Original: Japanese
Provisional translation

Analytical Method for Bicozamycin (Animal and Fishery Products)

1. Analyte

Bicozamycin

2. Applicable food

Animal and fishery products

3. Instrument

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

4. Reagents

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

Trimethylaminopropylsilanized silica gel/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg): A polyethylene tube of 12−13 mm inside diameter packed with 500 mg each of trimethylaminopropylsilanized silica gel in the upper layer and ethylenediamine-*N*-propylsilanized silica gel in the lower layer, or a cartridge equivalent to the specified one in separation capability.

Reference standard of bicozamycin: Contains not less than 95% of bicozamycin.

5. Procedure

1) Extraction

Add 50 mL of *n*-hexane to 10.0 g of the sample, homogenize, add 50 mL of acetonitrile saturated with *n*-hexane, and homogenize again. Centrifuge at 3,000 rpm for 10 min and collect the acetonitrile layer. Add 30 mL of acetonitrile saturated with *n*-hexane to the residue and the *n*-hexane layer, homogenize, and centrifuge as described above. Combine the resulting acetonitrile layers and add acetonitrile to make exactly 100 mL. Take exactly a 1 mL aliquot of the solution, concentrate at below 40°C, and remove the solvent. Dissolve the residue in a 5 mL mixture of acetone and *n*-hexane (4:1, v/v).

2) Clean-up

Inject 10 mL each of acetone and a mixture of acetone and *n*-hexane (4:1, v/v) into a trimethylaminopropylsilanized silica gel/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg) sequentially and discard each effluent. After transferring the solution obtained in 1) to the cartridge, add a 10 mL mixture of acetone and *n*-hexane (4:1, v/v), and discard the effluents. Then, add a 15 mL mixture of acetone and water (19:1, v/v), concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in a mixture of acetonitrile and water (9:1, v/v) to make exactly 5 mL and use this solution as the test solution.

6. Calibration curve

Prepare bicozamycin standard solutions (acetonitrile and water [9:1, v/v]) of several concentrations, inject each solution into LC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of bicozamycin in the test solution corresponding to 0.01 mg/kg in the sample results in 0.0002 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of bicozamycin from the calibration curve made in 6.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Sulfobetaine-group-bonded silica gel, 2.1 mm inside diameter, 150 mm in length, and 3.5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile and 0.1 vol% formic acid (9:1, v/v) to (1:1, v/v) in 10 min and hold for 10 min.

Ionization mode: ESI (−)

Major monitoring ion (*m/z*): Precursor ion 301, product ions 209, 184

Injection volume: 10 μL

Expected retention time: 5 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of bicozamycin from the sample with acetonitrile containing *n*-hexane, clean-up with a trimethylaminopropylsilanized silica gel/ethylenediamine-*N*-propylsilanized silica gel layered cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) When the analytical methods for bicozamycin using LC-MS/MS were developed, the following monitoring ions were used:

for quantitative ions *(m/z)*: precursor ion 301, product ion 184

for qualitative ions (*m/z*): precursor ion 301, product ion 209

ii) Food items used to develop the analytical method: cattle muscle, cattle fat, cattle liver, and yellowtail

12. References

None

13. Type

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