Original: Japanese
Provisional Translation

Analytical Method for Ractopamine (Animal and Fishery Products)

1. Analyte

Ractopamine

2. Instrument

Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents

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

Reference standard of ractopamine hydrochloride: Contains not less than 99% of ractopamine hydrochloride. Melting point of the standard is 163.9-164.6°C.

4. Procedure

1) Muscle, liver, kidney and other edible parts

For muscle, remove the fat layer as possible, homogenize, and weigh 5.00 g of the sample.

For liver, kidney and other edible parts, homogenize, and weigh 5.00 g of the sample.

Add 20 mL of ethyl acetate and 1 mL of 4 mol/L potassium carbonate solution, homogenize, centrifuge at 3,000 rpm for 10 minutes, and take the ethyl acetate layer. Add 20 mL of ethyl acetate to the residue in the centrifuge tube, shake for 5 minutes, centrifuge as described above, combine the obtained ethyl acetate layers, concentrate at below 40°C, and remove ethyl acetate. Dissolve 30 mL of acetonitrile to the residue, and transfer to a separating funnel. Add 30 mL of *n*-hexane saturated with acetonitrile to the separating funnel, shake, and discard the *n*-hexane layer. Repeat this step one more time. Concentrate the acetonitrile layer at below 40°C, and remove acetonitrile. Dissolve 1.0 mL of methanol to the residue, and use this solution as the test solution.

2) Fat

Remove the muscle layer as possible, homogenize, and weigh 5.00 g of the sample.

Add 30 mL each of acetonitrile and *n*-hexane saturated with acetonitrile, homogenize, centrifuge at 3,000 rpm for 10 minutes, take the acetonitrile layer, and transfer to a separating funnel. Add 30 mL of acetonitrile to the *n*-hexane layer and the residue in the centrifuge tube, shake for 5 minutes, and centrifuge as described above. Combine the obtained acetonitrile layers in the separating funnel, and add 30 mL of *n*-hexane saturated with acetonitrile. Shake vigorously for 5 minutes, let stand, take the acetonitrile layer, concentrate at below 40°C, and remove acetonitrile. Dissolve 1.0 mL of methanol to the residue, and use this solution as the test solution.

5. Calibration curve

Prepare 0.025-0.5 mg (as ractopamine)/L ractopamine hydrochloride standard solutions (methanol) of several concentrations. Inject each standard solution to LC-MS, and make

calibration curves by peak-height or peak-area method.

6. Quantification

Inject the test solution to LC-MS, and calculate the concentration of ractopamine from the calibration curve made in 5.

7. Measurement conditions

LC-MS

Column: Octadecylsilanized silica gel, stainless tube of 2.0-6.0 mm in inside diameter, 100-250 mm in length and 2-5 µm in particle diameter

Column temperature: 40°C

Mobile phase: acetonitrile/0.05% trifluoroacetic acid (1:4, v/v)

Major monitoring ion (*m/z*): 302 (ESI+)

Expected retention time: 4-6 min

8. Limit of quantification

0.01 mg/kg

9. Explanatory note

1) Outline of analytical method

The method consists of extraction of ractopamine from sample with ethyl acetate or acetonitrile, defatting by acetonitrile/hexane partitioning, and quantification and confirmation using LC-MS.

2) Notes

i) Ractopamine has 4 kinds of enantiomer. Depends on the column to use, the enantiomer may isolate from ractopamine, and several peaks may appear. In this case, make a calculation using the sum of each peak-height or peak-area.

ii) Optimum way of ionization and detected monitoring ions may differ by the instrument to use. Consider optimum condition for each instrument.

Identified ion besides the major monitoring ion in ESI (+) is 284 (m/z).

10. References

None

11. Type

C