Original: Japanese

Provisional Translation

Analytical Method for Cyflumetofen (Agricultural Products)

1. Analytes

Cyflumetofen

α,α,α-Trifluoro-*o*-toluic acid (hereafter referred to as the metabolite)

Glycoside of the metabolite

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 cyflumetofen: Contains not less than 96% of cyflumetofen. Melting point of the standard is 77–82 °C.

Reference standard of the metabolite: Contains not less than 99% of α,α,α- trifluoro-*o*-toluic acid. Melting point of the standard is 109–113 °C.

4. Procedure

1) Extraction

For fruits and vegetables, weigh 20.0 g of sample. For tea leaves, add 20 mL of water to 5.00 g of sample and let stand for 30 minutes.

Add 100 mL of acetonitrile/water (9:1, v/v), homogenize, and filter with suction. Add 50 mL of acetonitrile/water (9:1, v/v) to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetonitrile to make exactly 200 mL. Take a 20 mL (10 mL for tea leaves) aliquot of the extract, and concentrate to about 5 mL at below 40°C. Add 95 mL of water, and extract with shaking twice with 50 mL of ethyl acetate/*n*-hexane (1:9, v/v). Filter the extract through phase-separator filter paper.

Add 4 mL of hydrochloric acid to the remaining aqueous layer, heat under reflux for 1 hour, and cool. Extract with shaking twice with 50 mL of ethyl acetate/*n*-hexane (1:9, v/v). Filter the extract through phase-separator filter paper.

Combine the extracts, add 0.5 mL of 2% diethylene glycol-acetone solution, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetone/methanol (2:3, v/v).

2) Clean-up

i) Fruits and vegetables

Add 10 mL of acetone/methanol (2:3, v/v) to a graphitized carbon black cartridge (500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge, and elute with 15 mL of acetone/methanol (2:3, v/v). Collect the total eluate, add 0.5 mL of 2% diethylene glycol-acetone solution, concentrate at below 40°C, and remove the solvent. Dissolve the

residue in acetonitrile/water (2:3, v/v) to make exactly 2 mL, and use this solution as the test solution.

ii) Tea leaves

Clean-up the solution obtained in 1) with a graphitized carbon black cartridge as described in i), and dissolve the residue in 5 mL of ethyl acetate.

Add 10 mL of ethyl acetate to a trimethylaminopropylsilanized silica gel cartridge (1,000 mg) and discard the effluent. Transfer the solution described above to the cartridge, elute with 10 mL of ethyl acetate, and collect the eluate (Eluate I). Then, add 10 mL each of acetone and methanol to the cartridge sequentially, and discard the effluents. Elute with 15 mL of ammonia water/methanol (1:99, v/v), collect the eluate (Eluate II), and add 0.5 mL of 2% diethylene glycol-acetone solution. Concentrate both eluates at below 40°C, and remove the solvent. Dissolve the residues of Eluate I and Eluate II in acetonitrile/water (2:3, v/v) to make exactly 1 mL of each, and use these solutions as the test solutions of cyflumetofen and the metabolite respectively.

5. Calibration curve

Prepare 0.01–0.2 mg/L cyflumetofen and the metabolite standard solutions (acetonitrile/water (2:3, v/v)) of several concentrations. Inject 5 µL of each standard solution to LC-MS and make a calibration curves by peak-height or peak-area method.

6. Quantification

Inject 5 µL of the test solution to LC-MS and calculate the concentration of cyflumetofen and the metabolite from the calibration curves made in **5**. Use the following equation to calculate the concentration of cyflumetofen including the metabolite and glycoside of the metabolite.

Concentration (ppm) of cyflumetofen (including those of the metabolite and the glycoside of the metabolite) = A + B × 2.35

A: Concentration (ppm) of cyflumetofen

B: Concentration (ppm) of the metabolite (including that of glycoside)

7. Confirmation

Confirm using LC-MS.

8. Measurement conditions

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 150 mm in length and 5 µm in particle diameter

Mobile phase: Linear gradient from acetonitrile/0.1% formic acid containing 0.002 mol/L ammonium acetate (2:3, v/v) to (19:1, v/v) in 10 min and hold for 10 min.

Ionization mode: Cyflumetofen ESI (+), the metabolite ESI (-)

Major monitoring ions (*m/z*): Cyflumetofen 465, the metabolite 189

Expected retention time: Cyflumetofen 15 min, the metabolite 6 min

9. Limit of quantification

0.01 mg/kg for each analyte

0.04 mg/kg for each analyte (for tea leaves)

10. Explanatory note

1) Outline of analytical method

Cyflumetofen, the metabolite and the glycoside of the metabolite are extracted from sample with acetonitrile/water (9:1, v/v), and cyflumetofen is extracted with ethyl acetate/*n*-hexane (1:9, v/v). The metabolite and its glycoside remaining in the aqueous layer are hydrolyzed in acidic conditions (hydrochloric acid) under reflux, and are subsequently extracted with ethyl acetate/*n*-hexane (1:9, v/v). The combined extracts are cleaned up with a graphitized carbon black cartridge, and a trimethylaminopropylsilanized silica gel cartridge for tea leaves. Quantification and confirmation are performed using LC-MS. Cyflumetofen and the metabolite (including that of glycoside) are quantified individually, and the concentration of the metabolite is converted to the concentration of cyflumetofen by multiplying by a conversion factor. Regard the sum of the concentration of cyflumetofen and the metabolite as the analytical result of cyflumetofen.

2) Notes

i) This method is applicable to agricultural products with a low fat content (such as most of fruits, vegetables, and tea leaves). Its applicability to agricultural products with a high fat content (such as grains, legumes, nuts, seeds, and fruits like avocado) is under investigation.

ii) In this method, the glycoside of the metabolite is hydrolyzed in acidic conditions (hydrochloric acid) under reflux with heating, and extracted with ethyl acetate/*n*-hexane (1:9, v/v).

iii) For tea leaves, the recovery of cyflumetofen will be low unless cleaned up with a trimethylaminopropylsilanized silica gel cartridge.

iv) For agricultural products except tea leaves, if clean-up is inadequate, additional clean-up with the procedure for tea leaves or with a synthetic magnesium silicate cartridge (910 mg) is recommended. The procedure for the latter approach is as follows. Dissolve the residue after the clean-up with the graphitized carbon black cartridge in 5 mL of acetone/*n*-hexane (1:19, v/v). Transfer the solution to a synthetic magnesium silicate cartridge (conditioned with 5 mL of acetone/*n*-hexane (1:19, v/v)), add 5 mL of acetone/*n*-hexane (1:19, v/v), discard the effluent, and elute cyflumetofen with 10 mL of acetone/*n*-hexane (3:7, v/v). Then, add 10 mL of acetone to the cartridge, discard the effluent, and elute the metabolite with 20 mL of acetone/acetic acid/ethyl acetate (30:1:70, v/v/v).

v) Although phase-separator filter paper is used to dehydrate the solution after partitioning in this method, dehydration with anhydrous sodium sulfate is also applicable.

11. References

None

12. Type

C