Analytical Method for Lasalocid
(Animal Products)

1. Analyte

Lasalocid 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.

Formic acid-methanol test solution: Add a mixture of water and methanol (1:99, v/v) to 5 mL of formic acid to make 1,000 mL.

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

5. Procedure

1) Extraction

Add 100 mL of methanol to 10.0 g of the sample, homogenize, centrifuge at 3,000 rpm for 5 min, and collect the supernatant. After adding 50 mL of methanol to the residue and homogenizing, centrifuge as described above, and collect the supernatant. Combine the resulting supernatants and add methanol to make exactly 200 mL. Take exactly a 2 mL aliquot of the solution.

2) Clean-up

i) Trimethylaminopropylsilanized silica gel column chromatography

Inject 10 mL of methanol into a trimethylaminopropylsilanized silica gel cartridge (500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add 10 mL of methanol, and discard the effluent. Then, add 10 mL of formic acid-methanol test solution, concentrate the eluate at below 40℃, and remove the solvent. Dissolve the residue in 5 mL of water and methanol (9:1, v/v).

ii) Divinylbenzene-*N*-vinylpyrrolidone copolymer column chromatography

Inject 10 mL each of methanol and water into a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg) sequentially and discard each effluent. Transfer the solution obtained in i) to the cartridge, add 10 mL of water and a 10 mL mixture of water and methanol (1:1, v/v) sequentially, and discard each effluent. Then, add 20 mL of methanol, concentrate the eluate at below 40℃, and remove the solvent. Dissolve the residue in water and methanol (9:1, v/v) to make exactly 5 mL and use this solution as the test solution.

6. Calibration curve

Prepare lasalocid A sodium standard solutions (water and methanol [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 lasalocid in the test solution corresponding to 0.01 mg/kg (as lasalocid A) in the sample results in 0.0002 mg/L (as lasalocid A).

7. Quantification

Inject the test solution into LC-MS/MS, and calculate the concentration of lasalocid A 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, 150 mm in length, and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from 5 mmol/L ammonium acetate solution and 5 mmol/L ammonium acetate-methanol solution (9:1, v/v) to (1:99, v/v) in 10 min, and hold for 10 min.

Ionization mode: ESI (−)

Major monitoring ion (*m/z*): Precursor ion 589, product ions 235, 121

Injection volume: 10 μL

Expected retention time: 14 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of lasalocid A from the sample with methanol, clean-up with a trimethylaminopropylsilanized silica gel cartridge, followed by clean-up with a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

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

for quantitative ions *(m/z)*: precursor ion 589, product ion 235

for qualitative ions (*m/z*): precursor ion 589, product ion 121

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

12. References

None

13. Type

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