Original: Japanese
Provisional translation

Analytical Method for Kresoxim-methyl (Animal and Fishery Products)

1. Analytes

Kresoxim-methyl

2-[2-(4-hydroxy-2-methylphenoxymethyl)phenyl]-2-methoxyimino acetic acid (hereinafter referred to as metabolite M9)

2. Application

Animal and fishery 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 kresoxim-methyl: Contains not less than 97% of kresoxim-methyl.

Reference standard of metabolite M9: Contains not less than 98% of metabolite M9.

5. Procedure

1) Extraction

Add 100 mL of 0.5 vol% formic acid-acetone solution to 10.0 g of the sample, homogenize, and filter with suction. Add 50 mL of 0.5 vol% formic acid-acetone solution to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates and add 0.5 vol% formic acid-acetone solution to make exactly 200 mL. Take exactly a 2 mL aliquot of the solution.

2) Clean-up

i) Graphitized carbon black column chromatography

Inject 5 mL of 0.5 vol% formic acid-acetone solution into a graphitized carbon black cartridge (250 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add 10 mL of 0.5 vol% formic acid-acetone solution, and collect the total eluate. Add 10 mL of 0.1 vol% formic acid and concentrate to less than 10 mL at below 40℃.

ii) Octadecylsilanized silica gel column chromatography

Inject 5 mL each of methanol and 0.1 vol% formic acid into an octadecylsilanized silica gel cartridge (1,000 mg) sequentially and discard each effluent. Transfer the solution obtained in 1) to the cartridge, add 10 mL of 0.1 vol% formic acid and methanol (3:2, v/v), and discard the effluent. Then, add 10 mL of 0.1 vol% formic acid-methanol solution, collect the eluate, add 0.1 vol% formic acid-methanol solution to make exactly 10 mL, and use this solution as the test solution.

6. Calibration curve

Prepare stock standard solutions by dissolving the kresoxim-methyl reference standard and the metabolite M9 reference standard, respectively. Prepare several solutions by mixing each stock standard solution appropriately and diluting with 0.1 vol% formic acid-methanol solution. 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 kresoxim-methyl and metabolite M9 in the test solution corresponding to 0.01 mg/kg (The concentration of metabolite M9 is calculated as kresoxim-methyl.) in the sample results in 0.0001 mg/L (The concentration of metabolite M9 is calculated as kresoxim-methyl.) for each analyte.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of kresoxim-methyl and metabolite M9 from the calibration curve made in 6. Use the following equation to calculate the concentration of kresoxim-methyl including metabolite M9.

Concentration (ppm) of kresoxim-methyl (including metabolite M9) = A + B × 0.9938

A: Concentration (ppm) of kresoxim-methyl

B: Concentration (ppm) of metabolite M9

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 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile and 0.01 vol% formic acid (2:3, v/v) to (7:3, v/v) in 10 min and hold for 3 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*):

Kresoxim-methyl: Precursor ion 314, product ions 131, 116

Metabolite M9: Precursor ion 316, product ions 269, 116

Injection volume: 5 μL

Expected retention time:

Kresoxim-methyl: 12 min

Metabolite M9: 5 min

10. Limit of quantification

0.01 mg/kg for each analyte (The concentration of metabolite M9 is calculated as kresoxim-methyl.)

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of kresoxim-methyl and metabolite M9 from the sample with acetone under acidity using formic acid, clean-up with a graphitized carbon black cartridge and an octadecylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS. In the method, kresoxim-methyl and metabolite M9 are quantified respectively. For the concentration of kresoxim-methyl including metabolite M9, the concentration of metabolite M9 is converted to the concentration of kresoxim-methyl by multiplying by the conversion factor, and the sum of the concentrations of kresoxim-methyl and metabolite M9 is regarded as the analytical result of kresoxim-methyl.

2) Notes

i) When the analytical methods for kresoxim-methyl and metabolite M9 using LC-MS/MS were developed, the following monitoring ions were used:

Kresoxim-methyl

for quantitative ions (*m/z*): precursor ion 314, product ion 116

for qualitative ions (*m/z*): precursor ion 314, product ion 131

Metabolite M9

for quantitative ions (*m/z*): precursor ion 316, product ion 269

for qualitative ions (*m/z*): precursor ion 316, product ion 116

ii) Food items used to develop the analytical method: cattle muscle, cattle fat, cattle liver, milk, eel and *Corbicula* (freshwater clam)

12. References

None

13. Type

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