Original: Japanese
Provisional translation

Analytical Method for Ipfencarbazone (Animal and Fishery Products)

1. Analyte

Ipfencarbazone

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 ipfencarbazone: Contains not less than 98% of ipfencarbazone.

5. Procedure

1) Extraction

i) Muscle, fat, viscera, egg, milk, fish and shellfish

Weigh 10.0 g of sample (5.00 g for fat), add 70 mL of acetone and *n*-hexane (1:2) mixed solution, and homogenize. Centrifuge at 3,000 rpm for 5 min and collect the organic layer. Add 30 mL of acetone and *n*-hexane (1:2) mixed solution to the residue, homogenize and centrifuge as described above. Combine the organic layer with the previous organic layer and add acetone and *n*-hexane (1:2) mixed solution to make exactly 100 mL. Take exactly a 10 mL aliquot of the solution (exactly a 20 mL aliquot of the solution for fat), concentrate at below 40ºC, and remove the solvent. Add 20 mL of *n*-hexane to the residue and extract with shaking twice using 20 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40ºC and remove the solvent. Dissolve the residue in 5 mL of acetone and *n*-hexane (1:9) mixed solution.

ii) Honey

Dissolve 10.0 g of sample in 6 mL of water, add 70 mL of acetone and *n*-hexane (1:2) mixed solution, and homogenize. Centrifuge at 3,000 rpm for 5 min and collect the organic layer. Add 30 mL of acetone and *n*-hexane (1:2) mixed solution to the residue, homogenize and centrifuge as described above. Combine the organic layer with the previous organic layer and add acetone and *n*-hexane (1:2) mixed solution to make exactly 100 mL. Take exactly a 10 mL aliquot of the solution, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetone and *n*-hexane (1:9) mixed solution.

2) Clean-up

i) Ethylenediamine-*N*-propylsilanized silica gel column chromatography

Add 10 mL each of acetone and *n*-hexane to an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) sequentially and discard each effluent. Transfer the solution

obtained in 1) to the cartridge, add 5 mL of acetone and *n*-hexane (1:9) mixed solution, collect the total eluate including the transferred solutions, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetonitrile and water (3:7) mixed solution.

ii) Octadecylsilanized silica gel column chromatography

Inject 10 mL each of acetonitrile and water into an octadecylsilanized silica gel cartridge (500 mg) sequentially and discard each effluent. Transfer the solution obtained in i) to the cartridge, add 5 mL of acetonitrile and water (3:7) mixed solution and discard the effluent. Then, add 10 mL of acetonitrile and water (3:2) mixed solution, add acetonitrile and water (3:21) mixed solution to the eluate to make exactly 10 mL, and use this solution as the test solution.

6. Calibration curve

Prepare ipfencarbazone standard solutions (acetonitrile and water [3:2]) of several concentrations, inject each standard solution into 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 ipfencarbazone 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/MS and calculate the concentration of ipfencarbazone from the calibration curve prepared in **6.**

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel 2.1 mm inside diameter, 100 mm in length, 3.5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially, a mixed solution of 0.01 vol% acetic acid-acetonitrile solution and 0.01 vol% acetic acid (1:1) mixed solution for 0.5 min, followed by a linear gradient to (4:1) in 7.5 min, and hold for 4 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*):

Precursor ion 427, product ion 198

Precursor ion 429, product ion 198

Injection volume: 5 μL

Expected retention time: 6 min

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extracting ipfencarbazone from sample using acetone and *n*-hexane (1:2) mixed solution, defatting by acetonitrile/hexane partitioning, cleaning up with an ethylenediamine-*N*-propylsilanized silica gel cartridge and an octadecylsilanized silica gel cartridge and quantifying and confirming using LC-MS/MS.

2) Notes

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

for quantitative ions (*m*/*z*): precursor ion 427, product ion 198

for qualitative ions (*m*/*z*): precursor ion 429, product ion 198

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

12. Reference

None

13. Type

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