Original: Japanese
Provisional translation

Analytical Method for Cyflumetofen (Animal Products)

1. Analytes

Cyflumetofen

α,α,α-Trifluoro-*o*-toluic acid (hereafter referred to as metabolite B-1)

2. Applicable food

Animal products (except honey)

3. Instrument

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

4. Reagents

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

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

Reference standard of metabolite B-1: Contains not less than 95% of metabolite B-1.

5. Procedure

1) Extraction

Add 50 mL of methanol to 10.0 g of the sample, homogenize, centrifuge at 3,000 rpm for 10 min, and collect the supernatant. Add 25 mL of methanol to the residue, homogenize, centrifuge as described above, and collect the supernatant. Combine the resulting supernatants and add methanol to make exactly 100 mL. Take exactly a 25 mL aliquot of the solution and concentrate to approximately 1 mL at below 40°C. Add 0.1 vol% acetic acid to the solution to make 10 mL.

2) Clean-up

Add 5 mL each of methanol and 0.1 vol% acetic acid to a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (200 mg) sequentially and discard each effluent. After transferring the solution obtained in 1) to the cartridge, add 5 mL of water, and discard the effluent. Then add 10 mL of methanol, take the eluate, and concentrate to approximately 1 mL at below 40°C. Add a mixture of water and methanol (2:3, v/v) to the solution to make exactly 5 mL and use this solution as the test solution.

6. Calibration curve

Prepare stock standard solutions using cyflumetofen reference standard and metabolite B-1 reference standard, respectively. Mix each stock standard solution appropriately, dilute with a mixture of water and methanol (2:3, v/v), and prepare standard solutions of several concentrations. Inject each standard 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 of cyflumetofen and

metabolite B-1 in the test solution corresponding to 0.005 mg/kg (The concentration of metabolite B-1 is calculated as cyflumetofen.) in the sample results in 0.0025 mg/L (The concentration of metabolite B-1 is calculated as cyflumetofen.) for each analyte.

7. Quantification

Inject the test solution into LC-MS/MS, calculate the concentration of cyflumetofen and metabolite B-1 from the calibration curve made in 6. and calculate the concentration of cyflumetofen including metabolite B-1 using the following equation.

Concentration of cyflumetofen (including metabolite B-1) (ppm) = A + B × 2.354

A: Concentration of cyflumetofen (ppm)

B: Concentration of metabolite B-1 (ppm)

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.0 mm inside diameter, 150 mm in length, and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially, a mixture of 0.05 vol% acetic acid and 0.05 vol% acetic acid-methanol solution (2:3, v/v) for 1 min, followed by a linear gradient to (1:19, v/v) in 14 min.

Ionization mode

Cyflumetofen: ESI (+)

Metabolite B-1: ESI (−)

Major monitoring ion (*m/z*)

Cyflumetofen: Precursor ion 448, product ions 249, 173

Metabolite B-1: Precursor ion 189, product ions 145, 69

Injection volume: 10 μL

Expected retention time

Cyflumetofen: 14 min

Metabolite B-1: 4 min

10. Limit of quantification

0.005 mg/kg for each analyte (The concentration of metabolite B-1 is calculated as cyflumetofen.)

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of cyflumetofen and metabolite B-1 from the sample with methanol, clean-up with a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, and quantification and confirmation using LC-MS/MS. In the method, cyflumetofen and metabolite B-1 are quantified respectively. To calculate the concentration of cyflumetofen including metabolite B-1, the concentration of metabolite B-1 is converted to the

concentration of cyflumetofen by multiplying by a conversion factor, and the sum of the concentrations of cyflumetofen and metabolite B-1 is regarded as the analytical result of cyflumetofen.

2) Notes

i) To reduce the influence of matrix carryover in the sample in LC-MS/MS measurements, it is recommended to wash the cartridge by increasing the methanol concentration in the mobile phase after cyflumetofen is eluted.

ii) When the analytical methods for cyflumetofen and metabolite B-1 using LC-MS/MS were developed, the following monitoring ions were used:

Cyflumetofen:

for quantitative ions *(m/z)*: precursor ion 448, product ion 249

for qualitative ions (*m/z*): precursor ion 448, product ion 173

Metabolite B-1:

for quantitative ions *(m/z)*: precursor ion 189, product ion 145

for qualitative ions (*m/z*): precursor ion 189, product ion 69

iii) Metabolite B-1 is also included in the residue definition for compliance with MRLs of other agricultural chemicals.

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

12. References

None

13. Type

C