Original: Japanese

Provisional Translation

Analytical Method for 1-Naphthaleneacetic Acid (Agricultural Products)

1. Analyte

1-Naphthaleneacetic acid (includes conjugates)

2. Instrument

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

3. Reagents

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

Reference standard of 1-naphthaleneacetic acid: Contains not less than 95% of 1-naphthaleneacetic acid.

4. Procedure

1) Extraction

i) Grains, legumes, nuts and seeds

Add 20 mL of water to 10.0 g of sample and let stand for 30 minutes. Add 5 mL of 1 mol/L hydrochloric acid and 100 mL of acetone, homogenize, and filter with suction. Add 50 mL of acetone 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 20 mL aliquot of the extract, concentrate to about 3 mL at below 40°C, and add water to make about 10 mL.

ii) Fruits and vegetables

Add 5 mL of 1 mol/L hydrochloric acid and 100 mL of acetone to 20.0 g of sample, homogenize, and filter with suction. Add 50 mL of acetone 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 10 mL aliquot of the extract, concentrate to about 1 mL at below 40°C, and add water to make about 10 mL.

iii) Tea leaves

Add 20 mL of water to 5.00 g of sample and let stand for 30 minutes. Add 5 mL of 1 mol/L hydrochloric acid and 100 mL of acetone, homogenize, and filter with suction. Add 50 mL of acetone 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 to about 6 mL at below 40°C, and add water to make about 10 mL.

2) Hydrolysis

Add 5 mL of 3 mol/L hydrochloric acid and 5 mL of acetone to the solution obtained in 1), let stand for 18 hours at 80°C. Add 100 mL of 10 w/v% sodium chloride solution to the reaction

solution, and extract with shaking twice with 50 mL of diethyl ether. Combine the diethyl ether layers, and extract with shaking twice with 50 mL of 2 w/v% dipotassium hydrogen phosphate solution. Combine the aqueous layers, adjust the pH of the solution lower than 2 using 3 mol/L hydrochloric acid, and extract with shaking twice with 50 mL of diethyl ether. Dehydrate the extract with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, concentrate at below 40°C, and remove the solvent. Except for tea leaves, dissolve the residue in 5 mL of acetone/*n*-hexane (3:17, v/v). For tea leaves, dissolve the residue in 5 mL of acetone.

3) Clean-up

i) Except for tea leaves

Add 5 mL each of acetone and *n*-hexane to a silica gel cartridge (690 mg) sequentially, and discard the effluents. Transfer the solution obtained in 2) to the cartridge, add 5 mL of acetone/*n*-hexane (3:17, v/v), and discard the effluents. Elute with 20 mL of acetone/acetic acid/*n*-hexane (5:1:95, v/v/v), concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in 2 mmol/L ammonium acetate solution/methanol (7:3, v/v) to make exactly 2 mL, and use this solution as the test solution.

ii) Tea leaves

a) Graphitized carbon black column chromatography

Add 5 mL of acetone to a graphitized carbon black cartridge (250 mg) and discard the effluent. Transfer the solution obtained in 2) to the cartridge, elute with 25 mL of acetone, concentrate the total eluate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetone/*n*-hexane (3:17, v/v).

b) Silica gel column chromatography

Add 5 mL each of acetone and *n*-hexane to a silica gel cartridge (690 mg) sequentially, and discard the effluents. Transfer the solution obtained in a) to the cartridge, add 5 mL of acetone/*n*-hexane (3:17, v/v), and discard the effluents. Elute with 20 mL of acetone/acetic acid/*n*-hexane (5:1:95, v/v/v), concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in 2 mmol/L ammonium acetate solution/methanol (7:3, v/v) to make exactly 2 mL, and use this solution as the test solution.

5. Calibration curve

Prepare 1-naphthaleneacetic acid standard solutions (2 mmol/L ammonium acetate solution/methanol (7:3, v/v)) of several concentrations. Inject each standard solution to 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 sample containing 0.01 mg/kg of 1-naphthaleneacetic acid gives the test solution of 0.005 mg/L in concentration.

6. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of 1-naphthaleneacetic

acid from the calibration curve made in **5**.

7. Confirmation

Confirm using LC-MS/MS.

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 150 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially 2 mmol/L ammonium acetate solution/methanol (7:3, v/v) for 16 min, followed by a linear gradient to (3:7, v/v) in 0.1 min and hold for 4 min.

Ionization mode: ESI (-)

Major monitoring ions (*m/z*): Precursor ion 185, product ion 141

Injection volume: 10 μL

Expected retention time: 13 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory notes

1) Outline of analytical method

The method consists of extraction of 1-naphthaleneacetic acid (includes conjugates) from sample with acetone under acidic condition (hydrochloric acid). After hydrolysis of conjugates under acidic condition (hydrochloric acid), transferring into diethyl ether, transferring into dipotassium hydrogen phosphate solution, transferring into diethyl ether again under acidic condition (hydrochloric acid). And then clean-up with a graphitized carbon black cartridge (only for tea leaves) and a silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) Hydrolysis should be performed while sealed tightly.

ii) If the consecutive LC-MS/MS measurement is difficult because of interference from the former injected test solution, wash the column before the measurement.

iii) When the analytical method was developed, available monitoring ions for measurement using LC-MS/MS were not detected except the ions described in **8**. Use the following conditions for confirmation as necessary.

Column: Phenylsilanized silica gel, 2.1 mm in inside diameter, 150 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially 2 mmol/L ammonium acetate solution/acetonitrile (9:1, v/v) for 5 min, followed by a linear gradient to (1:1, v/v) in 10 min and hold for 4 min.

Ionization mode: ESI (-)

Major monitoring ions (*m/z*): Precursor ion 185, product ion 141

Expected retention time: 10 min

11. Reference

A summary of an agricultural chemical, 1-naphthaleneacetic acid

12. Type

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