Original: Japanese
Provisional translation

Analytical Method for Triclabendazole (Animal Products)

1. Analytes

Triclabendazole

Triclabendazole sulfoxide (hereinafter referred to as [metabolite A])

Triclabendazole sulfone (hereinafter referred to as [metabolite B])

Keto-triclabendazole (hereinafter referred to as [metabolite D])

Metabolites that are converted to metabolite D under acidic conditions (including metabolite A and 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.

Sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg): A polyethylene tube of 12–13 mm inside diameter packed with 500 mg of sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer or a cartridge equivalent to the specified one in separation capability.

Reference standard of triclabendazole: Contains not less than 98% of triclabendazole.

Reference standard of metabolite A: Contains not less than 98% of metabolite A.

Reference standard of metabolite B: Contains not less than 98% of metabolite B.

Reference standard of metabolite D: Contains not less than 98% of metabolite D.

5. Procedure

1) Hydrolysis

Weigh 10.0 g of sample, add 10 mL of 5 mol/L sodium hydroxide solution, mix, seal tightly, and heat at 100°C for 3 hrs. After returning it to room temperature, transfer the contents to another container and add 12 mL of 5 mol/L hydrochloric acid. Wash the container used for hydrolysis with 5 mL of methanol and combine the washing with the previous contents. Extract with shaking with 40 mL of ethyl acetate, centrifuge at 3,000 rpm for 5 min and collect the ethyl acetate layer. Then, add 40 mL of ethyl acetate to the aqueous layer, extract with shaking, centrifuge at 3,000 rpm for 5 min, collect the ethyl acetate layer, combine with the previously collected ethyl acetate layer and add ethyl acetate to make exactly 100 mL.

2) Defatting

Take exactly a 6 mL aliquot of the solution obtained in 1), concentrate at below 40°C, and

remove the solvent. Add 10 mL of *n*-hexane to the residue, extract with shaking with 10 mL of acetonitrile saturated with *n*-hexane, centrifuge at 3,000 rpm for 5 min, and collect the acetonitrile layer. Then, add 10 mL of acetonitrile saturated with *n*-hexane to the *n*-hexane layer, extract with shaking, centrifuge at 3,000 rpm for 5 min, collect the acetonitrile layer, and combine with the previously collected acetonitrile layer. Concentrate at below 40°C, remove the solvent and dissolve the residue in a mixed solution of ethanol and acetic acid (1:1) to make exactly 10 mL.

3) Oxidation reaction

Take exactly a 5 mL aliquot of the solution obtained in 2), add 25 µL of hydrogen peroxide, mix, seal tightly and heat at 90°C for 16 hrs. After returning it to room temperature, add 10 mL of water, extract with shaking with 15 mL of ethyl acetate and *n*-hexane (1:1) mixed solution, centrifuge at 3,000 rpm for 5 min and collect the organic layer. Then, add 15 mL of ethyl acetate and *n*-hexane (1:1) mixed solution to the aqueous layer, extract with shaking, centrifuge at 3,000 rpm for 5 min, collect the organic layer, and combine with the previously collected organic layer. Concentrate at below 40°C, remove the solvent and dissolve the residue in 2 mL of water and methanol (3:7) mixed solution.

4) Clean-up

Inject 5 mL of methanol and 5 mL of water and methanol (3:7) mixed solution into a sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg) sequentially and discard each effluent. Transfer the solution obtained in 3) to the cartridge, add 10 mL of water and methanol (3:7) mixed solution and discard the effluent. Then, add 20 mL of water and methanol (1:19) mixed solution, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in a mixed solution of acetonitrile and water (1:1) to make exactly 2 mL, and use this solution as the test solution.

6. Calibration curve

Prepare metabolite D standard solutions (acetonitrile and water [1:1]) of several concentrations, 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 metabolite D in the test solution corresponding to 0.01 mg/kg (equivalent to triclabendazole) in the sample is 0.0015 mg/L (equivalent to triclabendazole).

7. Quantification

Inject the test solution into LC-MS/MS, and calculate the concentration of metabolite D from the calibration curve prepared in **6**. The concentration of triclabendazole (including metabolites converted to metabolite D under acidic conditions) is calculated using the following equation.

Concentration of triclabendazole (including metabolites converted to metabolite D under acidic conditions) (ppm) = concentration of metabolite D (ppm) × 1.091

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: A mixed solution of 0.1 vol% formic acid and 0.1 vol% formic acid-acetonitrile solution (1:1)

Ionization mode: ESI (−)

Major monitoring ions (*m/z*): Precursor ion 327, product ions 182, 146

Injection volume: 5 μL

Expected retention time: 5 min

10. Limit of quantification

0.01 mg/kg (equivalent to triclabendazole)

11. Explanatory note

1) Outline of analytical method

The method consists of hydrolyzing the sample by heating in the presence of sodium hydroxide at 100°C for 3 hrs, acidifying it with hydrochloric acid, extracting triclabendazole metabolites that are converted to metabolite D under acidic conditions and triclabendazole with ethyl acetate, defatting by liquid-liquid partitioning using acetonitrile and hexane, dissolving in a mixture of ethanol and acetic acid, heating at 90°C for 16 hrs by adding hydrogen peroxide, oxidizing triclabendazole and its metabolites to metabolite D, extracting with a mixture of ethyl acetate and *n*-hexane from the solution after oxidation reaction, cleaning up with a sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, and quantifying and confirming using LC-MS/MS. In this method, metabolite D is quantified, and the concentration of metabolite D is converted to the concentration of triclabendazole (including the metabolites converted to metabolite D under acidic conditions) by multiplying it by the conversion factor and is regarded as the analytical result of triclabendazole.

2) Notes

i) For the oxidation reaction with hydrogen peroxide, confirm that the oxidation reaction to metabolite D has progressed sufficiently using the reference standards of triclabendazole, metabolite A, and metabolite B.

ii) A mixture of ethanol and acetic acid used in the oxidation reaction should be prepared just before use since it significantly affects its efficiency.

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

for quantitative ions (*m/z*): precursor ion 327, product ion 182

for qualitative ions (*m/z*): precursor ion 327, product ion 146

For reference, when the analytical methods for triclabendazole, metabolite A and

metabolite B using LC-MS/MS were developed, the following monitoring ions were used:

Triclabendazole

for quantitative ions (*m/z*): precursor ion 357, product ion 342

for qualitative ions (*m/z*): precursor ion 357, product ion 197

Metabolite A

for quantitative ions (*m/z*): precursor ion 373, product ion 358

for qualitative ions (*m/z*): precursor ion 373, product ion 181

Metabolite B

for quantitative ions (*m/z*): precursor ion 389, product ion 310

for qualitative ions (*m/z*): precursor ion 389, product ion 181

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

12. Reference

Determination of residues of triclabendazole in animal tissues by HPLC. Analytical procedure 193F.00, Novartis Animal Health Austrasia Pty. Ltd., Australia, 2004.

13. Type

C