Original: Japanese
Provisional translation

Analytical Method for Flavophospholipol (Animal Products)

1. Analyte

Moenomycin A

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.

Reference standard of moenomycin A: Contains not less than 95% of moenomycin A.

5. Procedure

1) Extraction

Add a 100 mL mixture of ammonia water and methanol (1:9, v/v) heated to 50°C to 10.0 g of the sample, homogenize, centrifuge at 3,000 rpm for 5 min, and collect the supernatant. Add an 80 mL mixture of ammonia water and methanol (1:9, v/v) heated to 50°C to the residue, homogenize, and centrifuge as described above. Collect the supernatant, combine with the previously obtained supernatant, and add methanol to make exactly 200 mL. Take exactly a 20 mL aliquot of the solution and concentrate to dryness at below 40°C. Dissolve the residue in a 20 mL mixture of ammonia water, water and methanol (1:60:40, v/v/v). Add 20 mL of ethyl acetate, shake, and repeat the operation of discarding the ethyl acetate layer twice after centrifuging at 3,000 rpm for 5 min. Concentrate the mixed layer of ammonia water, water and methanol to dryness at below 40°C. Dissolve the residue in a 2 mL mixture of formic acid, water and methanol (1:20:80, v/v/v).

2) Clean-up

Inject 5 mL of methanol and a 5 mL mixture of formic acid**,** water and methanol (1:20:80, v/v/v) into a trimethylaminopropylsilanized silica gel cartridge (500 mg) and discard each effluent. After transferring the solution obtained in 1) to the cartridge, add 5 mL of methanol, and discard the effluent. Following this, repeat the operation of washing the container containing the solution obtained in 1) with a 5 mL mixture of ammonia water and methanol (1:9, v/v) and transferring the washings to the previous cartridge twice, and collect the eluate. Additionally, add a 10 mL mixture of ammonia water and methanol (1:9, v/v) and combine the eluate with the previous eluate. Concentrate to approximately 1 mL at below 40°C, add a mixture of 0.3 vol% formic acid and 0.3 vol% formic acid-acetonitrile solution (3:2, v/v) to make exactly 5 mL, and use this solution as the

test solution.

6. Calibration curve

Dissolve the reference standard of moenomycin A in methanol to prepare stock standard solutions. Dilute the stock standard solutions with a mixture of 0.3 vol% formic acid and 0.3 vol% formic acid-acetonitrile solution (3:2, v/v) appropriately and prepare standard solutions 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 moenomycin A in the test solution corresponding to 0.01 mg/kg in the sample results in 0.002 mg/L.

7. Quantification

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

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octylsilanized silica gel, 2.1 mm inside diameter, 150 mm in length, and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially, 0.3 vol% formic acid and 0.3 vol% formic acid-acetonitrile solution (3:2, v/v) for 1 min, followed by a linear gradient from (3:2, v/v) to (1:3, v/v) in 9 min.

Ionization mode: ESI (−)

Major monitoring ion (*m/z*): Precursor ion 790, product ions 576, 554

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 moenomycin A from the sample with a mixture of ammonia water heated to 50°C and methanol (1:9, v/v), washing with ethyl acetate, clean-up with a trimethylaminopropylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) Using a glass centrifuge tube for homogenizing and extracting samples may cause the temperature of the extracts to decrease, which may result in inadequate recovery. Therefore, polypropylene containers are recommended when homogenizing and extracting.

ii) Depending on the sample, moenomycin A may not dissolve sufficiently in a mixture of formic acid, water, and methanol (1:20:80, v/v/v), which is the loading solvent for

trimethylaminopropylsilanized silica gel cartridge purification and may remain in the container. Therefore, wash the container thoroughly with a mixture of ammonia water and methanol (1:9, v/v), which is the elution solvent, inject the washings into the cartridge, and take the eluate.

iii) When the analytical method for moenomycin A using LC-MS/MS was developed, the following monitoring ions were used:

for quantitative ions *(m/z*): precursor ion 790, product ion 576

for qualitative ions (*m/z*): precursor ion 790, product ion 554

iv) Food items used to develop the analytical method: pig muscle, pig fat, pig liver, and chicken egg

12. References

None

13. Type

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