Original: Japanese
Provisional translation

Analytical Method for Prothioconazole (Animal Products)

1. Analyte

Metabolite M17 [2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1*H*-1,2,4-triazole-1-yl)-2-propanol] (including conjugates)

2. Applicable food

Animal products and milk

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 metabolite M17: Contains not less than 98% of metabolite M17.

5. Procedure

1) Extraction

Weigh 10.0 g of the sample (5.00 g for fat). Add 40 mL of acetonitrile and water (4:1, v/v) and 20 mL of *n*-hexane to the sample, homogenize, centrifuge at 3,000 rpm for 10 min, and collect the supernatant. Add 40 mL of acetonitrile and water (4:1, v/v) and 20 mL of *n*-hexane to the residue, homogenize, centrifuge as described above, and combine the resulting supernatants. Collect the mixed layer of acetonitrile and water, add 3 drops of defoaming silicone, concentrate at below 40°C, and remove the solvent.

2) Hydrolysis

Add 10 mL of water and 20 mL of 5 mol/L hydrochloric acid to the residue obtained in 1), connect to a reflux apparatus, heat under reflux for 2 hrs, and hydrolyze the conjugates of metabolite M17. Allow to cool and add water to the reaction solution to make exactly 100 mL.

3) Clean-up

Inject 5 mL each of acetonitrile and water into an octadecylsilanized silica gel cartridge (500 mg) sequentially and discard each effluent. Take exactly a 10 mL (20 mL for fat) aliquot of the solution obtained in 2), inject into the column, add 10 mL of acetonitrile and water (1:4, v/v), and discard the effluent. Then, add 10 mL of acetonitrile and water (4:1, v/v), add acetonitrile and water (4:1, v/v) to the eluate to make exactly 10 mL, and use this solution as the test solution.

6. Calibration curve

Prepare metabolite M17 standard solutions (acetonitrile and water [4:1, v/v]) of several concentrations, inject each standard solution into LC-MS/MS respectively, and make

calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, for muscle, fat and viscera, the concentration of metabolite M17 in the test solution corresponding to 0.01 mg/kg (equivalent to prothioconazole) in the sample results in 0.001 mg/L (equivalent to prothioconazole). For milk, the concentration of metabolite M17 in the test solution corresponding to 0.004 mg/kg (equivalent to prothioconazole) in the sample results in 0.0004 mg/L (equivalent to prothioconazole).

7. Quantification

Inject the test solution into LC-MS/MS, calculate the concentration of metabolite M17 from the calibration curve obtained in 6., and calculate the concentration of prothioconazole including metabolite M17 and its conjugates using the following equation.

Concentration of prothioconazole (including metabolite M17 and its conjugates) (ppm) = Concentration of metabolite M17 (ppm) × 1.103

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 acetonitrile (1:1, v/v) to (1:9, v/v) in 10 min, and hold for 5 min.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*): Precursor ion 312, product ions 125, 70

Injection volume: 4 μL

Expected retention time: 6 min

10. Limit of quantification

Muscle, fat and viscera: 0.01 mg/kg (equivalent to prothioconazole)

Milk: 0.004 mg/kg (equivalent to prothioconazole)

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of metabolite M17 and its conjugates from the sample using acetonitrile and water (4:1, v/v) containing *n*-hexane, hydrolysis of the conjugate of metabolite M17 to metabolite M17 using hydrochloric acid, clean-up using an octadecylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS. In the method, the concentration of metabolite M17 is converted to the concentration of prothioconazole by multiplying by the conversion factor, and is regarded as the analytical result of prothioconazole.

2) Notes

i) When the analytical methods for metabolite M17 using LC-MS/MS were developed, the

following monitoring ions were used:

for quantitative ions (*m/z*): precursor ion 312, product ion 70

for qualitative ions (*m/z*): precursor ion 312, product ion 125

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

12. References

- JAU6476 Independent Method Validation (Battelle Study Number A4-14-01-01)
- [Phenyl-UL-^{14}C] JAU6476-desthio Absorption, Distribution, Excretion, and Metabolism in the Lactating Goat

13. Type

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