Original: Japanese
Provisional translation

Analytical Method for Gamithromycin (Animal Products)

1. Analytes

Gamithromycin

2. Applicable food

Animal 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.

Sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (150 mg): A polyethylene tube of 12−13 mm inside diameter packed with 150 mg of sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer, or a cartridge equivalent to the specified one in separation capability.

5 mmol/L ammonium acetate solution (pH 4.0): Weigh 0.39 g of ammonium acetate, dissolve in about 950 mL of water, adjust pH to 4.0 using acetic acid, and add water to make 1 L.

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

5. Procedure

1) Extraction

Add 50 mL of acetone to 10.0 g (5.00 g for fat) of the sample, homogenize, centrifuge at 4,000 rpm for 10 min, and collect the supernatant. Add 25 mL of acetone to the residue, homogenize, centrifuge as described above, and collect the supernatant. Combine the resulting supernatants and add acetone to make exactly 100 mL. Take exactly a 5 mL aliquot of the solution, add 12.5 μL of acetic acid and 25 μL of 1 mol/L ammonium acetate solution, and mix thoroughly.

2) Clean-up

Inject 10 mL each of methanol and acetone into a sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (150 mg) sequentially and discard each effluent. After transferring the solution obtained in 1) to the cartridge, add 10 mL each of water, methanol, and acetonitrile sequentially, and discard each effluent. Then add 10 mL of acetonitrile and ammonia solution (20:1, v/v), concentrate the eluate at below 40ºC, and remove the solvent. Dissolve the residue in a 5 mmol/L ammonium acetate-methanol solution to make exactly 1 mL, and use this solution as the test solution.

6. Calibration curve

Prepare gamithromycin standard solution (5 mmol/L ammonium acetate-methanol) of several concentrations, inject each standard 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 gamithromycin in the test solution corresponding to 0.01 mg/kg in the sample results in 0.005 mg/L for muscle and liver and 0.0025 mg/L for fat.

7. Quantification

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

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.1 mm inside diameter, 100 mm in length, and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially, 5 mmol/L ammonium acetate solution (pH 4.0) and 5 mmol/L ammonium acetate-methanol solution (9:1, v/v) for 1 min, followed by a linear gradient to (1:19, v/v) in 8 min.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*): Precursor ion 778, product ions 620, 158, 116, 83

Injection volume: 5 μL

Expected retention time: 7 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of gamithromycin from the sample with acetone, clean-up with a sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) When the analytical method for gamithromycin was developed, the following monitoring ions were used:

for quantitative ions *(m/z)*: precursor ion 778, product ion 620

for qualitative ions (*m/z*): precursor ion 778, product ion 158, 116, 83

ii) Gamithromycin adheres to glass containers depending on the solvent to be dissolved. Therefore, plastic containers such as polypropylene or polytetrafluoroethylene should be used as much as possible.

iii) For the centrifuge used during the development of this analytical method, 4,000 rpm corresponds to approximately 3,430 × g.

iv) Food items used to develop the analytical method: cattle muscle, pig liver, cattle fat.

12. References

None

13. Type

C