ひと、くらし、みらいのために
厚生労働省
Ministry of Health, Labour and Welfare

Original: Japanese
Provisional translation

Analytical Method for Fluensulfone (Agricultural Products)

1. Analytes

Fluensulfone

3,4,4-trifluorobut-3-en-1-ylsulfonic acid (hereafter referred to as metabolite BSA)

2. Applicable foods

Vegetables and fruits (food which shows acidity)

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.

Trimethylammonium group-modified methacrylate polymer mini column (500 mg): A polyethylene column in inside diameter 12-13 mm packed with 500 mg of trimethylammonium group-modified methacrylate polymer, or a column equivalent to the specified one in separation capability.

Reference standard of fluensulfone: Contains not less than 95% of fluensulfone.

Reference standard of metabolite BSA sodium salt: Use the reference standard which clearly shows its purity.

5. Procedure

1) Extraction

i) Vegetables rich in chlorophyll

Add 100 mL of acetonitrile to 20.0 g of sample, homogenize, centrifuge at 3,000 rpm for 5 minutes, and collect the supernatant. Add 50 mL of acetonitrile to the residue, homogenize, centrifuge at 3,000 rpm for 5 minutes, combine the resulting supernatant, and add acetonitrile to make exactly 200 mL. Take a 20 mL aliquot of the solution accurately, and concentrate to about 5 mL at below 40°C.

ii) Other vegetables and fruits

Add 100 mL of acetonitrile to 20.0 g of sample, homogenize, centrifuge at 3,000 rpm for 5 minutes, and collect the supernatant. Add 50 mL of acetonitrile to the residue, homogenize, centrifuge at 3,000 rpm for 5 minutes, combine the resulting supernatant, and add acetonitrile to make exactly 200 mL. Take a 20 mL aliquot of the solution accurately, add 5 mL of water, and concentrate to about 2 mL at below 40°C.

2) Clean-up

i) Vegetables rich in chlorophyll

a. Graphitized carbon black column chromatography

Add 10 mL each of acetonitrile and acetonitrile/water (9:1, v/v) to a graphitized carbon black mini column (500 mg) sequentially, and discard the effluent. Transfer the solution obtained in "1), i)" to the column, and add 20 mL of acetonitrile/water (9:1, v/v). Collect the total eluate including the transferred solutions, add 5 mL of water, and concentrate to about 2 mL at below 40°C.

b. Trimethylammonium group-modified methacrylate polymer column chromatography

Add 10 mL each of acetonitrile and acetonitrile/water (1:4, v/v) to a trimethylammonium group-modified methacrylate polymer mini column (500 mg) sequentially, and discard the effluent. Transfer the solution obtained in "a" to the column, add 10 mL of acetonitrile/water (1:4, v/v), and discard the effluent. Then, add 10 mL of acetonitrile/water (2:3, v/v), collect the eluate, add water to make exactly 20 mL, and use this solution as the test solution of fluensulfone. Add 10 mL of acetonitrile to this column, and discard the effluent. Then, add 20 mL of acetonitrile/0.2 mol/L hydrochloric acid (9:1, v/v), collect the eluate, add 5 mL of acetonitrile/25% ammonia solution (9:1, v/v), and shake. Concentrate the solution at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile/water (1:4, v/v) to make exactly 20 mL, and use this solution as the test solution of metabolite BSA.

ii) Other vegetables and fruits

Add 10 mL each of acetonitrile and acetonitrile/water (1:4, v/v) to a trimethylammonium group-modified methacrylate polymer mini column (500 mg) sequentially, and discard the effluent. Transfer the solution obtained in "1), ii)" to the column, add 10 mL of acetonitrile/-water (1:4, v/v), and discard the effluent. Then, add 10 mL of acetonitrile/water (2:3, v/v), collect the eluate, add water to make exactly 20 mL, and use this solution as the test solution of fluensulfone. Add 10 mL of acetonitrile to this column, and discard the effluent. Then, add 20 mL of acetonitrile/0.2 mol/L hydrochloric acid (9:1, v/v), collect the eluate, add 5 mL of acetonitrile/25% ammonia solution (9:1, v/v), and shake. Concentrate the solution at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile/water (1:4, v/v) to make exactly 20 mL, and use this solution as the test solution of metabolite BSA.

6. Calibration curve

Prepare fluensulfone and metabolite BSA sodium salt standard solutions (acetonitrile /water (1:4, 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 0.005 mg/kg of each analyte gives the test solution of 0.0005 mg/L in concentration. (The concentration of metabolite BSA is calculated as fluensulfone.)

7. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of fluensulfone and metabolite BSA from the calibration curve made in 6. Use the following equation to calculate the concentration of fluensulfone including metabolite BSA.

Concentration (ppm) of fluensulfone (including metabolite BSA) = $A + B \times 1.534$

A: Concentration (ppm) of fluensulfone

B: Concentration (ppm) of metabolite BSA

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel with embedded polar groups (2.0 mm in inside diameter, 150 mm in length, 4 μm in particle diameter)

Column temperature: 40°C

Mobile phase: Initially acetonitrile/0.1 vol% formic acid (1:4, v/v) for 5 minutes, followed by a linear gradient from (1:4, v/v) to (99:1, v/v) in 10 minutes, and hold for 10 minutes.

Ionization mode:

Fluensulfone: ESI (＋)

Metabolite BSA: ESI (－)

Major monitoring ions (*m/z*):

Fluensulfone: Precursor ion 292, product ions 166, 109

Metabolite BSA: Precursor ion 189, product ions 81, 80

Injection volume: 10 μL

Expected retention time:

Fluensulfone: 15 minutes

Metabolite BSA: 10 minutes

10. Limit of quantification

0.005 mg/kg for each analyte (The concentration of metabolite BSA is calculated as fluensulfone.)

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of fluensulfone and metabolite BSA from sample with acetonitrile, for vegetables rich in chlorophyll, clean-up with a graphitized carbon black mini column, then clean-up with a trimethylammonium group-modified methacrylate polymer mini column, and quantification and confirmation using LC-MS/MS.

2) Notes

i) In order to retain fluensulfone in the trimethylammonium group modified methacrylate polymer mini column, it is necessary to remove acetonitrile as much as possible when loading. However, since fluensulfone is lost when dried, add 5 mL of water when adjusting the load liquid into the column, and concentrate to about 2 mL at below 40°C, being careful not let it dry.

ii) Note that the elution conditions from metabolite BSA may vary depending on the lot of the graphitized carbon black mini column.

iii) When the analytical method for fluensulfone and metabolite BSA using LC-MS/MS was developed, the following monitoring ions were used:

Fluensulfone

for quantitative ions (*m/z*): precursor ion 292, product ion 166
for qualitative ions (*m/z*): precursor ion 292, product ion 109

Metabolite BSA
for quantitative ions (*m/z*): precursor ion 189, product ion 81
for qualitative ions (*m/z*): precursor ion 189, product ion 80

iv) At the time of the development of the analytical method, the high-purity reference standard for metabolite BSA sodium salt was not available. Therefore, in “4. Reagents”, "Reference standard of metabolite BSA sodium salt: Use the reference standard which clearly shows its purity" is mentioned. However, if available, it is desirable to use the reference standard with a purity of 95% or higher.

v) The foods examined in the development of the analytical method: Spinach, cabbages, sweet potatoes, watermelons, strawberries

12. Reference

None

13. Type

C