Original: Japanese

Provisional Translation

Analytical Method for Pyrithiobac sodium (Agricultural Products)

1. Analyte

Pyrithiobac sodium

2. Instrument

High performance liquid chromatograph-ultraviolet spectrophotometric detector with column switching system (HPLC-UV (column switching))

Liquid chromatograph-mass spectrometer (LC-MS) or liquid chromatograph-tandem mass spectrumeter (LC-MS/MS)

3. Reagents

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

0.03 mol/L phosphate buffer (pH 3.0): Add 7.21 g of potassium dihydrogen phosphate, 0.2 g of sodium azide and 0.40 mL of phosphoric acid to 2000 mL of water, and adjust the pH of the solution to 3.00 with phosphoric acid or 50% sodium hydroxide solution.

Water (pH 2.4): Add phosphoric acid to water and adjust the pH of the solution to 2.40 (±0.05).

Octa- decylsilanized silica gel cartridge (2,000 mg): Polyethylene tube of 12–13 mm in inside diameter packed with 2,000 mg of octadecylsilanized silica gel, or other cartridge with equal separation characteristics.

Trimethylaminopropylsilanized silica gel cartridge (5,000 mg): Polyethylene tube of 19 mm in inside diameter packed with 5,000 mg of trimethylaminopropylsilanized silica gel, or other cartridge with equal separation characteristics.

Reference standard of pyrithiobac sodium: Contains not less than 98% of pyrithiobac sodium.

4. Procedure

1) Extraction

Add 100 mL of acetonitrile/0.01 mol/L ammonium carbonate solution (2:1, v/v) to 5.00 g of sample, homogenize, and centrifuge at 3,000 rpm for 15 minutes.

Take 60 mL of the supernatant, add 50 mL of dichloromethane, shake, and discard the dichloromethane layer. Add 0.05 g of sodium chloride and 50 mL of dichloromethane to the aqueous layer, shake, and discard the dichloromethane layer.

2) Clean-up

i) Trimethylaminopropylsilanized silica gel column chromatography

Add 20 mL each of methanol, water and 0.01 mol/L ammonium carbonate solution to a trimethylaminopropylsilanized silica gel cartridge (5,000 mg) sequentially, and discard the effluents. Transfer the aqueous layer obtained in 1) to the cartridge, add 5 mL of 0.01 mol/L ammonium carbonate solution, and discard the effluent.

ii) Octadecylsilanized silica gel column chromatography

Add 5 mL of methanol and 10 mL of water to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluents. Add 20 mL of 1 mol/L potassium citrate solution/methanol (3:1, v/v) to the cartridge, leave about 10 mL of the solvent above the top of the cartridge packing, and stop the flow through the cartridge. Connect the cartridge under the trimethylaminopropylsilanized silica gel cartridge described in i), add 20 mL of 1 mol/L potassium citrate solution/methanol (3:1, v/v) to the connected cartridges, and discard the effluent. Allow the cartridges to dry by passing air through them for 10 minutes to dry off adsorbed water.

Remove the trimethylaminopropylsilanized silica gel cartridge, add 10 mL each of water, water (pH 2.4), and water (pH 2.4)/methanol (7:3, v/v) to the octadecylsilanized silica gel cartridge sequentially, and discard the effluents. Dry the octadecylsilanized silica gel cartridge by allowing air to pass through it for 15 minutes. Elute with 10 mL of methanol, concentrate the eluate at below 50ºC, and remove the solvent. Dissolve the residue in 0.5 mL of acetonitrile/0.03 mol/L phosphate buffer (28:72, v/v), and use this solution as the test solution.

5. Calibration curve

Prepare pyrithiobac sodium stock standard solution (methanol), and prepare 0.03–0.8 mg/L standard solutions of several concentrations by diluting the stock standard solution with acetonitrile/0.03 mol/L phosphate buffer (28:72, v/v). Inject 100 µL of each standard solution to HPLC or LC-MS (LC-MS/MS), and make a calibration curve by peak-height or peak-area method.

6. Quantification

Inject 100 µL of the test solution to HPLC or LC-MS (LC-MS/MS), and calculate the concentration of pyrithiobac sodium from the calibration curve made in **5**.

7. Confirmation

Confirm using LC-MS or LC-MS/MS.

8. Measurement conditions

HPLC (column switching)

Detector: UV (wavelength 254 nm)

Column 1: Cyanopropylsilanized silica gel, 4.0 mm in inside diameter, 150 mm in length and 5 µm in particle diameter

Column 2: Octadecylsilanized silica gel, 4.6 mm in inside diameter, 250 mm in length and 5 µm in particle diameter

Column temperature: 40 ºC

Mobile phase:

A: Acetonitrile, B: 0.03 mol/L phosphate buffer (pH 3.0), C: Water

Inject the test solution to column 1, and elute with A/B (28:72, v/v) with flow rate of 1 mL/min. Connect column 2 to column 1 immediately before the retention time of pyrithiobac sodium (8–10 min after injection), and transfer pyrithiobac sodium from column 1 to column 2.

Separate column 1 from column 2 after pyrithiobac sodium is transferred to column 2. Wash column 1 with A/C (75:25, v/v) for 5 minutes with flow rate of 2 mL/min. Condition column 1

with A/B (43:57, v/v) for 10 minutes with flow rate of 2 mL/min, and the for 1 minute with flow rate of 1 mL/min.

Connect column 1 and column 2, elute pyrithiobac sodium with A/B (43:57, v/v) with flow rate of 1 mL/min.

Wash both columns with A/C (75:25, v/v) for 15 minutes with flow rate of 1 mL/min, and then with A/B (43:57, v/v) with flow rate of 1 mL/min.

Separate column 1 and column 2. Condition column 1 with A/B (28:72, v/v) for 5 minutes with flow rate of 2 mL/min, and then for1 minute with flow rate of 1 mL/min.

Injection volume: 100 µL

Expected retention time: 37 min

LC-MS/MS

Column: Octadecylsilanized silica gel, 4.6 mm in inside diameter, 150 mm in length and 5 µm in particle diameter

Column temperature: 30ºC

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

A: 1% Acetic acid

B: Acetonitrile

Time (min)	A (%)	B (%)
0	85	15
1	85	15
17	40	60
18	10	90
25	10	90
35	finish	

Flow rate: 1.0 mL/min

Ionization mode: APCI(+)

Major monitoring ion (*m/z*): Precursor ion 327, product ion 309

Injection volume: 100 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 pyrithiobac sodium from sample with acetonitrile/0.01 mol/L ammonium carbonate solution (2:1, v/v), washing with dichloromethane, clean-up with a trimethylaminopropylsilanized silica gel cartridge and an octadecylsilanized silica gel cartridge, quantification using HPLV-UV with column switching system or LC-MS (LC-MS/MS), and confirmation using LC-MS (LC-MS/MS).

2) Notes

i) This method was developed for the analysis of cotton seeds by DuPont company. Sumpter, S. R., et al. described the HPLC with column switching method. The measurement conditions for LC-MS/MS are quoted from a report by Bramble, F. Q., et al.

ii) The 0.03 mol/L phosphate buffer (pH 3.0) should be filtered daily before use with a 0.45-μm pore filter.

iii) The residue before dissolving in acetonitrile/0.03 mol/L phosphate buffer (28:72, v/v) can be stored in refrigerator.

11. References

Bramble, F. Q., et al., Analytical method for the determination of pyrithiobac sodium in cotton gin trash using ASE extraction and LC-MS/MS analysis, Report by DuPont company, http://www.epa.gov/oppbead1/methods/rammethods/2001_035M.tif

12. Type

D (Sumpter, S. R., et al., Improved analytical enforcement method for the determination of KIH-2031(DPX-PE350) residues in cottonseed using column-switching liquid chromatography, Report by DuPont company, http://www.epa.gov/oppbead1/methods/rammethods/1994_001M.tif)