Analytical Method for Cefquinome (Animal and Fishery Products)

1. Analyte

Cefquinome

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.

Styrene-divinylbenzene copolymer cartridge (500 mg): polyethylene tube of 10−12 mm in inside diameter packed with 500 mg of styrene-divinylbenzene copolymer, or other cartridge with equal separation characteristics.

Reference standard of cefquinome sulfate: Contains not less than 95% of cefquinome sulfate.

4. Procedure

1) Extraction

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

For muscle, liver, kidney, milk, egg, fish and shellfish, weigh 10.0 g of sample. For honey, weigh 10.0 g of sample and dissolve in 70 mL of water. Add 50 mL of acetonitrile/formic acid/water (900:1:100, v/v/v) and 50 mL of *n*-hexane to the sample, homogenize, and filter with suction. Add 30 mL of acetonitrile/formic acid/water (900:1:100, v/v/v) and 30 mL of *n*-hexane to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, collect the lower layer, and add acetonitrile/formic acid/water (900:1:100, v/v/v) to make exactly 200 mL. Take a 40 mL aliquot of the extract, concentrate to less than 4 mL (less than 18 mL for honey) at below 40°C, and add 20 mL of water, and mix well by ultrasonication.

ii) Fat

Add 50 mL of acetonitrile/formic acid/water (900:1:100, v/v/v) and 50 mL of *n*-hexane to 5.00 g of sample, homogenize and filter with suction. Add 30 mL of acetonitrile/formic acid/water (900:1:100, v/v/v) and 30 mL of *n*-hexane to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrate, collect the lower layer, and add acetonitrile/formic acid/water (900:1:100, v/v/v) to make exactly 200 mL. Take a 80 mL aliquot of the extract, concentrate to less than 8 mL at below 40°C, add 20 mL of water, and mix well by ultrasonication.

2) Clean-up

Add 10 mL each of acetonitrile and water to a styrene-divinylbenzene copolymer cartridge (500 mg) sequentially, and discard the effluent. Transfer the extract obtained in 1) to the cartridge, add 10 mL each of water and acetonitrile/water (1:19, v/v) sequentially, and

discard the effluent. Elute with 10 mL of acetonitrile/water (1:4, v/v). Add 10 μL of formic acid to the eluate, add acetonitrile/water (1:4, v/v) to make exactly 10 mL, and use this solution as the test solution.

5. Calibration curve

Prepare cefquinome standard solutions of several concentrations (acetonitrile/formic acid/water (200:1:800, v/v/v)). Inject 5 μL of 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, cefquinome concentration in the test solution corresponding to limit of quantification is 0.002 mg/L.

6. Quantification

Inject 5 μL of the test solution to LC-MS/MS and calculate the concentration of cefquinome 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 3 μm in particle diameter

Column temperature: 40°C.

Mobile phase: Linear gradient from acetonitrile/5 mmol/L ammonium acetate solution (1:9, v/v) to (3:7, v/v) in 10 min and hold for 2 min.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*):

Precursor ion: 529

Product ion: 396, 134

Expected retention time: 9 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of cefquinome from sample with acetonitrile/formic acid/water (900:1:100, v/v/v) under the existence of *n*-hexane, clean-up with a styrene-divinylbenzene copolymer cartridge, quantification and confirmation using LC-MS/MS.

2) Notes

i) Because cefquinome is unstable in methanol, take due precautions.

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

for quantification (*m/z*): precursor ion 529, product ion 134

for confirmation (*m/z*): precursor ion 529, product ion 396

iii) In the extraction step, insoluble substances appear in some food samples by addition of 20 mL of water after concentration of the extract. In such cases, mix well by ultrasonication to promote dissolution of cefquinome, and transfer the suspension to the styrene-divinylbenzene copolymer cartridge.

11. References

None

12. Type

C