Original: Japanese
Provisional translation

Analytical Method for Albendazole (Animal Products)

1. Analyte

Metabolite I [5-Propylsulfonyl-1*H*-benzimidazol-2-amine] (Including compounds converted to metabolite I by hydrolysis under acidic conditions using hydrochloric acid.)

2. Application

Animal products

3. Instrument

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

4. Reagents

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

Sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg): A polyethylene tube of 12–13 mm inside diameter packed with 500 mg of sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer or a cartridge equivalent to the specified one in separation capability.

Reference standard of metabolite I: Contains not less than 98% of metabolite I.

5. Procedure

1) Extraction

Add 10 mL of 6 mol/L hydrochloric acid to 10.0 g of sample, seal tightly, and heat in an oil bath at 110°C for 1 hr while shaking occasionally. After cooling, add 30 mL of ethyl acetate and *n*-hexane (1:1) mixed solution, shake, centrifuge at 3,000 rpm for 5 min, discard the organic layer, and repeat the process total of two times. Add 50 mL of acetonitrile to the aqueous layer and the residue, homogenize, centrifuge at 3,000 rpm for 10 min and collect the supernatant. Add 25 mL of acetonitrile to the residue, homogenize, centrifuge as described above and collect the supernatant. Combine the resulting supernatants, add 4 g of sodium carbonate, homogenize, centrifuge at 3,000 rpm for 5 min and collect the organic layer. Add 50 mL of acetonitrile to the aqueous layer and the residue, homogenize, centrifuge as described above and collect the organic layer. Combine the resulting organic layers and add acetonitrile to make exactly 200 mL.

2) Clean-up

Inject 5 mL of acetonitrile and 5 mL of water into a sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg) sequentially and discard each effluent. Transfer exactly 4 mL of the solution obtained in i) to the cartridge, add 5 mL of water, 5 mL of acetonitrile, and 10 mL of acetonitrile and ammonia solution (49:1) mixed solution sequentially and discard each effluent. Then, inject 12 mL of acetonitrile and ammonia

solution (9:1) mixed solution, concentrate the eluate 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.

6. Calibration curve

Prepare metabolite I standard solutions (methanol) 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 was prepared following the above procedure, the concentration of metabolite I in the test solution corresponding to 0.01 mg/kg in the sample is 0.0005 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS, and calculate the concentration of metabolite I from the calibration curve prepared 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 a mixed solution of 0.05 vol% formic acid-acetonitrile solution and 0.05 vol% formic acid (1:19) to (2:3) in 15 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 240, product ions 198, 133

Injection volume: 2 μL

Expected retention time: 10 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of heating the sample under acidic conditions using hydrochloric acid, defatting with ethyl acetate and *n*-hexane (1:1) mixed solution, extracting metabolite I with acetonitrile, salting out under alkaline conditions, cleaning up with a sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge and quantifying and confirming using LC-MS/MS.

2) Notes

i) When the analytical method for metabolite I with LC-MS/MS was developed, the following monitoring ions were used:

for quantitative ions (*m/z*): precursor ion 240, product ion 133

for qualitative ions (*m/z*): precursor ion 240, product ion 198

ii) When homogenized with acetonitrile after defatting operation and centrifuged, three

phases may be formed: an acetonitrile layer, a water layer and a solid layer. In that case, the acetonitrile and aqueous layers are taken.

iii) Confirm that the solution is pH at 6 or higher using such as pH test paper after homogenizing with sodium carbonate.

iv) The cartridge should be washed by increasing the acetonitrile concentration in the mobile phase after eluting metabolite I to reduce the influence of matrix carryover in the sample in LC-MS/MS measurements.

v) Food items used to develop the analytical method: cattle muscle, cattle fat, cattle liver, and cattle milk.

12. Reference

None

13. Type

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