Original: Japanese
Provisional translation

Analytical Method for Silafluofen
(Animal and Fishery Products)

1. Analytes

Silafluofen

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 silafluofen: Contains not less than 98% of silafluofen.

5. Procedure

1) Extraction

For muscle, fat, viscera, fish and shellfish, add 10 mL of water to 10.0 g of each sample and homogenize.

For milk and eggs, weigh 10.0 g of each sample.

Add 50 mL of acetone and *n*-hexane (1:2, v/v) to the sample, homogenize, centrifuge at 3,000 rpm for 5 min, and collect the organic layer. Add 30 mL of *n*-hexane to the residue, homogenize, and centrifuge as described above. Combine the resulting organic layers and add *n*-hexane to make exactly 100 mL. Take exactly a 10 mL aliquot of the solution, concentrate at below 40°C, and remove the solvent. Add 10 mL of *n*-hexane to the residue, extract with shaking twice using 20 mL each of acetonitrile saturated with *n*-hexane, and further extract with shaking using 10 mL of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of *n*-hexane.

2) Clean-up

Add 10 mL of *n*-hexane to an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge and add 10 mL of *n*-hexane. Collect the total eluate including the transferred solutions, concentrate at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile to make exactly 5 mL and use this solution as the test solution.

6. Calibration curve

Prepare silafluofen standard solutions (acetonitrile) of 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 silafluofen in the test solution corresponding to 0.01 mg/kg in the sample results in 0.002 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of silafluofen from the calibration curve made in 6.

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: Initially 20 mmol/L ammonium acetate solution and 20 mmol/L ammonium acetate-methanol solution (2:3, v/v) for 2 min, followed by a linear gradient to (1:49, v/v) in 3 min, and hold for 9 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 426, product ions 287, 181, 168

Injection volume: 2 μL

Expected retention time: 11 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of silafluofen from the sample with a mixture of acetone and *n*-hexane (1:2, v/v), further extraction with *n*-hexane, defatting by acetonitrile/hexane partitioning, clean-up with an ethylenediamine-*N*-propylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) When the analytical methods for silafluofen using LC-MS/MS were developed, the following monitoring ions were used:

for quantitative ions *(m/z*): precursor ion 426, product ion 287

for qualitative ions (*m/z*): precursor ion 426, product ions 181, 168

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

12. References

None

13. Type

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