Original: Japanese

Provisional Translation

Analytical Method for Diniconazole (Animal and Fishery 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) Muscle, fat, liver, kidney, fish/shellfish, milk and egg

For muscle, liver, kidney and fish/shellfish, weigh 20.0 g (5.00 g for fat) of sample. Add 20 mL of water and homogenize. For milk and egg, weigh 20.0 g of sample. Add 100 mL of acetone/*n*-hexane (1:2, v/v), homogenize, centrifuge at 2,500 rpm for 5 minutes and take the organic layer. Add 50 mL of *n*-hexane to the residue, homogenize, and centrifuge as described above. Combine the obtained organic layers, dehydrate 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.

ii) Honey

Dissolve 20.0 g of sample in 20 mL of water. Add 100 mL of acetone/*n*-hexane (1:2, v/v), homogenize, centrifuge at 2,500 rpm for 5 minutes and take the organic layer. Add 50 mL of *n*-hexane to the residue, homogenize, and centrifuge as described above. Combine the obtained organic layers, dehydrate 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

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 (1 mL for fat), 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/*n*-hexane (1:2, v/v), defatting by acetonitrile/hexane partitioning (omitted for honey), clean-up with a synthetic magnesium silicate cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

None

11. References

None

12. Type

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