Original: Japanese
Provisional translation

Analytical Method for Flufenacet (Agricultural Products)

1. Analytes

Flufenacet

[(4-fluorophenyl) (1-methylethyl) amino] oxo-acetic acid (hereinafter referred to as metabolite W)

[*N*-(4-fluorophenyl)-*N*-(1-methylethyl) acetamide]-2-sulfinylacetic acid (hereinafter referred to as metabolite P1)

2. Application

Grains, legumes, nuts, seeds and vegetables

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.

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

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

Reference standard of metabolite W: Contains not less than 98% of metabolite W.

Reference standard of metabolite P1: Contains not less than 98% of metabolite P1.

5. Procedure

1) Extraction

i) Grains, legumes, nuts and seeds

Add 20 mL of water to 10.0 g of the sample 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 with suction. Combine the resulting filtrates and add methanol to make exactly 200 mL. Take exactly a 20 mL aliquot of the solution, and concentrate to approximately 1 mL at below 40°C. Add 4 mL of 0.1 vol% formic acid to the residue.

ii) Fruits and vegetables

Add 100 mL of methanol to 20.0 g of the sample, homogenize, and filter with suction. Add 50 mL of methanol to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates and add methanol to make exactly 200 mL. Take exactly a 20 mL aliquot of the solution, and concentrate to approximately 1 mL at below

40°C. Add 4 mL of 0.1 vol% formic acid to the residue.

2) Clean-up

Inject 5 mL of methanol and 5 mL of 0.1 vol% formic acid into an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard each effluent. Inject 5 mL of methanol 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 octadecylsilanized silica gel cartridge, inject 5 mL of 0.1 vol% formic acid and methanol (4:1) mixed solution, and discard the effluent. Connect the graphite carbon/ethylenediamine-*N*-propylsilanized silica gel layered cartridge to the lower part of the octadecylsilanized silica gel cartridge, inject 5 mL of methanol, and collect the eluate. Then, detach the octadecylsilanized silica gel cartridge, inject 7 mL of ammonia solution and methanol (1:99) mixed solution into the graphite carbon/ethylenediamine-*N*-propylsilanized silica gel layered cartridge, combine the eluate with the previous eluate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in methanol to make exactly 2 mL for grains, legumes, nuts and seeds, and exactly 4 mL for vegetables, and use these solutions as the test solution.

6. Calibration curve

Prepare stock standard solutions of reference standards of flufenacet, metabolite W and metabolite P1. Prepare standard solutions of several concentrations by mixing each stock standard solution appropriately and diluting with methanol. Inject each solution into LC-MS/MS and make calibration curves by peak-height or peak-area method. When the test solutions are prepared following the above procedure, the concentration of flufenacet in the test solution corresponding to 0.01 mg/kg in the sample is 0.005 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate each concentration of flufenacet, metabolite W and metabolite P1 from the calibration curve prepared in **6**. Use the following equation to calculate the concentration of flufenacet including metabolite W and metabolite P1.

Concentration (ppm) of flufenacet (including metabolite W and metabolite P1) = A + B × 1.613 + C × 1.206

A: Concentration (ppm) of flufenacet

B: Concentration (ppm) of metabolite W

C: Concentration (ppm) of metabolite P1

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: Linear gradient from a mixed solution of 5 mmol/L ammonium acetate solution and 5 mmol/L ammonium acetate-methanol solution (9:1) to (1:9) in 14 min, and hold at (1:9) for 2 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*)

Flufenacet: Precursor ion 364, product ions 194, 152

Metabolite W: Precursor ion 226, product ions 138, 110

Metabolite P1: Precursor ion 302, product ions 284, 1

Injection volume: 2 μL

Expected retention time

Flufenacet: 14 min

Metabolite W: 8 min

Metabolite P1: 8 min

10. Limit of quantification

0.01 mg/kg for each analyte (The concentration of metabolite W and metabolite P1 is calculated as flufenacet)

11. Explanatory note

1) Outline of analytical method

The method consists of extracting flufenacet, metabolite W and metabolite P1 from samples with methanol, cleaning up with an octadecylsilanized silica gel cartridge and a graphite carbon/ethylenediamine-*N*-propylsilanized silica gel layered cartridge, and quantifying and confirming using LC-MS/MS. In this method, flufenacet, metabolite W and metabolite P1 are quantified respectively. For the concentration of flufenacet including metabolite W and metabolite P1, the concentration of metabolite W and metabolite P1 is converted to the concentration of flufenacet by multiplying by the conversion factor, and the sum of the concentrations of flufenacet, metabolite W and metabolite P1 is regarded as the analytical result of flufenacet.

2) Notes

i) If suction filtration cannot be achieved during extraction, centrifugation should be used. When developing the analytical method, centrifugation was performed at 3,000 rpm for 5 min as soybeans were clogged during suction filtration.

ii) If insoluble matter precipitates and adheres to the concentration container during the concentration of the extract, the container should be washed twice with 1 mL each out of 5 mL of methanol when connecting the octadecylsilanized silica gel cartridge and the graphite carbon/ethylenediamine-*N*-propylsilanized silica gel layered cartridge and injecting methanol.

iii) As moisture remains in the concentration operation after clean-up, concentrate the solution until the ammonia odor disappears (approximately 1 mL or less), then use a

measuring flask to make the solution up to the specified volume.

iv) When the analytical methods for flufenacet, metabolite W and metabolite P1 were developed, the following monitoring ions were used:

Flufenacet

for quantitative ions (*m/z*): precursor ion 364, product ion 194

for qualitative ions (*m/z*): precursor ion 364, product ion 152

Metabolite W

for quantitative ions (*m/z*): precursor ion 226, product ion 138

for qualitative ions (*m*/*z*): precursor ion 226, product ion 110

Metabolite P1

for quantitative ions (*m/z*): precursor ion 302, product ion 284

for qualitative ions (*m*/*z*): precursor ion 302, product ion 111

v) Food items used to develop the analytical method: wheat, soybean, potato and tomato.

12. Reference

None

13. Type

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