Original: Japanese

Provisional Translation

Analytical Method for Azocyclotin and Cyhexatin (Agricultural 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.

Sodium L-ascorbate (GR)

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

4. Procedure

1) Extraction

i) Grains, fruits and vegetables

For grains, add about 0.5 g of sodium L-ascorbate and 20 mL of water to 10.0 g of sample, and let stand for 30 minutes. For fruits and vegetables, add about 0.5 g of sodium L-ascorbate to 20.0 g of sample.

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, and add acetone to make exactly 200 mL.

Take a 40 mL aliquot of the extract, add 200 mL of 10% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Combine the extracts, dehydrate with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. For grains, dissolve the residue in 1 mL of *n*-hexane. For fruits and vegetables, dissolve the residue in *n*-hexane to make exactly 2 mL, and take a 1 mL aliquot of the solution.

ii) Legumes, nuts and seeds

Add about 0.5 g of sodium L-ascorbate and 20 mL of water to 10.0 g of sample, and let stand for 30 minutes. 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, and add acetone to make exactly 200 mL. Take a 40 mL aliquot of the extract, 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, 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 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 1 mL of *n*-hexane.

iii) Tea leaves

Add about 0.5 g of sodium L-ascorbate and 20 mL of water to 5.00 g of sample, and let stand for 30 minutes. 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, and add acetone to make exactly 200 mL. Take an 80 mL aliquot of the extract, concentrate to about 40 mL at below 40°C.

Add 200 mL of 10% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Combine the extracts, dehydrate 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 5 mL of acetonitrile/toluene (3:1, v/v).

2) Clean-up (for tea leaves only)

Add 10 mL of acetonitrile/toluene (3:1, v/v) to a graphitized carbon black cartridge (500 mg) and discard the effluent. Transfer the solution obtained in 1) iii) to the cartridge, elute with 15 mL of acetonitrile/toluene (3:1, v/v), collect the total eluate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 1 mL of *n*-hexane.

3) Ethylation

Add 1 mL of 3 mol/L ethylmagnesium bromide-diethyl ether solution to the solution obtained in 1) or 2) 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.

4) 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 3) 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** 3), 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 foods (e.g. legumes, nuts and seeds), transferring into *n*-hexane, clean-up with a graphitized carbon black cartridge (if necessary), 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) For the clean-up procedure using a graphitized carbon black cartridge, centrifugation is effective if the residue does not completely dissolve and cannot be applied to the cartridge.

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

iv) For high-fat fruits and vegetables such as avocado, alkaline degradation should be performed as is described for legumes, nuts and seeds.

v) Sodium L-ascorbate is added to inhibit oxidative degradation during extraction.

11. References

None

12. Type

C