Spinetoram CIPAC 5249/m analytical method for TC and SC, WG and DT formulations

Outline of CIPAC Method: Spinetoram is determined by reversed phase high performance liquid chromatography (HPLC) using UV detection at 250nm and external standardization.

Reagents

Spinetoram reference standard with known Spinetoram-J and Spinetoram-L purities

Water HPLC Grade

Acetonitrile HPLC Grade

Methanol HPLC Grade

Buffer pH 5.5, 2g/L Ammonium Acetate in water

Solvent Mix A Acetonitrile – Methanol 80 + 20 (v/v)

Eluent Solvent Mix A – Buffer pH 5.5 80 + 20 (v/v)[1]

Calibration Solution Weigh in duplicate (to the nearest 0.1mg) about 43mg of the Spinetoram standard (s mg) into separate volumetric flasks (100mL). Add water (about 10mL) and swirl briefly to disperse. Add methanol (50mL) and shake to dissolve. Adjust to mark with methanol and mix well (Solutions C_1 and C_2).

Apparatus

High performance liquid chromatograph equipped with an ultraviolet spectrophotometric detector and an injection system capable to inject 10μL

Column Phenomenex Luna C8(2) 3μ 150x4.6mm, or equivalent material with the same selectivity

Electronic Integrator

Mechanical Shaker

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[1] If mixed online this ratio is equivalent to Acetonitrile – Methanol – Buffer 64 + 16 + 20 (v/v/v)

Disposable filters, solvent compatible, porosity 0.45μm Nylon

Procedure

a. Chromatographic Conditions (typical)

Parameter	Specification
Column Temperature	30 ºC
Flow Rate	1.0 mL/min
Measuring Wavelength	250 nm
Injection Volume	10 µL
Run Time	Approx. 20min
Retention Time	Spinetoram-J: 10- 12min Spinetoram-L: 12-15min

b. *Equilibration of the system*
Pump sufficient eluent through the column to equilibrate the system. Inject 10µL portions of the calibration solution C_1 and repeat the injections until retention times and peak areas vary by less than ±0.5% of the mean for three successive injections.

c. *Sample preparation (TC, SC, WG)*
Weigh accurately, to the nearest 0.1 mg, sufficient sample to contain about 40mg spinetoram into a 100 mL volumetric flask. Add 10.0 mL of purified water with a volumetric pipette and briefly swirl sample to disperse. Add 50 mL of methanol and shake to dissolve. Adjust to 100.0mls with Methanol. Filter through a 0.45 µm nylon syringe filter for LC analysis, taking care to discard to waste the first few filtered drops.

d. *Sample Preparation* (DT)

For analysis of production or market samples at least 5 tablets from each batch should be milled or ground and then sub-samples taken for analysis as described below.

Weigh accurately, to the nearest 0.1 mg, sufficient sample of ground tablet to contain about 10 mg spinetoram into a 25 mL volumetric flask. Add 2.5 mL of purified water with a volumetric pipette and briefly swirl sample to disperse. Add 15 mL of methanol and shake to dissolve. Adjust to 25.0mls with Methanol. Filter through a 0.45 µm nylon syringe filter for LC analysis, taking care to discard to waste the first few filtered drops.

e. *Determination*

Inject 10µL portions of the calibration solutions (C_1 and C_2) and of the sample solutions (S_1, S_2, ..., etc.) in the following sequence:

C_1, S_1, C_2, S_2, ...

Determine the peak area of each Spinetoram component (Spinetoram-J and Spinetoram-L) and calculate the response factors (*f*) from the calibration solutions bracketing the injections of the sample solutions. Average the response factors of the calibration solutions preceding and following the sample solution injections. The results for each individual component **must** be calculated separately and then added together for the total Spinetoram content. Do not add the areas together first.

Calculations

Amount Spinetoram-J or Spinetoram-L (mg) = $W_a \times P$

Where*:*
W_a = weight of respective component in calibration solution
P = Purity, decimal

RF = Amount Spinetoram* ÷ Area Spinetoram*

Where:
RF = Response factor for respective component J or L
*Amount Spinetoram** = calculated amount of respective J or L component (mg) in calibration solution
*Area Spinetoram** = Peak area for respective J or L component in calibration solution

Weight % = Area Spinetoram* × RF ÷ S × 100%

Where:
Weight % = Weight % of respective J or L component
*Area Spinetoram** = Peak area of respective J or L component in sample solution
RF = Response factor calculated for respective J or L component
S = Sample weight, mg

The total amount of Spinetoram is the sum of the weight % of Spinetoram-J and Spinetoram-L.