D peak areas for the sample
H_s = average of the sum of the spinosyn A plus D peak areas for the calibration solution
s = mass of spinosad in the calibration solution (mg)
w = mass of the sample taken (mg)
P = purity of spinosad (spinosyn A plus D) (g/kg)

Repeatability r = 32 g/kg at 900 g/kg active ingredient content
37 g/kg at 884 g/kg active ingredient content
Reproducibility R = 38 g/kg at 900 g/kg active ingredient content
38 g/kg at 884 g/kg active ingredient content

SPINOSAD SUSPENSION CONENTRATES
*636/SC/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for **636**/TC/(M)/2.1.
2.2 LC-MS. As for **636**/TC/(M)/2.2.

3 Spinosad. As for **636**/TC/(M)/3 except:
(b) Sample preparation. Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) sufficient sample to contain about 25 mg of spinosad (*w* mg). Add water (5 ml) and methanol (90 ml), mix and allow to cool to room temperature. Fill to the mark with methanol. Filter through 0.2 µm PTFE membrane before injection.

Repeatability r = 14 g/kg at 432 g/kg active ingredient content
17 g/kg at 458 g/kg active ingredient content
41 g/kg at 461 g/kg active ingredient content
Reproducibility R = 22 g/kg at 432 g/kg active ingredient content
31 g/kg at 458 g/kg active ingredient content
41 g/kg at 461 g/kg active ingredient content

* Provisional CIPAC method 2005. Prepared by a committee chaired by D Heim. Based on a method supplied by Dow Agroscience–LLC, USA.

4 Suspensibility

REAGENTS AND APPARATUS As for **636**/TC/(M)/3 and MT 184 except add at:
Calibration solution. Dilute with methanol to the anticipated spinosad concentration.

PROCEDURE

(a) Preparation of suspension. MT 184 *(a)(ii).*

(b) Determination of sedimentation. MT 184 *(b).*

(c) Determination of spinosad in the bottom 25 ml of suspension. After removal of the top 225 ml of suspension, transfer the remaining 25 ml of suspension quantitatively to a volumetric flask (50 ml) with methanol, add methanol to within a few cm below the mark and mix. Allow to attain room temperature and fill to the mark with methanol. Mix thoroughly. Determine the mass of spinosad (*Q* g) by **636**/TC/(M)/3.

(d) Calculation

$$\text{Suspensibility} = \frac{111(c - Q)}{c}\ \%$$

where:
c = mass of spinosad in the sample taken for the preparation of suspension (g)
Q = mass of spinosad in the bottom 25 ml of suspension (g)

SPINOSAD GRANULES
*636/GR/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for **636**/TC/(M)/2.1.
2.2LC-MS. As for **636**/TC/(M)/2.2.

* Provisional CIPAC method 2005. Prepared by a committee chaired by D Heim. Based on a method supplied by Dow Agroscience–LLC, USA.

3 Spinosad. As for **636**/TC/(M)/3 except:

(b) Sample preparation. Weigh (to the nearest 0.1 mg) into a ground glass stoppered flask (150 ml) sufficient sample to contain about 25 mg of spinosad (*w* mg). Add by pipette methanol (100 ml) and shake for 1 hour. Allow to cool to room temperature. Filter through a 0.2 µm PTFE membrane before injection.

Repeatability r = 0.57 to 0.76 g/kg at 5.7 to 6.4 g/kg active ingredient content

Reproducibility R = 0.69 to 0.83 g/kg at 5.7 to 6.4 g/kg active ingredient content