Analytical Method for Cyenopyrafen (Agricultural Products)

1. Analyte

Cyenopyrafen

2. Instrument

Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents

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

Reference standard of cyenopyrafen: Contains not less than 98% of cyenopyrafen. Melting point of the standard is 107–108°C.

4. Procedure

1) Extraction

For fruits and vegetables, weigh 20.0 g of sample. For grains, legumes, nuts and seeds, weigh 10.0 g of sample, and for tea leaves, weigh 5.00 g of sample, add 20 mL of water and let stand for 30 minutes.

Add 100 mL of acetonitrile/water (4:1, v/v), homogenize, and filter with suction. Add 50 mL of acetonitrile/water (4:1, v/v) to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add acetonitrile to make exactly 200 mL. Take a 2 mL (4 mL for tea leaves) aliquot of the exact, add 10 mL of water, and concentrate to about 10 mL at below 40°C.

2) Clean-up

i) Octadecylsilanized silica gel column chromatography and graphitized carbon black column chromatography

Add 5 mL each of acetonitrile and water to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluent. Add 5 mL of acetonitrile/water (4:1, v/v) to a graphitized carbon black cartridge (500 mg), and discard the effluent. Transfer the extract obtained in 1) to the octadecylsilanized silica gel cartridge, add 10 mL of acetonitrile/water (3:2, v/v), and discard the effluent. Connect the graphitized carbon black cartridge at the bottom of the octadecylsilanized silica gel cartridge, and elute with 15 mL of acetonitrile/water (4:1, v/v). Concentrate the eluate at below 40°C and remove the solvent. Dissolve the residue in 5 mL of ethyl acetate/*n*-hexane (1:19, v/v).

ii) Silica gel column chromatography

Add 5 mL of ethyl acetate/*n*-hexane (1:19, v/v) to a silica gel cartridge (690 mg), and discard the effluent. Transfer the solution obtained in i), add 5 mL of ethyl acetate/*n*-hexane (1:19, v/v), and discard the effluent. Elute with 10 mL of ethyl acetate/*n*-hexane (1:4, v/v), and concentrate the eluate at below 40°C and remove the

solvent. Dissolve the residue in acetonitrile/water (3:1, v/v) to make exactly 4 mL for fruits and vegetables, 2 mL for grains, legumes, nuts and seeds, and tea leaves, and use this solution as the test solution.

5. Calibration curve

Prepare 0.0005–0.01 mg/L cyenopyrafen standard solutions (acetonitrile/water (3:1, v/v)). Inject 5 μL of each standard solution to LC-MS, and make a calibration curve by peak-height or peak-area method.

6. Quantification

Inject 5 μL of the test solution to LC-MS and calculate the concentration of cyenopyrafen from the calibration curve made in **5**.

7. Confirmation

Confirm using LC-MS.

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 150 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially mobile phase A/mobile phase B (1:3, v/v) for 15 min, followed by a linear gradient to (1:19, v/v) in 0.5 min and hold for 8 min.

Mobile phase A: 0.1 vol% formic acid

Mobile phase B: 0.1 vol% formic acid-acetonitrile solution

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): 395, 394

Expected retention time: 11 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of cyenopyrafen from sample with acetonitrile/water (4:1, v/v), clean-up with an octadecylsilanized silica gel cartridge, a graphitized carbon black cartridge and a silica gel cartridge, quantification and confirmation using LC-MS.

2) Notes

i) For orange peel, use 10.0 g of sample and make the test solution volume to 2 mL.

ii) When the analytical method for cyenopyrafen using LC-MS was developed, the following monitoring ions were used:

for quantification (*m/z*): 394

for confirmation (*m/z*): 395

11. References

None

12. Type

C