Original: Japanese
Provisional translation

# Analytical Method for Flubendazole (Animal Products)

**1. Analytes**

Flubendazole

(2-Amino-1*H*-benzoimidazol-5-yl)-(4-fluorophenyl)-methanone [hereinafter referred to as metabolite R35475])

**2. Application**

Animal products

**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.

Reference standard of flubendazole: Contains not less than 95% of flubendazole.

Reference standard of metabolite R35475: Contains not less than 95% of metabolite R35475.

**5. Procedure**

1) Extraction

Add 50 mL of acetone to 10.0 g of the sample, homogenize, and filter with suction. Add 25 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 100 mL.

2) Clean-up

Add 5 mL of acetone to a benzenesulfonylpropylsilanized silica gel cartridge (500 mg) and discard the effluent. Take exactly a 2 mL aliquot of the solution obtained in 1), transfer to the cartridge, add 5 mL each of acetone, water and acetonitrile sequentially, and discard each effluent. Then, add 10 mL of acetonitrile and ammonia water (97:3, v/v), concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in methanol to make exactly 4 mL and use this solution as the test solution.

**6. Calibration curve**

Prepare several mixed methanol solutions of the reference standards of flubendazole and metabolite R35475, inject each solution into 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 concentration in the test solution corresponding to 0.005 mg/kg in the sample results in 0.00025 mg/L.

**7. Quantification**

Inject the test solution into LC-MS/MS and calculate the concentration of flubendazole and metabolite R35475 from the calibration curve prepared in 6.

**8. Confirmation**

Confirm using LC-MS/MS.

**9. Measurement conditions**

(Example)

Column: Octadecylsilanized silica gel, 2.1 mm inside diameter, 150 mm in length and 3 μm in particle diameter.

Column temperature: 40°C

Mobile phase: Linear gradient from 5 mmol/L ammonium acetate solution and 5 mmol/L ammonium acetate-methanol solution (3:2, v/v) to (1:19, v/v) in 20 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*):

Flubendazole: precursor ion 314, product ions 123, 95

Metabolite R35475: precursor ion 256, product ions 123, 95

Injection volume: 5 μL

Expected retention time:

Flubendazole: 15 min

Metabolite R35475: 12 min

**10. Limit of quantification**

Flubendazole: 0.005 mg/kg

Metabolite R35475: 0.005 mg/kg

**11. Explanatory note**

1) Outline of analytical method

The method consists of extraction of flubendazole and metabolite R35475 from the sample with acetone, clean-up with a benzenesulfonylpropylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) When the analytical methods for flubendazole and metabolite R35475 using LC-MS/MS were developed, the following monitoring ions were used:

Flubendazole

for quantitative ions (*m/z*): precursor ion 314, product ion 123

for qualitative ions (*m/z*): precursor ion 314, product ion 95

Metabolite R35475

for quantitative ions (*m/z*): precursor ion 256, product ion 123

for qualitative ions (*m/z*): precursor ion 256, product ion 95

ii) Since each reference standard of flubendazole and metabolite R35475 is difficult to dissolve in solvents, ensure that they are completely dissolved when preparing the standard solution.

iii) Food items used to develop the analytical method: cattle muscle, cattle fat, cattle liver, milk,

and chicken egg

**12. References**

None

**13. Type**

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