Original: Japanese

Provisional Translation

Analytical Method for Flusilazole (Animal and Fishery Products)

1. Analytes

Flusilazole

[Bis(4-fluorophenyl)methyl]silanol (hereafter referred to as metabolite D)

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 flusilazole: Contains not less than 98% of flusilazole.

Reference standard of metabolite D: Contains not less than 90% of metabolite D.

4. Procedure

1) Extraction

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

Add 100 mL of acetone to 10.0 g of sample, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 20 mL aliquot of the extract, concentrate to about 4 mL at below 40°C, and add 10 mL of water.

ii) Honey

Dissolve 10.0 g of sample in 20 mL of water. Add 100 mL of acetone, homogenize, and filter with suction. Add 10 mL of water 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 20 mL aliquot of the extract, concentrate to about 4 mL at below 40°C, and add 10 mL of water.

iii) 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 as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 40 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 resulting extracts, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 4 mL of acetone and add 10 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 effluents. Transfer the solution obtained in 1) to the cartridge, add 10 mL of acetonitrile/water (2:3, v/v), and discard the effluent. Elute with 10

mL of acetonitrile/water (3:2, v/v), add acetonitrile/water (3:2, v/v) to the eluate to make exactly 10 mL, and use this solution as the test solution.

5. Calibration curve

Prepare flusilazole and metabolite D standard solutions (acetonitrile/water (3:2, v/v)) of several concentrations. Inject each standard solution to LC-MS/MS, and make calibration curves 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 flusilazole or metabolite D gives the test solution of 0.001 mg/L in concentration. The concentration of metabolite D is calculated as that of flusilazole.

6. Quantification

Inject the test solution to LC-MS/MS and calculate the concentration of flusilazole and metabolite D from the calibration curves made in **5**. Use the following equation to calculate the concentration of flusilazole including that of the metabolite D.

Concentration (ppm) of flusilazole (including that of the metabolite D)

$= A + B \times 1.260$

A: Concentration (ppm) of flusilazole

B: Concentration (ppm) of metabolite D

7. Confirmation

Confirm using LC-MS/MS.

8. Measurement conditions

Example

Column: 1-diisopropylsilyl-3-myristoylamidated silica gel, 2.1 mm in inside diameter, 150 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/5 mmol/L ammonium acetate solution (3:7, v/v) to (19:1, v/v) for 15 min.

Ionization mode

Flusilazole: ESI (+)

Metabolite D: ESI (-)

Major monitoring ions (*m/z*)

Flusilazole: precursor ion 316, product ion 247, 165

Metabolite D: precursor ion 249, product ion 153, 173

Injection volume: 10 μL

Expected retention time

Flusilazole: 9 min

Metabolite D: 10 min

9. Limit of quantification

0.01 mg/kg for each analyte (The concentration of metabolite D is calculated as flusilazole)

10. Explanatory note

1) Outline of analytical method

This method consists of extraction of flusilazole and metabolite D from sample with acetone, defatting with acetonitrile/hexane partitioning (for fat only), clean-up with an octadecyl-silanized silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) Because metabolite D is likely to be lost during concentration under reduced pressure, remove the solvent under a gentle nitrogen stream after concentrating to about 1 mL.

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

Flusilazole

for quantification (*m/z*): precursor ion 316, product ion 247

for confirmation (*m/z*): precursor ion 316, product ion 165

Metabolite D

for quantification (*m/z*): precursor ion 249, product ion 153

for confirmation (m/z): precursor ion 249, product ion 173

iii) When the analytical method for flusilazole was developed, the purity of the available metabolite D standard was 90%. Therefore, the specification in **3** was set as, “Reference standard of metabolite D: Contains not less than 90% of metabolite D.” However, a standard with purity of not less than 95% should be used, if available.

11. References

None

12. Type

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