Original: Japanese
Provisional translation

Analytical Method for Flutianil (Agricultural Products)

1. Analyte

Flutianil

2. Application

Agricultural products

3. Instrument

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

4. Reagents

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

Graphite carbon/ethylenediamine-*N*-propylsilanized silica gel layered cartridge: A polyethylene tube of 12–13 mm in inside diameter packed with 500 mg each of graphite carbon in the upper layer and ethylenediamine-*N*-propylsilanized silica gel in the lower layer, or other cartridges with equal separation characteristics.

0.5 mol/L phosphate buffer solution (pH 7.0): Weigh 52.7 g of dipotassium hydrogen phosphate (K_2HPO_4) and 30.2 g of potassium dihydrogen phosphate (KH_2PO_4), dissolve in approximately 500 mL of water, adjust pH to 7.0 using 1 mol/L sodium hydroxide solution or 1 mol/L hydrochloric acid and add water to make 1 L.

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

5. 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 min. Add 50 mL of acetonitrile, homogenize, and filter with suction. Add 20 mL of acetonitrile to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add acetonitrile to make exactly 100 mL. Take exactly a 20 mL aliquot of the solution, add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer solution (pH 7.0), and shake for 10 min. Let stand and discard the separated aqueous layer.

Inject 10 mL of acetonitrile into an octadecylsilanized silica gel cartridge (1,000 mg) and discard the effluent. Transfer the acetonitrile layer described above to the cartridge, add another 2 mL of acetonitrile, collect the total eluate, dehydrate with anhydrous sodium sulfate, filter out anhydrous sodium sulfate, concentrate the filtrate at below

40°C and remove the solvent. Dissolve the residue in 2 mL of acetonitrile and toluene (3:1) mixed solution.

ii) Fruits, vegetables and herbs

Add 50 mL of acetonitrile to 20.0 g of sample, homogenize, and filter with suction. Add 20 mL of acetonitrile to the residue on the filter paper, homogenize and filter with suction. Combine the resulting filtrates, and add acetonitrile to make exactly 100 mL. Take exactly a 20 mL aliquot of the solution, add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer solution (pH 7.0) and shake for 10 min. Let stand and discard the separated aqueous layer. Dehydrate the acetonitrile layer with anhydrous sodium sulfate, filter out anhydrous sodium sulfate, concentrate the filtrate at below 40°C and remove the solvent. Dissolve the residue in 2 mL of acetonitrile and toluene (3:1) mixed solution.

iii) Tea and hops

Add 20 mL of water to 5.00 g of sample and let stand for 30 min. Add 50 mL of acetonitrile, homogenize, and filter with suction. Add 20 mL of acetonitrile to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates and add acetonitrile to make exactly 100 mL. Take exactly a 5 mL aliquot of the solution and add 15 mL of acetonitrile, 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer solution (pH 7.0), and shake for 10 min. Let stand and discard the separated aqueous layer.

Inject 10 mL of acetonitrile into an octadecylsilanized silica gel cartridge (1,000 mg) and discard the effluent. Transfer the acetonitrile layer described above to the cartridge, add another 2 mL of acetonitrile, collect the total eluate, dehydrate with anhydrous sodium sulfate, filter out anhydrous sodium sulfate, concentrate the filtrate at below 40°C and remove the solvent. Dissolve the residue in 2 mL of acetonitrile and toluene (3:1) mixed solution.

2) Clean-up

i) Grains, legumes, nuts, seeds, fruits, vegetables and herbs

Inject 10 mL of acetonitrile and toluene (3:1) mixed solution into a graphite carbon/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add another 20 mL of acetonitrile and toluene (3:1) mixed solution, collect the total eluate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in methanol to make exactly 4 mL for grains, legumes, nuts and seeds or exactly 8 mL for fruits, vegetables and herbs, and use these solutions as the test solution.

ii) Tea and hops

Inject 10 mL of acetonitrile and toluene (3:1) mixed solution into a graphite carbon/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add another 20 mL of acetonitrile and toluene (3:1) mixed solution, collect the total eluate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in methanol to make exactly 1 mL and use this solution as the test solution.

6. Calibration curve

Prepare flutianil standard solutions (methanol) of 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 flutianil in the test solution corresponding to 0.01 mg/kg in the sample is 0.005 mg/L for grains, legumes, nuts, seeds, fruits, vegetables and herbs or 0.0025 mg/L for tea and hops.

7. Quantification

Inject the test solutions into LC-MS/MS, and calculate the concentration of flutianil from the calibration curves prepared in **6**.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel 2.1 mm inside diameter, 150 mm in length and 3.5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: 5 mmol/L ammonium acetate solution

Mobile phase B: 5 mmol/L ammonium acetate-methanol solution

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	85	15
1	60	40
3.5	60	40
6	50	50
8	45	55
17.5	5	95
30	5	95
30	85	15

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 427, product ions 411, 201, 192, 132

Injection volume: 5 μL

Expected retention time: 17 min.

10. Limit of quantification

0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extracting flutianil from sample with acetonitrile, dehydrating by salting out, leaving as they are for fruits, vegetables, and herbs, or cleaning up with an octadecylsilanized silica gel cartridge for grains, legumes, nuts, seeds, tea and hops, followed by cleaning up with a graphite carbon/aminopropylsilanized silica gel layered cartridge for grains, legumes, nuts, seeds, fruits, vegetables, and herbs, or cleaning up with a graphite carbon/ethylenediamine-*N*-propylsilanized silica gel layered cartridge for tea and hops and quantifying and confirming with LC-MS/MS.

2) Notes

i) This analytical method conforms to "Multi-residue method I for agricultural chemicals by LC-MS (Agricultural Products)".

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

for quantitative ions *(m/z*): precursor ion 427, product ion 411

for qualitative ions (*m/z*): precursor ion 427, product ion 192

iii) Under the measurement conditions of this method, leading appears on the peak shape of flutianil, although it does not affect its quantitative analysis. In addition, it has been reported that the peak shape can be improved by using acetonitrile in the mobile phase.

iv) Food items used to develop the analytical method: brown rice, soybean, pumpkin, spinach, eggplant, potato, orange, strawberry, and tea.

12. Reference

MHLW Director Notice (Syoku-An No.1129002 November 29, 2005) "Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives and Veterinary Drugs in Food (Partial Amendment)", "Multi-residue Method I for Agricultural Chemicals by LC-MS (Agricultural Products)"

13. Type

C