Original: Japanese
Provisional translation

Analytical Method for Cycloprothrin (Fishery Products)

1. Analyte

Cycloprothrin

2. Applicable food

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 cycloprothrin: Contains not less than 95% of cycloprothrin

5. Procedure

1) Extraction

Add 50 mL of acetone to 10.0 g of the sample, homogenize, and filter with suction. Add 25 mL of acetone to the residue on the filter paper, homogenize, and filter with suction as described above. Combine the resulting filtrates and add acetone to make exactly 100 mL. Take exactly a 20 mL aliquot of the solution, add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice using 50 mL and 25 mL of *n*-hexane. Combine the resulting extracts, dehydrate with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrates at below 40℃ and remove the solvent. Add 30 mL of *n*-hexane to the residue and extract with shaking twice using 30 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts and concentrate to 1 mL or less at below 40℃.

2) Clean-up

i) Octadecylsilanized silica gel column chromatography

Inject 10 mL of acetonitrile into an octadecylsilanized silica gel cartridge (2,000 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge and add 5 mL of acetonitrile. Concentrate the total eluate including the transferred solution at below 40℃ and remove the solvent. Dissolve the residue in a 1 mL mixture of acetone and *n*-hexane (1:19, v/v).

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

Inject a 5 mL mixture of acetone and *n*-hexane (1:19, v/v) into an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge and add a 10 mL mixture of acetone and *n*-hexane (1:19, v/v). Concentrate the total eluate including the transferred solution 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°C

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 acetone, transfer into *n*-hexane for re-dissolution, defatting by acetonitrile/hexane partitioning, clean-up with an octadecylsilanized silica gel cartridge and an ethylenediamine-*N*-propylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) 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

ii) Food items used to develop the analytical method: eel and *Corbicula* (freshwater clam)

12. Reference

None

13. Type

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