Analytical Method for Amitrol (Agricultural Products)

1. Analyte

Amitrol

2. Instruments

High performance liquid chromatograph-fluorometric detector (HPLC-FL)

Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents

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

Acetate buffer: Add 0.05 mol/L sodium acetate solution to 800 mL of 0.05 mol/L acetic acid to make exactly 1,000 mL solution.

Weakly acidic cation-exchange resin: Wash a weakly acidic cation exchange resin, prepared for column chromatography, with 1 mol/L hydrochloric acid, and then wash with 2.8% aqueous ammonia. Wash again with 1 mol/L hydrochloric acid, and then wash with water until the washing is neutral.

Phosphate buffer: Add 10% phosphoric acid to 0.05 mol/L sodium dihydrogen phosphate solution, and adjust the pH to 3.0.

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

4. Procedure

1) Extraction

i) Grains, legumes, nuts and seeds, fruits, vegetables, powdered tea and hop

Add 80 mL of ethanol to 30.0 g of sample, homogenize, and filter with suction. Add 40 mL of 60 vol% ethanol to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and measure the volume. Take a 10 mL aliquot of the filtrate, and add 1 mL of hydrogen peroxide solution. Connect a reflux condenser, and heat the solution in a 75°C water bath for 30 minutes, and allow to cool.

ii) Tea leaves except powdered tea

Immerse 10.0 g of sample in 600 mL of water at 100 °C, and let stand for 5 minutes at room temperature, filter, and cool. Take a 12 mL aliquot of the filtrate, and add 1 mL of hydrogen peroxide solution. Connect a reflux condenser, and heat the solution in a 75°C water bath for 30 minutes, and allow to cool.

2) Clean-up

i) Strongly acidic cation-exchange resin chromatography

Place 1 mL of strongly acidic cation-exchange resin (0.063–0.156 mm in particle diameter) suspended in water in a chromatographic tube (10 mm in inside diameter), and let flow out water to the extent that only a small quantity of water remains on the top of

the column. Add 5 mL of water to the column, and discard the effluent. Transfer the solution obtained in 1) to the column, add 10 mL of water, and discard the effluent. Then, elute with 12 mL of 2.8% aqueous ammonia, add 30 mL of 1-propanol to the eluate, concentrate at below 45°C and remove the solvent. Dissolve the residue in 5 mL of water.

ii) Weakly acidic cation-exchange resin chromatography

Place 5 mL of weakly acidic cation-exchange resin (0.33–0.50 mm in particle diameter) suspended in water in a chromatographic tube (10 mm in inside diameter), and let flow out water to the extent that only a small quantity of water remains on the top of the column. Add 10 mL of water to the column, and discard the effluent. Transfer the solution obtained in 2) i) to the column, add 50 mL of water, and discard the effluent. Then, elute with 35 mL of 2.8% aqueous ammonia, add 100 mL of 1-propanol to the eluate, concentrate at below 45°C and remove the solvent. Dissolve the residue in acetate buffer to make exactly 2 mL.

3) Fluorescence derivatization

Add 100 μL of 0.25 w/v% fluorescamine-acetone solution to 1 mL of the solution obtained in 2) ii), mix well by shaking, and let stand for 1 hour.

Add 0.5 mL of 0.05 mol/L sodium borate solution, mix well, and use this solution as the test solution.

5. Calibration curve

Prepare 0.02–2 mg/L amitrol standard solutions (acetate buffer), and prepare derivative solutions following **4** 3). Inject 10 μL of each derivative solution to HPLC, and make a calibration curve by peak-height or peak-area method.

6. Quantification

Inject 10 μL of the test solution to HPLC and calculate the concentration of amitrol from the calibration curve made in **5**.

7. Confirmation

Confirm using LC-MS.

8. Measurement conditions

Example

HPLC

Detector: FL (excitation wavelength is 380 nm, emission wavelength is 484 nm)

Column: Octadecylsilanized silica gel, 4.6 mm in inside diameter, 150 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: acetonitrile/phosphate buffer (3:7, v/v)

Expected retention time: 15 min

9. Limit of quantification

0.025 mg/kg (0.1 mg/kg for tea leaves)

10. Explanatory note

1) Outline of analytical method

The method consists of extraction amitrol from sample with ethanol and 60 vol% ethanol, heating under reflux with hydrogen peroxide, clean-up with strongly acidic cation-exchange resin and weakly acidic cation-exchange resin. Then, fluorescence derivatization with fluorescamine, quantification using HPLC-FL, and confirmation using LC-MS.

2) Notes

i) Elution patterns from strongly and weakly acidic cation-exchange resin vary among the several resins. Elution patterns should be confirmed before use with reference standards.

ii) The optimal pH for fluorescence derivatization is between 4.1 and 4.4.

iii) Fluorescent derivative in the test solution decomposes gradually, and thus, it is recommended to perform the measurement quickly.

11. References

None

12. Type

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