Original: Japanese
Provisional translation

Analytical Method for EPTC
(Animal and Fishery Products)

1. Analyte

EPTC

2. Application

Animal and fishery products

3. Instruments

Gas chromatograph-flame thermionic detector (GC-FTD) or gas chromatograph-nitrogen phosphorus detector (GC-NPD)

Gas chromatograph-mass spectrometer (GC-MS)

4. Reagents

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

Synthetic magnesium silicate cartridge (1,000 mg): A polyethylene tube of 12–13 mm in inside diameter packed with 1,000 mg of synthetic magnesium silicate or other cartridges with equal separation characteristics.

Reference standard of EPTC: Contains not less than 98% of EPTC.

5. Procedure

1) Extraction

i) Muscle, fat, liver, kidney, fish and shellfish

Weigh 20.0 g of sample when other than fat. For fat, weigh 5.00 g of sample.

Add 20 mL of water to the sample, homogenize, add 100 mL of acetone and *n*-hexane (1:2) mixed solution, homogenize further, centrifuge at 2,500 rpm for 5 min and collect the organic layer. Add 50 mL of *n*-hexane to the residue, homogenize and centrifuge as described above. Combine the obtained organic layers, dehydrate with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Add 2 mL of 2 vol% diethylene glycol-acetone solution to the filtrate and concentrate to approximately 2 mL at below 40ºC. After removing the solvent under a nitrogen stream, weigh the residue and take this as the extracted fat weight.

When other than fat, dissolve the residue in acetone and cyclohexane (1:4) mixed solution to make exactly 20 mL. (If the extracted fat weight exceeds 2.0 g, increase the liquid volume so that the extracted fat concentration in the solution is 0.10 g/mL or less.) For fat, dissolve the residue in acetone and cyclohexane (1:4) mixed solution so that the extracted fat concentration in the solution is 0.10 g/mL or less.

ii) Milk and egg

Add 100 mL of acetone and *n*-hexane (1:2) mixed solution to 20.0 g of sample,

homogenize, centrifuge at 2,500 rpm for 5 min, and collect the organic layer. Add 50 mL of *n*-hexane to the residue for eggs, or 50 mL of acetone and *n*-hexane (1:4) mixed solution for milk, homogenize, and centrifuge as described above. Combine the obtained organic layers, dehydrate with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Add 2 mL of 2 vol% diethylene glycol-acetone solution to the filtrate and concentrate to approximately 2 mL at below 40ºC. After removing the solvent under a nitrogen stream, weigh the residue and take this as the extracted fat weight. Dissolve the residue in acetone and cyclohexane (1:4) mixed solution to make exactly 20 mL. (If the extracted fat weight exceeds 2.0 g, increase the liquid volume so that the extracted fat concentration in the solution is 0.10 g/mL or less.)

iii) Honey

Dissolve 20.0 g of sample in 20 mL of water. Add 100 mL of acetone and *n*-hexane (1:2) mixed solution to the sample, homogenize, centrifuge at 2,500 rpm for 5 min, and collect the organic layer. Add 50 mL of *n*-hexane to the residue, homogenize and centrifuge as described above. Combine the obtained organic layers, dehydrate with anhydrous sodium sulfate and filter out the anhydrous sodium sulfate. Concentrate the filtrate to approximately 2 mL at below 40ºC and add *n*-hexane to make exactly 20 mL.

2) Clean-up

i) Muscle, fat, liver, kidney, fish, shellfish, milk and egg

a) Gel permeation chromatography

Centrifuge the solution obtained in 1) at 3,000 rpm for 5 min, transfer 5.0 mL of the supernatant to a column for gel permeation chromatography (a styrene-divinylbenzene copolymer column) and elute with acetone and cyclohexane (1:4) mixed solution. Collect the solution eluted from the retention time of acrinathrin to the end of tricyclazole elution, concentrate to approximately 2 mL at below 40ºC, add 5 mL of *n*-hexane, and mix.

b) Synthetic magnesium silicate column chromatography

Inject 5 mL of ether and 15 mL of *n*-hexane into a synthetic magnesium silicate cartridge (1,000 mg) sequentially and discard each effluent. Transfer the solution obtained in a) to the cartridge, add another 5 mL of ether and *n*-hexane (3:7) mixed solution, concentrate the total eluate to approximately 1 mL at below 40ºC, add acetone to make exactly 5 mL and use this solution as the test solution.

ii) Honey

Inject 5 mL of ether and 15 mL of *n*-hexane into a synthetic magnesium silicate cartridge (1,000 mg) sequentially and discard each effluent. Transfer exactly a 5 mL aliquot of the solution obtained in 1) to the cartridge, add another 5 mL of ether and *n*-hexane (3:7) mixed solution, concentrate the total eluate to approximately 1 mL at below 40ºC, add acetone to make exactly 5 mL, and use this solution as the test solution.

6. Calibration curve

Prepare EPTC standard solutions (acetone) of several concentrations, inject each standard solution into GC-FTD or GC-NPD respectively, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of EPTC in the test solution corresponding to 0.01 mg/kg in the sample (except for fat, etc.) is 0.01 mg/L.

7. Quantification

Inject the test solution into GC-FTD or GC-NPD, and calculate the concentration of EPTC from the calibration curves prepared in **6**.

8. Confirmation

Confirm using GC-MS.

9. Measurement conditions

(Example)

GC

Detector: NPD

Column: 5% phenyl-methyl silicone, 0.25 mm inside diameter, 30 mm in length and 0.25 μm in film thickness

Column temperature: 60°C (2 min) → 15°C/min → 200°C (2 min) → 5°C/min → 280°C (10 min)

Inlet temperature: 250°C

Detector temperature: 300°C

Carrier gas: Helium

Injection volume: 1 μL

Expected retention time: 10 min

GC-MS

Column: 5% phenyl-methyl silicone, 0.25 mm inside diameter, 30 m in length and 0.25 μm in film thickness

Column temperature: 50°C (1 min) → 25°C/min → 125°C (0 min) → 10°C/min → 300°C (10 min)

Inlet temperature: 250°C

Carrier gas: Helium

Ionization mode (Ionized energy): EI (70 eV)

Major monitoring ions (*m/z*): 189, 160, 132, 128

Injection volume: 1 μL

Expected retention time: 8 min

10. Limit of quantification

0.01 mg/kg (0.02 mg/kg for fat)

11. Explanatory note

1) Outline of analytical method

The method consists of extracting EPTC from sample with a mixed solution of acetone and *n*-hexane (1:2), cleaning up with gel permeation chromatography and a synthetic magnesium silicate cartridge (gel permeation chromatography is omitted for honey), quantifying with GC-FTD or GC-NPD, and confirming with GC-MS.

2) Notes

i) EPTC is easily volatilized during concentration as it has a high vapor pressure. Add diethylene glycol as a keeper in the vacuum concentration after extraction, leave approximately 2 mL of solvent, and gently remove the solvent using a nitrogen stream. Be very careful not to dry out during the vacuum concentration following the clean-up process.

ii) In step "**5**, 2), i), b)", wash the container of a) twice with 1 mL of *n*-hexane when injecting the solution obtained in a) into the cartridge.

iii) The following is an example of the conditions for gel permeation chromatography.

Column: A styrene-divinylbenzene copolymer column (20 mm in inside diameter and 300 mm in length), connected to a styrene-divinylbenzene copolymer column (20 mm in inside diameter and 100 mm in length) as a guard column, or other column with equal characteristics.

Mobile phase: A mixed solution of acetone and cyclohexane (1:4)

Flow rate: 5 mL/min

Column temperature: 40°C

Injection volume: 5.0 mL

Monitoring wavelength: 254 nm

Collection range: Determine in advance using the following method.

Prepare a 5 mg/L mixed solution of acrinathrin and tricyclazole in mobile phase, transfer 5.0 mL of the solution to a column for gel permeation chromatography, and monitor at 254 nm to confirm the elution position. Other appropriate methods may be used, such as fractionating the eluate at appropriate intervals and measuring with GC or GC-MS.

An example of the collection range (from the retention time of acrinathrin to the end of tricyclazole elution) is shown in Figure 1.

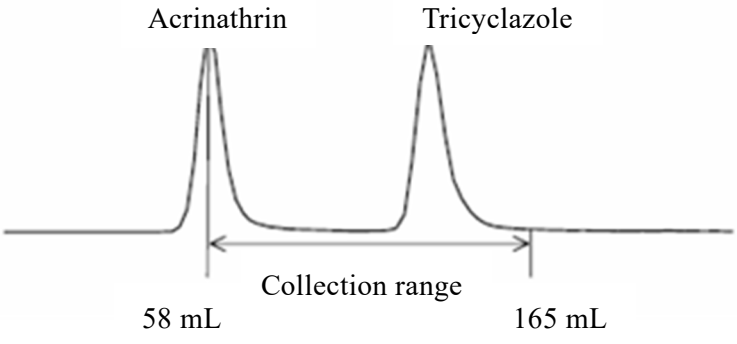

Figure 1. An example of the collection range

(Example) 58–165 mL (total volume: 107 mL)

iv) Samples with high fat content would have a lower concentration rate in the test solution. If the target measurement sensitivity cannot be achieved, repeat gel permeation chromatography and subsequent operations multiple times using the extracted fat, and combine the eluate obtained after clean-up using a synthetic magnesium silicate cartridge to make test solutions. Moreover, adjust the concentration rate of the test solution appropriately if necessary. For example, in the case of fat, the extracted fat weight exceeds 4 g, therefore, dissolve the residue in a mixed solution of acetone and cyclohexane (1:4) after measuring the weight to make exactly 50 mL. Repeat gel permeation chromatography and subsequent operations twice for this solution and combine the eluate obtained after clean-up using a synthetic magnesium silicate cartridge.

v) Food items used to develop the analytical method: cattle muscle, cattle fat, cattle liver, pig muscle, salmon, eel, *Corbicula* (freshwater clam), cattle milk, chicken egg and honey.

12. Reference

None

13. Type

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