Original: Japanese

Provisional Translation

Analytical Method for Pyrifluquinazon (Agricultural Products)

1. Analytes

Pyrifluquinazon

1,2,3,4-tetrahydro-3-[(3-pyridylmethyl)amino]-6-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl] quinazoline-2-one (hereafter referred to as metabolite B)

2. Instrument

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

3. Reagents

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

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

Reference standard of metabolite B: Contains not less than 95% of metabolite B.

4. 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 minutes. Add 100 mL of acetone, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 10 mL aliquot of the extract and add 20 mL of water.

ii) Fruits and vegetables

Add 100 mL of acetone to 20.0 g of sample, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 5 mL aliquot of the extract and add 10 mL of water.

iii) Tea leaves

Add 20 mL of water to 5.00 g of sample and let stand for 30 minutes. Add 100 mL of acetone, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 10 mL aliquot of the solution and add 20 mL of water.

2) Clean-up

Add 10 mL each of acetonitrile and water to a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg) sequentially, and discard the effluents. Transfer the solution obtained in 1) to the cartridge, add 10 mL of acetonitrile/water (3:7, v/v), and discard the effluent. Elute with 10 mL of acetonitrile/water (1:1, v/v), add acetonitrile/water (1:1, v/v) to the eluate to make exactly 10 mL, and use this solution as the test solution.

5. Calibration curve

Prepare pyrifluquinazon and metabolite B standard solutions (acetonitrile/water (1:1, v/v)) of several concentrations. Inject each standard solution to 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 sample of grains, legumes, nuts, seeds, fruits or vegetables containing 0.01 mg/kg of pyrifluquinazon or metabolite B gives the test solution of 0.0005 mg/L in concentration, and the sample of tea leaves containing 0.02 mg/kg of pyrifluquinazon or metabolite B gives the test solution of 0.0005 mg/L in concentration. The concentration of metabolite B is calculated as that of pyrifluquinazon.

6. Quantification

Inject the test solution to LC-MS/MS and calculate the concentration of pyrifluquinazon and metabolite B from the calibration curves made in **5**. Use the following equation to calculate the concentration of pyrifluquinazon including metabolite B.

Concentration (ppm) of pyrifluquinazon (including metabolite B)

$= A + B \times 1.100$

A: Concentration (ppm) of pyrifluquinazon

B: Concentration (ppm) of metabolite B

7. Confirmation

Confirm using LC-MS/MS.

8. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 150 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: acetonitrile/5 mmol/L ammonium acetate solution (1:1, v/v)

Ionization mode: ESI (+)

Major monitoring ions (*m/z*)

Pyrifluquinazon: precursor ion 465, product ion 423, 107, 93, 92

Metabolite B: precursor ion 423, product ion 107, 93, 92

Injection volume: 10 μL

Expected retention time

Pyrifluquinazon: 8 min

Metabolite B: 6 min

9. Limit of quantification

0.01 mg/kg for each analyte (The concentration of metabolite B is calculated as pyrifluquinazon.)

For tea leaves, 0.02 mg/kg for each analyte (The concentration of metabolite B is calculated as pyrifluquinazon.)

10. Explanatory note

1) Outline of analytical method

This method consists of extraction of pyrifluquinazon and metabolite B from sample with acetone, clean-up with a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) Using methanol may transform some pyrifluquinazon to metabolite B.

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

Pyrifluquinazon

for quantification (*m/z*): precursor ion 465, product ion 92

for confirmation (*m/z*): precursor ion 465, product ion 423, 107, 93

Metabolite B

for quantification (*m/z*): precursor ion 423, product ion 92

for confirmation (*m/z*): precursor ion 423, product ion 107, 93

11. References

None

12. Type

C