Analytical Method for Pyrimisulfan (Agricultural Products)

1. Analyte

Pyrimisulfan

2. Instrument

Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents

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

Silica gel cartridge (800 mg): Polyethylene tube of 8–9 mm in inside diameter packed with 800 mg of silica gel prepared for column chromatography, or other cartridge with equal separation characteristics.

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

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 with suction. Combine the resulting filtrates and add acetone to make exactly 200 mL. Take a 20 mL aliquot of the extract, concentrate to about 2 mL at below 40°C, and add 10 mL of 0.05 vol% formic acid solution.

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 with suction. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 10 mL aliquot of the extract, concentrate to about 1 mL at below 40°C, and add 10 mL of 0.05 vol% formic acid solution.

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 with suction. Combine the resulting filtrates, and add acetone to make exactly 200 mL.

2) Clean-up

i) Graphitized carbon black column chromatography (for tea leaves only)

Add 5 mL of acetone to a graphitized carbon black cartridge (500 mg), and discard the effluent. Transfer 20 mL of the extract obtained in 1) iii) to the cartridge, and elute with 10 mL of acetone. Combine the eluates, concentrate to about 2 mL at below 40°C, and

add 10 mL of 0.05 vol% formic acid solution.

ii) Octadecylsilanized silica gel column chromatography

Add 5mL each of acetonitrile and 0.05 vol% formic acid to an octadecylsilanized silica gel cartridge (1,000 mg), and discard the effluent. Transfer the extract obtained in 1) (or the solution obtained in 2) i) for tea leaves) to the cartridge. Add 10 mL of acetonitrile/0.05 vol% formic acid (3:7, v/v), and discard the effluent. Elute with 10 mL of acetonitrile/0.05 vol% formic acid (3:2, v/v), and add acetonitrile/0.05 vol% formic acid (3:2, v/v) to the eluate to make exactly 10 mL. Take a 5 mL aliquot of the resultant solution, concentrate at below 40°C and remove the solvent. Dissolve the residue in 1 mL of acetone, and add 19 mL of *n*-hexane.

iii) Silica gel column chromatography

Add 5 mL of acetone/*n*-hexane (1:19, v/v) to silica gel cartridge (800 mg), and discard the effluent. Transfer the solution obtained in 2) ii) to the cartridge, and discard the effluent. Elute with 20 mL of acetone/*n*-hexane (3:7, v/v), concentrate at below 40°C and remove the solvent. Dissolve the residue in acetonitrile/0.05 vol% formic acid (3:2, v/v) to make exactly 5 mL solution (2.5 mL for tea leaves), and use this solution as the test solution.

5. Calibration curve

Prepare pyrimisulfan standard solutions of several concentrations (acetonitrile/0.05 vol% formic acid (3:2, v/v)). Inject each standard solution to LC-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 0.01 mg/kg of pyrimisulfan gives the test solution of 0.001 mg/L in concentration.

6. Quantification

Inject the test solution to LC-MS and calculate the concentration of pyrimisulfan from the calibration curve made in **5**.

7. Confirmation

Confirm using LC-MS.

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 150 mm in length and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/0.05 vol% formic acid (1:3, v/v) to (3:1, v/v) in 15 min and hold for 3 min.

Ionization mode: ESI (−) and ESI (+)

Major monitoring ion (*m/z*): 418 [ESI (−)], 420 [ESI (+)]

Injection volume: 5 μL

Expected retention time: 15 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of pyrimisulfan from sample with acetone, clean-up with a graphitized carbon black cartridge (for tea leaves only), an octadecylsilanized silica gel cartridge and a silica gel cartridge, quantification and confirmation using LC-MS.

2) Notes

i) To dissolve the residue after clean-up with the octadecylsilanized silica gel cartridge, add acetone and then *n*-hexane, because the solubility of pyrimisulfan in *n*-hexane is low. Insufficient dissolution of pyrimisulfan results in low recovery and/or large variation, and thus ultrasonication is recommended.

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

for quantification (*m/z*): 418 [ESI (−)]

for confirmation (*m/z*): 420 [ESI (+)]

Conditions for LC-MS/MS are shown below:

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 420, product ions 370 and 255

iii) It has been reported that pyrimisulfan was lost when pyrimisulfan solution in *n*-hexane or in ethyl acetate was dehydrated with anhydrous sodium sulfate.

11. References

None

12. Type

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