Original: Japanese

Provisional Translation

Analytical Method for Diniconazole (Agricultural Products)

1. Analyte

Diniconazole

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.

Synthetic magnesium silicate cartridge (1,000 mg): Polyethylene tube of 12-13 mm in inside diameter packed with 1,000 mg of synthetic magnesium silicate, or other cartridge with equal separation characteristics.

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

4. Procedure

1) Extraction

i) 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 concentrate to about 15 mL at below 40°C. Add 100 mL of 10 w/v% sodium chloride solution, extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Dehydrate the extract with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. Dissolve the residue in *n*-hexane to make exactly 10 mL.

ii) 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 concentrate to about 15 mL at below 40°C. Add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Dehydrate the extract with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. Add 30 mL of *n*-hexane to the residue, and extract with shaking three times with 30 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40°C and remove the solvent. Dissolve the residue in *n*-hexane to make exactly 10 mL.

iii) Tea

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 concentrate to about 15 mL at below 40°C. Add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Dehydrate the extract with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. Dissolve the residue in *n*-hexane to make exactly 10 mL.

2) Clean-up

i) Fruits, vegetables, grains, legumes, nuts and seeds

Add 20 mL of *n*-hexane to a synthetic magnesium silicate cartridge (1,000 mg), and discard the effluent. Transfer 1 mL of solution obtained in 1) to the cartridge, add 20 mL of *n*-hexane, and discard the effluent. Elute with 10 mL of acetone/*n*-hexane (2:3, v/v), concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile/water (1:1, v/v) to make exactly 4 mL for fruits and vegetables, 2 mL for grains, legumes, nuts and seeds, and use this solution as the test solution.

ii) Tea

Add 20 mL of *n*-hexane to a synthetic magnesium silicate cartridge (1,000 mg), and discard the effluent. Add 20 mL of acetone/*n*-hexane (2:3, v/v) to a graphitized carbon black cartridge (500 mg), and discard the effluent. Transfer 1 mL of solution obtained in 1) to the synthetic magnesium silicate cartridge, add 20 mL of *n*-hexane, and discard the effluent. Connect the graphitized carbon black cartridge to the bottom of the synthetic magnesium silicate cartridge, elute with 10 mL of acetone/*n*-hexane (2:3, v/v), and collect the eluate. Detach the synthetic magnesium silicate cartridge, elute with 10 mL of acetone/*n*-hexane (2:3, v/v) from the graphitized carbon black cartridge, and combine the eluate with the first eluate. Concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile/water (1:1, v/v) to make exactly 1 mL, and use this solution as the test solution.

5. Calibration curve

Prepare diniconazole standard solutions (acetonitrile/water (1:1, v/v)) of several concentrations. Inject each standard solution to LC-MS/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 diniconazole gives the test solution of 0.005 mg/L in concentration.

6. Quantification

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

7. Confirmation

Confirm using LC-MS/MS.

8. Measurement conditions

Example

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

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/0.1 vol% formic acid (1:9, v/v) to (9:1, v/v) in 10 min and hold for 10 min

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 326, product ion 159, 70

Injection volume: 10 μL

Expected retention time: 12 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory notes

1) Outline of analytical method

The method consists of extraction of diniconazole from sample with acetone, transferring into *n*-hexane, defatting by acetonitrile/hexane partitioning (for grains, legumes, nuts and seeds), clean-up with a synthetic magnesium silicate cartridge, and additional clean-up with a graphitized carbon black cartridge (for samples containing high amounts of chlorophyll such as tea), and quantification and confirmation using LC-MS/MS.

2) Notes

For fruits and vegetables, if the clean-up (of pigments, for example) is insufficient, additional clean-up using a graphitized carbon black cartridge is recommended. The procedure is described below. The clean-up for tea described in 2) ii) can be also used for the additional clean-up.

Add 20 mL of acetone/n-hexane (2:3, v/v) to a graphitized carbon black cartridge (500 mg), and discard the effluent. Concentrate the eluate obtained by the clean-up using the synthetic magnesium silicate cartridge (1,000 mg) at below 40°C, and remove the solvent. Dissolve the residue in 2 mL of acetone/n-hexane (2:3, v/v), and transfer to a graphitized carbon black cartridge. Elute with 20 mL of acetone/n-hexane (2:3, v/v), concentrate at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile/water (1:1, v/v) to make exactly 4 mL, and use this solution as the test solution.

11. References

None

12. Type

C