Analytical Method for Rafoxanide (Animal and Fishery Products)

1. Analyte

Rafoxanide

2. Instrument

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

3. Reagents

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

Reference standard of rafoxanide: Contains not less than 97% of rafoxanide.

4. Procedure

1) Extraction

i) Muscle, liver, kidney, milk, egg, fish, shellfish and honey

Add 100 mL of acetone to 10.0 g of sample (for honey, dissolve 10.0 g of sample in 20 mL of water, and add 100 mL of acetone), homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper (for honey, dissolve the residue on the filter paper in 10 mL of water, and add 50 mL of acetone), homogenize, and filter with suction. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 2 mL aliquot of the extract, and add 2 mL of water.

ii) Fat

Add 100 mL of acetone to 5.00 g of sample, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 4 mL aliquot of the extract, concentrate at below 40°C and remove the solvent. Add 30 mL of *n*-hexane to the residue, and extract with shaking twice with 30 mL of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40°C and remove the solvent. Dissolve the residue in 2 mL of acetone and add 2 mL of water.

2) Clean-up

Add 5 mL each of acetonitrile and water to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add 10 mL of acetonitrile/water (7:3, v/v), and discard the effluent. Elute with 10 mL of acetonitrile, concentrate the eluate at below 40°C and remove the solvent. Add acetonitrile to the residue to make exactly 4 mL, and use this solution as the test solution.

5. Calibration curve

Prepare rafoxanide standard solutions of several concentrations (acetonitrile). 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 rafoxanide gives the test solution of 0.0025 mg/L in concentration.

6. Quantification

Inject the test solution to LC-MS/MS and calculate the concentration of rafoxanide from the calibration curve made in **5**.

7. Confirmation

Confirm using LC-MS/MS.

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 150 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: acetonitrile/0.1 vol% formic acid (17:3, v/v)

Ionization mode: ESI (−)

Major monitoring ions (*m/z*): Precursor ion 624, product ions 345, 127

Injection volume: 4 μL

Expected retention time: 10 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of rafoxanide from sample with acetone, defatting with acetonitrile/hexane partitioning (only for fat), clean-up with an octadecylsilanized silica gel cartridge, quantification and confirmation using LC-MS/MS.

2) Notes

i) Elution of rafoxanide from the octadecylsilanized silica gel cartridge varies widely among the types of cartridges, and for this reason, take due precautions.

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

for quantification (*m/z*): precursor ion 624, product ion 127

for confirmation (*m/z*): precursor ion 624, product ion 345

11. References

None

12. Type

C