Original: Japanese

Provisional Translation

Analytical Method for Spinosad (Animal and Fishery Products)

1. Analytes

Spinosyn A

Spinosyn D

2. Instrument

Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents

Use the reagents listed in Section 3 of the General Rules, except the following.

Reference standard of spinosyn A: Contains not less than 90% of spinosyn A.

Reference standard of spinosyn D: Contains not less than 90% of spinosyn D.

4. Procedure

1) Extraction

i) Muscle, fat, liver, kidney, fish and shellfish

For muscle, liver, kidney, fish and shellfish, weigh 20.0 g of sample. For fat, weigh 5.00 g of sample.

Add 20 mL of 1 mol/L dipotassium hydrogen phosphate, and homogenize. Add 100 mL of acetone/*n*-hexane (1:2, v/v), homogenize again, centrifuge at 3,000 rpm for 5 minutes, and collect the organic layer. Add 50 mL of *n*-hexane to the residue, homogenize, and centrifuge as described above. Combine the organic layers, dehydrate with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. Dissolve the residue in *n*-hexane to make exactly 20 mL.

ii) Milk, egg and honey

Weigh 10.0 g of sample.

Add 10 mL of 1 mol/L dipotassium hydrogen phosphate, and homogenize. Add 100 mL of acetone/*n*-hexane (1:2, v/v), homogenize again, centrifuge at 3,000 rpm for 5 minutes, and collect the organic layer. Add 50 mL of *n*-hexane to the residue, homogenize, and centrifuge as described above. Combine the organic layers, dehydrate with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. For milk and egg, dissolve the residue in 10 mL of *n*-hexane. For honey, dissolve the residue in acetone/*n*-hexane (1:1, v/v) to make exactly 10 mL.

2) Clean-up

i) Muscle, fat, liver, kidney, fish, shellfish, milk and egg

a) Porous diatomaceous earth column chromatography

Transfer 10 mL (total volume for milk and egg) of the solution obtained in 1) to the porous diatomaceous earth cartridge (to hold 20 mL of solution). Let stand for 10 minutes, elute

with 80 mL of acetonitrile saturated with *n*-hexane, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in acetone/*n*-hexane (1:1, v/v) to make exactly 10 mL.

b) Trimethylaminopropylsilanized silica gel column chromatography and ethylenediamine-*N*-propylsilanized silica gel column chromatography

Connect an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) under a trimethylaminopropylsilanized silica gel cartridge (500 mg), add 10 mL of acetone/*n*-hexane (1:1, v/v), and discard the effluent. Transfer 2 mL of the solution obtained in a) to the cartridge, elute with 10 mL of acetone/*n*-hexane (1:1, v/v), collect the total eluate, concentrate at below 40°C and remove the solvent. Dissolve the residue in methanol to make exactly 4 mL (1 mL for fat), and use this solution as the test solution.

ii) Honey

Connect an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) under a trimethylaminopropylsilanized silica gel cartridge (500 mg), add 10 mL of acetone/*n*-hexane (1:1, v/v), and discard the effluent. Transfer 2 mL of the solution obtained in 1) to the cartridge, elute with 10 mL of acetone/*n*-hexane (1:1, v/v), collect the total eluate, concentrate at below 40°C and remove the solvent. Dissolve the residue in methanol to make exactly 4 mL, and use this solution as the test solution.

5. Calibration curve

Prepare spinosyn A and spinosyn D standard solutions (methanol) of several concentrations. Inject each standard solution to LC-MS, and make a calibration curve by peak-height or peak-area method. When the test solution is prepared following the above procedure, the sample containing 0.01 mg/kg of spinosyn A or spinosyn D gives the test solution of 0.005 mg/L in concentration.

6. Quantification

Inject the test solution to LC-MS. Calculate the concentration of spinosyn A and spinosyn D from the calibration curves made in **5**, and regard the sum of the results as the analytical result of spinosad.

7. Confirmation

Confirm using LC-MS or LC-MS/MS.

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 100 mm in length and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Acetonitrile/10 mmol/L ammonium acetate solution (3:1, v/v)

Ionization mode: ESI (+)

Major monitoring ion (*m/z*)

Spinosyn A: 733, 732, 142

Spinosyn D: 747, 746, 142

Injection volume: 5 μL

Expected retention time: Spinosyn A 8 min, Spinosyn D 10 min

9. Limit of quantification

Spinosyn A 0.01 mg/kg

Spinosyn D 0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of spinosyn A and spinosyn D from sample with acetone/*n*-hexane (1:2, v/v) under weak basic condition, defatting with porous diatomaceous earth cartridge (omitted for honey), clean-up with connected cartridges of trimethylaminopropyl-silanized silica gel and ethylenediamine-*N*-propylsilanized silica gel, quantification and confirmation using LC-MS.

2) Notes

i) Quantify spinosyn A and spinosyn D individually, and regard the sum of the results as the analytical result of spinosad.

ii) Because spinosyn A and spinosyn D in some samples are not extracted into the organic layer, extraction should be performed under neutral to weak basic conditions.

iii) For milk, the *n*-hexane layer obtained in the second extraction sometimes turns into a solid mass. In such case, stir well with a spatula and then centrifuge instead of homogenizing the sample. The majority of spinosyn A and spinosyn D is extracted during the first extraction.

iv) In the dehydration procedure, wash the anhydrous sodium sulfate twice with 20 mL of acetone/*n*-hexane (1:2, v/v).

v) In the procedure of defatting with a porous diatomaceous earth column, a portion of spinosyn A and spinosyn D may not be eluted when the flow rate is too fast. Therefore, adjust the flow rate to less than 4 mL/min.

vi) Trimethylaminopropylsilanized silica gel/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg) can be used.

vii) When the analytical method for spinosyn A and spinosyn D using LC-MS was developed, the following monitoring ions were used:

Spinosyn A

for quantification (*m/z*) 732

for confirmation (*m/z*) 142

Spinosyn D

for quantification (*m/z*) 746

for confirmation (*m/z*) 142

When LC-MS/MS is used, the major monitoring ions are as follows:

Spinosyn A

for quantification (*m/z*): precursor ion 732, product ion 142

for confirmation (*m/z*): precursor ion 732, product ion 98

Spinosyn D

for quantification (*m/z*): precursor ion 746, product ion 142

for confirmation (*m/z*): precursor ion 746, product ion 98

11. References

None

12. Type

C