Original: Japanese
Provisional translation

Analytical Method for Ethiprole (Animal and Fishery Products)

1. Analyte

Ethiprole

2. Application

Animal and fishery products

3. Instruments

Liquid chromatograph-mass spectrometer (LC-MS) or 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 ethiprole: Contains not less than 98% of ethiprole.

5. Procedure

1) Extraction

i) Except for honey

Add 100 mL of acetone to 10.0 g of sample, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates and add acetone to make exactly 200 mL. Take exactly a 40 mL aliquot of the solution and concentrate to approximately 2 mL at below 40℃. Add 100 mL of 10 w/v% sodium chloride solution and extract with shaking twice with 100 mL and 50 mL each of ethyl acetate and *n*-hexane (1:1) mixed solution. Combine the extracts, dehydrate with anhydrous sodium sulfate, filter out anhydrous sodium sulfate, concentrate at below 40℃, and remove the solvent. Add 30 mL of *n*-hexane to the residue and extract with shaking twice with 30 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40℃, and remove the solvent. Dissolve the residue in acetonitrile to make exactly 6 mL. Take exactly a 3 mL aliquot of the solution and add 7 mL of water.

ii) Honey

Dissolve 10.0 g of sample in 10 mL of water. Add 100 mL of acetone to the sample, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates and add acetone to make exactly 200 mL. Take exactly a 40 mL aliquot of the solution and concentrate to approximately 2 mL at below 40°C. Add 100 mL of 10 w/v% sodium chloride solution and extract with shaking twice with 100 mL and 50 mL each of ethyl acetate and *n*-hexane (1:1) mixed solution. Combine the extracts, dehydrate with anhydrous sodium sulfate, filter out anhydrous sodium sulfate, concentrate at below 40℃, and remove the solvent. Dissolve the

residue in acetonitrile to make exactly 6 mL. Take exactly a 3 mL aliquot of the solution and add 7 mL of water.

2) Clean-up

Inject 5 mL each of acetonitrile and water into an octadecylsilanized silica gel cartridge (500 mg) sequentially and discard the effluent. Transfer the solution obtained in 1) to the cartridge and discard the effluent. Then, inject 10 mL of acetonitrile and water (1:1) mixed solution, add acetonitrile and water (1:1) mixed solution to the eluate to make exactly 10 mL, and use this solution as the test solution.

6. Calibration curve

Prepare ethiprole standard solutions (acetonitrile and water [1:1]) of several concentrations, inject each standard solution into LC-MS or LC-MS/MS and make a calibration curve by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of ethiprole in the test solution corresponding to 0.01 mg/kg in the sample is 0.001 mg/L.

7. Quantification

Inject the test solution into LC-MS or LC-MS/MS and calculate the concentration of ethiprole from the calibration curve prepared in **6.**

8. Confirmation

Confirm using LC-MS or LC-MS/MS.

9. Measurement conditions

(Example)

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

Column temperature: 40°C

Mobile phase: Linear gradient from a mixed solution of acetonitrile and 2 mmol/L ammonium acetate solution (3:7) to (9:1) in 22 min and hold at (9:1) for 3 min.

Ionization mode:

1) LC-MS: ESI (−), ESI (+)
2) LC-MS/MS: ESI (−)

Major monitoring ions (*m/z*):

1) LC-MS: 395 (−), 397 (+)
2) LC-MS/MS: Precursor ion 395, product ions 330, 262, 250

Injection volume: 5 μL

Expected retention time: 14 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extracting ethiprole from sample with acetone, transferring it into ethyl acetate and *n*-hexane (1:1) mixed solution for re-dissolution, defatting by

acetonitrile/hexane partitioning, cleaning up with an octadecylsilanized silica gel cartridge, and quantifying and confirming using LC-MS or LC-MS/MS.

2) Notes

i) When the analytical method for ethiprole using LC-MS or LC-MS/MS was developed, the following monitoring ions were used:

LC-MS

for quantitative ion (*m*/*z*): 395 (−)

for qualitative ion (*m*/*z*): 397 (+)

LC-MS/MS

for quantitative ions (*m*/*z*): precursor ion 395, product ion 330

for qualitative ions (*m*/*z*): precursor ion 395, product ions 262, 250

ii) In LC-MS, *m/z* 397 in ESI (−) and *m/z* 399 in ESI (+) are confirmed besides the major monitoring ions.

iii) The sensitivity may be low in ESI (+) mode depending on the type of equipment used. In such cases, it is possible to measure ethiprole with high sensitivity using the following conditions.

Measurement conditions

Mobile phase: Linear gradient from a mixed solution of 5 mmol/L ammonium acetate solution and 5 mmol/L ammonium acetate-methanol solution (7:3) to (1:99) in 15 min and hold at (1:99) for 5 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*)

LC-MS: 399, 397

LC-MS/MS: Precursor ion 397, product ions 351, 255

Injection volume: 5 μL

Expected retention time: 14 min

iv) Food items used to develop the analytical method: cattle muscle, cattle fat, cattle liver, cattle milk, chicken egg, honey, eel, salmon and *Corbicula* (freshwater clam).

12. Reference

MHLW Director Notice, Syoku-An No.0124004, [Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food] (Analytical Method for Ethiprole), (January 24, 2005)

13. Type

C