Original: Japanese
Provisional translation

Analytical Method for Ipfencarbazone (Agricultural Products)

1. Analyte

Ipfencarbazone

2. Applicable food

Agricultural 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) Grains, legumes, nuts and seeds

Add 20 mL of water to 10.0 g of the sample and let stand for 30 min. Add 100 mL of acetone, 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 20 mL aliquot of the solution, add 20 mL of saturated sodium chloride solution, and extract with shaking using 20 mL of *n*-hexane. Then, centrifuge at 3,000 rpm for 5 min and collect the supernatant. Add 20 mL of *n*-hexane to the aqueous layer, extract with shaking, centrifuge as described above, and collect the supernatant. Combine the resulting supernatant with the previously obtained supernatant, concentrate at below 40℃, and remove the solvent. Dissolve the residue in 5 mL of acetone and *n*-hexane (1:9, v/v).

ii) Fruits and vegetables

Add 100 mL of acetone to 20.0 g of 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 10 mL aliquot of the solution, add 10 mL of saturated sodium chloride solution, and extract with shaking using 10 mL of *n*-hexane. Then, centrifuge at 3,000 rpm for 5 min, and collect the supernatant. Add 10 mL of *n*-hexane to the aqueous layer, extract with shaking, centrifuge as described above, and collect the supernatant. Combine the resulting supernatant with the previously obtained supernatant, concentrate at below 40℃, and remove the solvent. Dissolve the residue in 5 mL of acetone and *n*-hexane (1:9, v/v).

iii) Tea leaves

Add 20 mL of water to 5.00 g of the sample and let stand for 30 min. Add 100 mL of acetone,

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, add 40 mL of saturated sodium chloride solution, and extract with shaking using 40 mL of *n*-hexane. Then, centrifuge at 3,000 rpm for 5 min, and collect the supernatant. Add 40 mL of *n*-hexane to the aqueous layer, extract with shaking, centrifuge as described above, and collect the supernatant. Combine the resulting supernatant with the previously obtained supernatant, concentrate at below 40℃, and remove the solvent. Dissolve the residue in 5 mL of acetone and *n*-hexane (1:9, v/v).

2) Clean-up

i) Graphitized carbon black/ethylenediamine-*N*-propylsilanized silica gel layered column chromatography

Add 10 mL each of acetone and *n*-hexane to a graphitized carbon black/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg) sequentially and discard each effluent. Transfer the solution obtained in 1), add 5 mL of acetone and *n*-hexane (1:9, v/v), collect the total eluate including the transferred solutions, concentrated the eluate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetonitrile and water (3:7, v/v).

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 1) to the cartridge, add 5 mL of acetonitrile and water (3:7, v/v), and discard the effluent. Then, add 10 mL of acetonitrile and water (3:2, v/v), add acetonitrile and water (3:2, v/v) 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, v/v]) 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 results in 0.001 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of ipfencarbazone 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, 100 mm in length, 3.5 μm in particle diameter

Column temperature: 40°C

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

Ionization mode: ESI (+)

Major monitoring ions (*m/z*):

Precursor ion 427, product ions 198

Precursor ion 429, product ions 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 extraction of ipfencarbazone from the sample with acetone, transfer into *n*-hexane for re-dissolution, clean-up with a graphitized carbon black/ethylenediamine-*N*-propylsilanized silica gel layered cartridge and an octadecylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) When the analytical methods for ipfencarbazone using LC-MS/MS were 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) The foods examined in the development of the analytical method: brown rice, soybeans, spinach, cabbage, potatoes, oranges, apples and tea leaves

12. Reference

None

13. Type

C