Original: Japanese
Provisional translation

Analytical Method for Cycloprothrin (Agricultural Products)

1. Analyte

Cycloprothrin

2. Applicable food

Grains

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.

0.5 mol/L phosphate buffer solution (pH 7.0): Weigh 52.7 g of dipotassium hydrogen phosphate (K_2HPO_4) and 30.2 g of potassium dihydrogen phosphate (KH_2PO_4). Dissolve in approximately 500 mL of water, adjust pH to 7.0 using 1 mol/L sodium hydroxide solution or 1 mol/L hydrochloric acid, and add water to make 1 L.

Reference standard of cycloprothrin: Contains not less than 95% of cycloprothrin

5. Procedure

1) Extraction

Add 20 mL of water to 10.0 g of the sample and let stand for 30 min. Add 50 mL of acetonitrile, homogenize, and filter with suction. Add 20 mL of acetonitrile to the residue on the filter paper, homogenize, and filter with suction as described above. Combine the resulting filtrates and add acetonitrile to make exactly 100 mL. Take exactly a 20 mL aliquot of the solution, add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer solution (pH 7.0), and shake for 5 min. Let stand and discard the separated aqueous layer.

Inject 5 mL of acetonitrile into an octadecylsilanized silica gel cartridge (1,000 mg) and discard the effluent. Transfer the acetonitrile layer described above to the cartridge and add 5 mL of acetonitrile. Concentrate the total eluates including the transferred solutions at below 40°C and remove the solvent. Dissolve the residue in a 2 mL mixture of acetonitrile and toluene (3:1, v/v).

2) Clean-up

Inject a 5 mL mixture of acetonitrile and toluene (3:1, v/v) into a graphitized carbon/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge and add a 20 mL mixture of acetonitrile and toluene (3:1, v/v). Concentrate the total eluate including the transferred solutions at below 40℃ and remove the solvent. Dissolve the residue in methanol to make exactly 1 mL and use this solution as the test solution.

6. Calibration curve

Prepare cycloprothrin standard solutions (methanol) 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 cycloprothrin in the test solution corresponding to 0.01 mg/kg in the sample results in 0.02 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of cycloprothrin 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, 3.5 μm in particle diameter

Column temperature: 40℃

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: 5 mmol/L ammonium acetate solution

Mobile phase B: 5 mmol/L ammonium acetate-methanol solution

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	90	10
3	30	70
13	10	90
15	10	90

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 499, product ions 257, 181

Injection volume: 2 μL

Expected retention time: 13 min

10 Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of cycloprothrin from the sample with acetonitrile, dehydration by salting out, clean-up with an octadecylsilanized silica gel cartridge and a graphitized carbon/aminopropylsilanized silica gel layered cartridge, and quantification and confirmation using LC-MS/MS.

"5.Procedure" of this analytical method conforms to "Multi-residue method I for agricultural chemicals by LC-MS (Agricultural Products)", except for the sample concentration rate.

2) Notes

i) Considering the possibility of simultaneous analysis with other pesticides, a 20 mL mixture of acetonitrile and toluene (3:1, v/v) was used for the elution of cycloprothrin from a graphitized carbon/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg), in conformity with "Multi-residue method I for agricultural chemicals by LC-MS (Agricultural Products)". When analyzing only cycloprothrin, the volume of acetonitrile and toluene (3:1, v/v) can be reduced. Although the study was conducted using standard solutions only, it has been confirmed that cycloprothrin could be eluted with 5 mL of acetonitrile and toluene (3:1, v/v) from a graphitized carbon/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg).

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

for quantitative ions *(m/z*): precursor ion 499, product ion 181

for qualitative ions (*m/z*): precursor ion 499, product ion 257

iii) Food items used to develop the analytical method: brown rice

12. Reference

MHLW Director Notice, Seishoku No. 1-0620, multi-residue method I for agricultural chemicals by LC-MS (Agricultural Products) (June 20, 2017)

13. Type

C