Original: Japanese
Provisional translation

Analytical Method for Amitrole
(Agricultural Products)

1. Analyte

Amitrole

2. Application

Grains, legumes, nuts, seeds and fruits

3. Instrument

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

4. Reagents

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

Sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (150 mg): A polyethylene tube of 12−13 mm inside diameter packed with 150 mg of sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer, or a cartridge equivalent to this specified one in separation capability.

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

5. Procedure

1) Extraction

i) Grains, legumes, nuts and seeds

Add 20 mL of water to 10.0 g of sample (5.00 g for tea leaves) and let stand for 30 min. Add 100 mL of methanol, homogenize, and filter with suction. Add 50 mL of methanol to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates and add methanol to make exactly 200 mL. Take exactly an 8 mL aliquot of the solution and add 10 mL of 2 vol% formic acid.

ii) Fruits and vegetables

Add 100 mL of methanol to 20.0 g of sample, homogenize, and filter with suction. Add 50 mL of methanol to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates and add methanol to make exactly 200 mL. Take exactly an 8 mL aliquot of the solution and add 10 mL of 2 vol% formic acid.

2) Clean-up

i) Grains, legumes, nuts and seeds

a) Sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer column

chromatography

Inject 5 mL each of methanol and 2 vol% formic acid into a sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (150 mg) sequentially, and discard each effluent. Transfer the solution obtained in 1) to the cartridge, add 5 mL of water and 10 mL of methanol, and discard each effluent. Then, add 5 mL of ammonia solution and methanol (1:99) mixed solution, concentrate the eluate at below 40℃, and remove the solvent. Dissolve the residue in 1 mL of methanol and add 4 mL of ethyl acetate.

b) Aminopropylsilanized silica gel column chromatography

Inject 5 mL of ethyl acetate and methanol (4:1) mixed solution into an aminopropylsilanized silica gel cartridge (360 mg) and discard the effluent. Transfer the solution obtained in a) to the cartridge, add 10 mL of ethyl acetate and methanol (4:1) mixed solution, concentrate the eluate at below 40℃, and remove the solvent. Dissolve the residue in acetonitrile and methanol (3:1) mixed solution to make exactly 2 mL, and use this solution as the test solution.

ii) Fruits and vegetables

Inject 5 mL each of methanol and 2 vol% formic acid into a sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (150 mg) sequentially, and discard each effluent. Transfer the solution obtained in 1) to the cartridge, add 5 mL of water and 10 mL of methanol, and discard each effluent. Then, add 5 mL of ammonia solution and methanol (1:99) mixed solution, concentrate the eluate at below 40℃, and remove the solvent. Dissolve the residue in a mixed solution of acetonitrile and methanol (3:1) to make exactly 4 mL, and use this solution as the test solution.

6. Calibration curve

Prepare amitrole standard in a mixed solution of acetonitrile and methanol (3:1) at several concentrations, inject each solution into LC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of amitrole in the test solution corresponding to 0.01 mg/kg in the sample is 0.002 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS, and calculate the concentration of amitrole from the calibration curve prepared in **6**.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

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

Column temperature: 40°C

Mobile phase: A mixed solution of acetonitrile and water (19:1) containing 10 mmol/L ammonium acetate.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*): Precursor ion 85, product ions 57, 43

Injection volume: 10 μL

Expected retention time: 4 min

10. Limit of quantification

0.01 mg/kg

11. **Explanatory note**

1) Outline of analytical method

The method consists of extracting amitrole from sample with methanol, cleaning up with a sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge and then further cleaning up with an aminopropylsilanized silica gel cartridge in the case of grains, legumes, nuts and seeds, followed by quantifying and confirming using LC-MS/MS.

2) Notes

i) When the analytical method for amitrole using LC-MS/MS was developed, the following monitoring ions were used:

for quantitative ions (*m/z*): precursor ion 85, product ion 57

for qualitative ions (*m/z*): precursor ion 85, product ion 43

ii) Food items used to develop the analytical method: apple, mume plum (*Prunus mume*), grape, wheat and rapeseed.

12. Reference

None

13. Type

C