Original: Japanese

Provisional Translation

Analytical Method for Halosulfuron Methyl (Animal and Fishery Products)

1. Analyte

Halosulfuron methyl

2. Instruments

Liquid chromatograph-mass spectrometer (LC-MS)

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

3. Reagents

Use the reagents listed in Section 3 of the General Rules, except the following.

Reference standard of halosulfuron methyl: Contains not less than 97% of halosulfuron methyl.

4. Procedure

1) Extraction

Weigh 10.0 g (5.00 g for fat) of sample. Add 50 mL of acetone/*n*-hexane (1:2, v/v), 6 mL of 0.1 mol/L hydrochloric acid and 8 g of sodium chloride, homogenize, centrifuge at 3,000 rpm for 5 minutes, and take the upper acetone/*n*-hexane layer. Add 50 mL of *n*-hexane to the lower acetone/water layer, homogenize, centrifuge at 3,000 rpm for 5 minutes. Combine the upper acetone/*n*-hexane layer with the former acetone/*n*-hexane layer, and make exactly 100 mL. Take a 10 mL (20 mL for fat) aliquot of the solution, concentrate at below 40°C, and remove the solvent. Add 20 mL of *n*-hexane to the residue, extract with shaking with 20 mL of acetonitrile saturated with *n*-hexane. Concentrate the acetonitrile layer at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetone/*n*-hexane (3:1, v/v).

2) Clean-up

Add 10 mL each of acetone/methanol (1:1, v/v) and acetone/*n*-hexane (3:1, v/v) to a trimethylaminopropylsilanized silica gel cartridge (500 mg) sequentially, and discard the effluents. Transfer the solution obtained in 1) to the cartridge, add 10 mL of acetone/*n*-hexane (3:1, v/v), and discard the effluent. Elute with 10 mL of acetone/methanol (1:1, v/v), 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.

5. Calibration curve

Prepare halosulfuron methyl standard solutions (methanol) of several concentrations. Inject each standard solution to 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 sample containing 0.01 mg/kg of halosulfuron methyl gives the test solution of 0.002 mg/L in concentration.

6. Quantification

Inject the test solution to LC-MS or LC-MS/MS, and calculate the concentration of halosulfuron methyl from the calibration curve made in **5**.

7. Confirmation

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

8. Measurement conditions

Example

1) LC-MS

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 100 mm in length and 3 µm in particle diameter

Column temperature: 40°C

Mobile phase: 0.01 vol% acetic acid/0.01 vol% acetic acid-methanol solution (1:4, v/v)

Ionization mode: ESI (-)

Major monitoring ions (*m/z*): 433, 254, 252

Injection volume: 10 µL

Expected retention time: 5 min

2) LC-MS/MS

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 100 mm in length and 3 µm in particle diameter

Column temperature: 40°C

Mobile phase: Initially 0.01 vol% acetic acid/0.01 vol% acetic acid-methanol solution (17:3, v/v) for 0.5 min, followed by a linear gradient to (1:9, v/v) in 3.5 min and hold for 5 min.

Ionization mode: ESI (-)

Major monitoring ions (*m/z*): Precursor ion 435, product ion 254

Precursor ion 433, product ion 252

Injection volume: 2 µL

Expected retention time: 8 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory notes

1) Outline of analytical method

The method consists of extraction of halosulfuron methyl from sample with acetone/*n*-hexane (1:2, v/v) under acidic condition (hydrochloric acid), defatting by acetonitrile/hexane partitioning, clean-up with a trimethylaminopropylsilanized silica gel cartridge, and quantification and confirmation using LC-MS or LC-MS/MS.

2) Notes

i) When the analytical method for halosulfuron methyl was developed, the following monitoring ions were used:

LC-MS

for quantification (*m/z*): 252

for confirmation (*m/z*): 433, 254

LC-MS/MS

for quantification (*m/z*): precursor ion 433, product ion 252

for confirmation (*m/z*): precursor ion 435, product ion 254

ii) In the testing of egg and milk, the solution may gelate in the extraction process if hydrochloric acid is added first. Therefore, add acetone/*n*-hexane to the sample before adding hydrochloric acid.

iii) For honey, defatting by acetonitrile/hexane partitioning can be omitted because honey has almost no fat.

11. References

None

12. Type

C