Original: Japanese
Provisional Translation

Analytical Method for Zilpaterol (Animal Products)

1. Analytes

Zilpaterol

2. Applicable foods

Animal Products, Milk, and Egg

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.

0.6 mol/L hydrochloric acid･ethanol/water (1:1, v/v) solution: Add ethanol/water (1:1, v/v) in 50 mL of hydrochloric acid to make 1,000 mL.

Reference standard of Zilpaterol: Contains not less than 98% of Zilpaterol

5. Procedure

1) Extraction

For muscle, fat, liver, kidney, and egg, weigh sample accurately, add half weight of 0.6 mol/L hydrochloric acid･ethanol/water (1:1, v/v) solution (1:2, the solution/the sample weight) to the sample, homogenize, and then take the sample equivalent to 10.0 g. For milk, weigh 10.0 g of sample, and 2.5 mL of 1.2 mol/L hydrochloric acid to the sample. Add 50 mL of *n*-hexane to the sample, homogenize, add 50 mL of acetonitrile saturated with *n*-hexane, homogenize, centrifuge at 3,000 rpm for 5 minutes, and collect the acetonitrile layer. Combine the residue and the *n*-hexane layer, add 25 mL of acetonitrile saturated with *n*-hexane, homogenize, centrifuge as described above, collect the resulting acetonitrile layer, and add acetonitrile to make exactly 100 mL.

2) Clean-up

Add 10 mL of acetonitrile/water (1:1, v/v) to octadecylsilanized silica gel cartridge (1,000 mg) and benzenesulfonic-propylsilanized silica gel cartridge (500 mg) respectively, and discard the effluent. Connect the benzenesulfonic-propylsilanized silica gel to the lower part of the octadecylsilanized silica gel cartridge, transfer 5 mL of the solution obtained in 1) to the cartridge, add 10 mL of acetonitrile/water (1:1, v/v), and discard the effluent. Detach the octadecylsilanized silica gel cartridge, add 20 mL of 25% ammonia water/acetonitrile (1:99, v/v) to benzenesulfonic-propylsilanized silica gel cartridge, and concentrate the eluate at below 40°C and remove the solvent. Dissolve the residue in acetonitrile/water (1:9, v/v) to make exactly 2 mL, and use this solution as the test solution.

6. Calibration curve

Prepare zilpaterol standard solutions (acetonitrile/water (1:9v/v)) of several concentrations,

inject each standard solution to LC-MS/MS, and make a calibration curve by peak-height or peak-area method. When the test solution is prepared following the above procedure, the sample containing 0.01 mg/kg of zilpaterol gives the test solution of 0.0025 mg/L in concentration.

7. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of zilpaterol 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 in inside diameter, 150 mm in length, 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/0.1 vol% formic acid (1:49, v/v) to (4:1, v/v) in 10 min, and hold for 10 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*) Precursor ion 262, product ion 244, 202, 185

Injection volume: 5 μL

Expected retention time: 6 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of zilpaterol from sample with n-hexane using acetonitrile, clean-up with an octadecylsilanized silica gel cartridge and a benzenesulfonic-propylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

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

for quantification (*m/z*): precursor ion 262, product ion 185

for confirmation (*m/z*): precursor ion 262, product ion 244, 202

ii) Since zilpaterol appears to degrade in the sample such as liver and kidney, add hydrochloric acid when preparing the sample to prevent degradation. In addition, to avoid degradation during sample preparation as much as possible, use the sample homogenized with a kitchen knife etc. Also add ethanol to homogenize the sample. Since milk sample can be homogenized without adding ethanol, add only hydrochloric acid.

Add hydrochloric acid to all samples to harmonize the procedure, although it can be tested without adding hydrochloric acid in samples except liver and kidney.

iii) In the centrifugation operation for the extraction of 5-1), if residues are contained in collecting the acetonitrile layer, filter with cotton plug if necessary.

iv) The foods examined in the development of the analytical method: Beef muscle, beef fat, beef liver, beef kidney, milk, chicken egg

12. Reference

None

13. Type

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