Original: Japanese
Provisional translation

Analytical Method for Isoxaflutole (Animal Products)

1. Analytes

Isoxaflutole

2-Cyano-3-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylphenyl)propan-1,3-dione (hereinafter referred to as metabolite B)

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 isoxaflutole: Contains not less than 95% of isoxaflutole.

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

5. Procedure

1) Extraction

Weigh the sample accurately. Add a mixture of 4 mol/L hydrochloric acid and ethanol (1:1, v/v), which is half the weight of the sample, grind and homogenize, and weigh the sample equivalent to 10.0 g. Add 50 mL of acetonitrile saturated with *n*-hexane, 50 mL of *n*-hexane and 20 g of anhydrous sodium sulfate, and homogenize. Centrifuge at 3,000 rpm for 5 min, discard the *n*-hexane layer, and collect the acetonitrile layer. Add 50 mL of acetonitrile saturated with *n*-hexane and 50 mL of *n*-hexane to the residue and homogenize. Centrifuge as described above, combine the resulting acetonitrile layer with the previously collected acetonitrile layer, and add acetonitrile to make exactly 100 mL. Take exactly a 4 mL aliquot of the solution and add 16 mL of 0.1 mol/L hydrochloric acid and 2 g of sodium chloride. Then, add a 20 mL mixture of ethyl acetate and *n*-hexane (3:7, v/v), extract with shaking, centrifuge at 3,000 rpm for 5 min, and collect the organic layer. Add a 20 mL mixture of ethyl acetate and *n*-hexane (3:7, v/v) to the aqueous layer and extract with shaking. Centrifuge as described above, combine the resulting organic layers, concentrate at below 40℃, and remove the solvent. Dissolve the residue in a 2 mL mixture of acetic acid, water and methanol (1:20:30, v/v/v).

2) Clean-up

Inject 5 mL of methanol and a 5 mL mixture of acetic acid, water and methanol (1:20:30, v/v/v) into a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg) and discard each effluent. Transfer the solution obtained in 1) to the cartridge, add a 10 mL mixture of acetic acid, water and methanol (1:20:30, v/v/v), and discard the effluent. Then, add a 20 mL mixture

of acetic acid, water and methanol (1:5:45, v/v/v), collect the eluate, and concentrate the eluate to approximately 1 mL at below 40℃. Add 0.05 vol% acetic acid and 0.05 vol% acetic acid-acetonitrile solution (7:3, v/v) to make exactly 2 mL and use this solution as the test solution.

6. Calibration curve

Prepare stock standard solutions by dissolving the isoxaflutole reference standard in 1 vol% acetic acid-acetonitrile solution and the metabolite B reference standard in acetonitrile. Prepare standard solutions of several concentrations by mixing each stock standard solution appropriately and diluting with 0.05 vol% acetic acid and a mixture of 0.05 vol% acetic acid-acetonitrile solution (7:3, v/v) appropriately. 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 of isoxaflutole and metabolite B in the test solution corresponding to 0.01 mg/kg in the sample results in 0.002 mg/L for each analyte.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of isoxaflutole and metabolite B from the calibration curve made in 6. Use the following equation to calculate the concentration of isoxaflutole including metabolite B.

Concentration (ppm) of isoxaflutole (including metabolite B) = A + B × 1.000

A: Concentration (ppm) of isoxaflutole

B: Concentration (ppm) of metabolite B

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: Initially 0.05 vol% acetic acid and 0.05 vol% acetic acid-acetonitrile solution (7:3, v/v) for 5 min, followed by a linear gradient from (7:3, v/v) to (9:11, v/v) in 15 min.

Ionization mode: ESI (−)

Major monitoring ions (*m/z*):

Isoxaflutole: Precursor ion 358, product ions 278, 79

Metabolite B: Precursor ion 358, product ions 278, 79

Injection volume: 5 μL

Expected retention time:

Isoxaflutole: 19 min

Metabolite B: 5 min

10. Limit of quantification

0.01 mg/kg for each analyte

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of isoxaflutole and metabolite B from a ground and homogenized sample with a mixture of 4 mol/L hydrochloric acid and ethanol (1:1, v/v) using acetonitrile containing *n*-hexane, transfer into a mixture of ethyl acetate and *n*-hexane (3:7, v/v) for re-dissolution, clean-up with a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, and quantification and confirmation using LC-MS/MS. In the method, isoxaflutole and metabolite B are quantified respectively. For the concentration of isoxaflutole including metabolite B, the concentration of metabolite B is converted to the concentration of isoxaflutole by multiplying by the conversion factor, and the sum of the concentrations of isoxaflutole and metabolite B is regarded as the analytical result of isoxaflutole.

2) Notes

i) Since isoxaflutole and metabolite B are unstable when exposed to light, they should be handled in the shade as much as possible, such as using brown glassware.

ii) On the eluate from the cartridge, metabolite B is lost when the solvent is completely removed by blowing nitrogen gas after the vacuum concentration step. Therefore, approximately 1 mL of the eluate should be left after concentration.

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

Isoxaflutole

for quantitative ions (*m/z*): precursor ion 358, product ion 79

for qualitative ions (*m/z*): precursor ion 358, product ion 278

Metabolite B

for quantitative ions (*m/z*): precursor ion 358, product ion 79

for qualitative ions (*m/z*): precursor ion 358, product ion 278

iv) 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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