Original: Japanese

Provisional Translation

Analytical Method for Pyraclonil (Agricultural Products)

1. Analyte

Pyraclonil

2. Instrument

Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents

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

Reference standard of pyraclonil: Contains not less than 98% of pyraclonil. Melting point of the standard is 93–95°C.

4. Procedure

1) Extraction

For grains, legumes, nuts and seeds, weigh 10.0 g of sample, add 20 mL of water and let stand for 30 minutes. For fruits and vegetables, weigh 20.0 g of sample. For tea leaves, weigh 5.00 g of sample, add 20 mL of water and let stand for 30 minutes.

Add 100 mL of acetonitrile, homogenize, and filter with suction. Add 50 mL of acetonitrile to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetonitrile to make exactly 200 mL. Take a 4 mL aliquot of the extract for grains, legumes, nuts, seeds, fruits and vegetables, or a 8 mL aliquot for tea leaves, add 10 mL of water, and concentrate to about 10 mL at below 40°C.

2) Clean-up

Add 5 mL each of acetonitrile and water to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluents. Add 10 mL each of acetonitrile and water to a graphitized carbon black cartridge (500 mg) sequentially, and discard the effluents.

Transfer the solution obtained in 1) to the octadecylsilanized silica gel cartridge, add 10 mL of acetonitrile/water (3:7, v/v), and discard the effluent. Connect the graphitized carbon black cartridge under the octadecylsilanized silica gel cartridge, add 10 mL of acetonitrile/water (1:1, v/v), and discard the effluent. Remove the octadecylsilanized silica gel cartridge, elute with 10 mL of acetonitrile from the graphitized carbon black cartridge, collect the eluate, concentrate at below 40°C and remove the solvent. Dissolve the residue in methanol to make exactly 2 mL for grains, legumes, nuts, seeds, and tea leaves, or exactly 4 mL for fruits and vegetables, and use this solution as the test solution.

5. Calibration curve

Prepare 0.001–0.02 mg/L pyraclonil standard solutions (methanol) of several concentrations. Inject 5 μL of each standard solution to LC-MS, and make a calibration curve by peak-height or peak-area method.

6. Quantification

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

7. Confirmation

Confirm using LC-MS.

8. Measurement conditions

Column: Octadecylsilanized silica gel, 2.0–2.1 mm in inside diameter, 150 mm in length and 3–3.5 μm in particle diameter

Mobile phase: Initially 0.005 mol/L ammonium acetate solution/0.005 mol/L ammonium acetate-methanol solution (3:2, v/v) for 2 min, followed by a linear gradient to (1:4, v/v) in 13 min and hold for 5 min.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*): 315

Expected retention time: 13 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of pyraclonil from sample with acetonitrile, clean-up with an octadecylsilanized silica gel cartridge and a graphitized carbon black cartridge, and quantification and confirmation using LC-MS.

2) Notes

i) It has been confirmed that "Multi-residue Method I for Agricultural Chemicals by LC-MS (Agricultural Products)" was applicable to pyraclonil in rice. The applicability of the method to other agricultural products has not been investigated.

ii) It is recommended that an additional clean-up procedure using a silica gel cartridge (690 mg) is used if the clean-up procedure described above does not produce an extract that is sufficiently clean for LC-MS analysis.

Outline of the procedure: Dissolve the residue after the clean-up with the graphitized carbon black cartridge in 5 mL of ethyl acetate/*n*-hexane (1:4, v/v). Add 5 mL of ethyl acetate/n-hexane (1:4, v/v) to a silica gel cartridge (690 mg) and discard the effluent. Transfer the solution obtained above to the cartridge, add 5 mL of ethyl acetate/*n*-hexane (1:4, v/v), discard the effluent, and then elute with 10 mL of ethyl acetate/*n*-hexane (2:3, v/v).

11. References

None

12. Type

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