Original: Japanese

Provisional Translation

Analytical Method for Azocyclotin and Cyhexatin (Animal and Fishery Products)

1. Analytes

Azocyclotin

Cyhexatin

2. Instrument

Gas chromatograph-flame photometric detector (with interference filter for tin, wavelength 610 nm) (GC-FPD(Sn))

3. Reagents

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

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

4. Procedure

1) Extraction

i) Except for honey

Add 100 mL of acetone/acetic acid (99:1, v/v) to 10.0 g (5.00g for fat) of sample, homogenize, and filter with suction. Add 50 mL of acetone/acetic acid (99:1, v/v) to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, concentrate at below 40°C, and remove the solvent.

Add 50 mL of ethanol and 10 mL of 10 mol/L potassium hydroxide solution to the residue, shake vigorously for 30 minutes. Then, add 50 mL of 10% sodium chloride solution and extract with shaking twice with 50 mL each of *n*-hexane. Combine the extracts, wash twice with 100 mL each of 10% sodium chloride solution, dehydrate the extract with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. Dissolve the residue in *n*-hexane to make exactly 5 mL (2.5 mL for fat).

ii) Honey

Dissolve 10.0 g of sample in 20 mL of water. Add 100 mL of acetone/acetic acid (99:1, v/v), homogenize, and filter with suction. Add 50 mL of acetone/acetic acid (99:1, v/v) to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, concentrate at below 40°C, and remove the solvent.

Add 100 mL of 10% sodium chloride solution, and extract with shaking twice with 50 mL each of *n*-hexane. Combine the extracts, dehydrate the extract with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. Dissolve the residue in *n*-hexane to make exactly 5 mL.

2) Ethylation

Take a 1 mL aliquot of the solution obtained in 1), add 1 mL of 3 mol/L ethylmagnesium bromide-diethyl ether solution and let stand for 20 minutes at room temperature. Add 10 mL of 0.5 mol/L sulfuric acid to the solution gradually, add 10 mL of water, and extract with shaking twice with 10 mL and 5 mL of *n*-hexane. Combine the extracts, dehydrate with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate to about 1 mL at below 40°C.

3) Clean-up

Add 10 mL of *n*-hexane to a synthetic magnesium silicate cartridge (910 mg) and discard the effluent. Transfer the solution obtained in 2) to the cartridge, elute with 15 mL of *n*-hexane, collect the total eluate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in *n*-hexane to make exactly 1 mL, and use this solution as the test solution.

5. Calibration curve

Prepare a 20 mg/L cyhexatin standard solution (acetone). Take a 1 mL aliquot of the solution, remove the solvent under a stream of nitrogen, and dissolve in 1 mL of *n*-hexane. Perform the same procedure as **4** 2), make up the volume and dilute the solution with *n*-hexane, and then prepare several standard solutions for calibration curve. Inject each standard solution to GC-FPD, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the sample containing 0.01 mg/kg of azocyclotin and cyhexatin gives the test solution of 0.02 mg/L in concentration.

6. Quantification

Inject the test solution to GC-FPD, and calculate the concentration of azocyclotin and cyhexatin from the calibration curves made in **5**.

7. Confirmation

Confirm using GC-FPD.

8. Measurement conditions

Example

1) GC-FPD (for quantification)

Detector: FPD (Sn)

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

Column temperature: 100°C (1 min) - 30°C/min heating - 280°C (10 min)

Inlet temperature: 250°C

Detector temperature: 250°C

Carrier gas: Helium

Injection volume: 2 μL

Expected retention time: 8 min

2) GC-FPD (for confirmation)

Detector: FPD (Sn)

Column: (14% cyanopropyl-phenyl)methyl silicone, 0.25 mm in inside diameter, 30 m in length and 0.25 µm in film thickness

Column temperature: 50°C (1 min) - 30°C/min heating - 280°C (5 min)

Inlet temperature: 250°C

Detector temperature: 250°C

Carrier gas: Helium

Injection volume: 2 µL

Expected retention time: 10 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of azocyclotin and cyhexatin from sample with acetone acidified with acetic acid, removing fat by alkaline degradation for high fat animal and fishery products, transferring into *n*-hexane, ethylation, clean-up with a synthetic magnesium silicate cartridge, and quantification and confirmation using GC-FPD.

2) Notes

i) Ethylation converts azocyclotin to the same compound as cyhexatin.

ii) During ethylation, gradually add the 0.5 mol/L sulfuric acid solution in order to prevent a vigorous reaction.

iii) For low-fat animal and fishery products, except honey, the alkaline degradation step can be omitted.

11. References

None

12. Type

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