Original: Japanese
Provisional translation

Analytical Method for Hexythiazox (Animal Products)

1. Analytes

Hexythiazox

Metabolites converted to *trans*-5-(4-chlorophenyl)-4-methylthiazolidin-2-one (hereinafter referred to as PT-1-3) by hydrolysis under alkaline conditions.

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 hexythiazox: Contains not less than 98% of hexythiazox.

Reference standard of PT-1-3: Contains not less than 98% of PT-1-3.

5. Procedure

1) Extraction

Add 100 mL of acetone to 10.0 g of sample, homogenize, centrifuge at 3,000 rpm for 10 min, and collect the supernatant. Add 50 mL of acetone to the residue, homogenize, centrifuge as described above, combine the resulting supernatant and add acetone to make exactly 200 mL. Take exactly a 2 mL aliquot of the solution, concentrate at below 40°C, and remove the solvent. Add 30 mL of *n*-hexane to the residue, and extract with shaking three times with 30 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts and concentrate to approximately 2 mL at below 40°C.

2) Clean-up and hydrolysis

Inject 10 mL of acetonitrile into an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) and discard the effluent. Load the concentrated solution obtained in 1) to the cartridge, add 10 mL of acetonitrile, collect the total eluate including the loaded solution, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 2 mL of methanol, add 20 mL of 0.1 mol/L sodium hydroxide solution, mix, seal tightly and heat at 60°C for 80 min. Allow the heated solution to cool until it reaches room temperature.

3) Clean-up

Inject 10 mL each of methanol and water into a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg) sequentially and discard each effluent. Transfer the hydrolyzed solution obtained in 2) to the cartridge, add 10 mL of water and discard the effluent. Then, add 10 mL of methanol, concentrate the eluate at below 40°C, and remove

the solvent. Dissolve the residue in methanol to make exactly 5 mL and use this solution as the test solution.

6. Calibration curve

Prepare PT-1-3 standards in methanol solution at 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 PT-1-3 in the test solution corresponding to 0.01 mg/kg in the sample is 0.0002 mg/L (equivalent to hexythiazox).

7. Quantification

Inject the test solution into LC-MS/MS, calculate the concentration of PT-1-3 from the calibration curve prepared in **6** and calculate the concentration of hexythiazox using the following equation.

Concentration of hexythiazox (including metabolites converted to PT-1-3 by hydrolysis under alkaline conditions) (ppm) = concentration of PT-1-3 (ppm) × 1.550

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 a mixed solution of 0.1 vol% formic acid and methanol (9:1) to (1:99) in 10 min and hold for 10 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 228, product ions 168, 116

Injection volume: 10 μL

Expected retention time: 12 min

10. Limit of quantification

0.01 mg/kg (equivalent to hexythiazox)

11. Explanatory note

1) Outline of analytical method

The method consists of extracting hexythiazox and its metabolites from sample with acetone, defatting by acetonitrile/hexane partitioning, cleaning up with an ethylenediamine-*N*-propylsilanized silica gel cartridge, converting hexythiazox and its metabolites to PT-1-3 by adding sodium hydroxide solution, cleaning up with a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge and quantifying and confirming using LC-MS/MS.

2) Notes

i) When the analytical methods for hexythiazox and PT-1-3 using LC-MS/MS were developed, the following monitoring ions were used:

Hexythiazox

for quantitative ions (*m/z*): precursor ion 353, product ion 228

for qualitative ions (*m/z*): precursor ion 353, product ion 168

ii) In hydrolysis, prolonged heating may reduce the recovery rate. Therefore, ensure that hexythiazox has been converted to PT-1-3 using a hexythiazox standard before testing, and perform the test with the optimal heating time.

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

12. Reference

None

13. Type

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