Original: Japanese
Provisional translation

Analytical Method for Norflurazon (Agricultural Products)

1. Analytes

Norflurazon

4-Chloro-5-(amino)-2-(α,α,α-trifluoro-*m*-tolyl) -3(2*H*)-pyridazinone (hereinafter referred to as metabolite B)

2. Applications

Grains, legumes, nuts, seeds, fruits 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.

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 norflurazon: Contains not less than 98% of norflurazon.

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

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 3 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 and vegetables

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.

2) Clean-up

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, concentrate the total eluate at below 40°C, and remove the solvent. Dissolve the residue in methanol to make exactly 2 mL for grains, legumes, nuts and seeds, or exactly 4 mL for fruits and vegetables, etc., and use these solutions as the test solution.

6. Calibration curve

Dissolve each reference standard of norflurazon and metabolite B in methanol respectively to make stock standard solutions. Mix each stock standard solution appropriately, dilute with methanol, and prepare standard solutions 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 norflurazon and metabolite B in the test solution corresponding to 0.005 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 norflurazon and metabolite B from the calibration curve prepared in **6**. Use the following equation to calculate the concentration of norflurazon including metabolite B.

Concentration (ppm) of norflurazon (including metabolite B) = A + B × 1.048

A: Concentration (ppm) of norflurazon

B: Concentration (ppm) of metabolite B

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.1 mm inside diameter, 100 mm in length and 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

Ionization mode: ESI (+)

Major monitoring ions (*m/z*)

Norflurazon: Precursor ion 304, product ions 284, 102

Metabolite B: Precursor ion 290, product ions 270, 160

Injection volume: 5 µL

Expected retention time

Norflurazon: 11 min

Metabolite B: 9 min

10. Limit of quantification

0.005 mg/kg for each analyte (Metabolite B is calculated as norflurazon.)

11. Explanatory note

1) Outline of analytical method

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

2) Notes

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

Norflurazon

for quantitative ions (*m/z*): precursor ion 304, product ion 284

for qualitative ions (*m/z*): precursor ion 304, product ion 102

Metabolite B

for quantitative ions (*m/z*): precursor ion 290, product ion 270

for qualitative ions (*m/z*): precursor ion 290, product ion 160

ii) Food items used to develop the analytical method: peanut, asparagus, orange and apple.

12. Reference

MHLW Director Notice (Syoku-An No.0526001-Section 2. May 26, 2006) “Multi-residue Method I for Agricultural Chemicals by LC-MS (Agricultural Products)”

13. Type

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