Original: Japanese

Provisional Translation

Analytical Method for Dinotefuran (Agricultural Products)

1. Analytes

Dinotefuran

2. Instruments

High performance liquid chromatograph-ultraviolet spectrophotometric detector (HPLC-UV)

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 dinotefuran: Contains not less than 99% of dinotefuran. Melting point of the standard is 107.5°C.

4. Procedure

1) Extraction

For grains, legumes, nuts and seeds, add 20 mL of water to 10.0 g of sample, and let stand for 2 hours. For fruits, vegetables and herbs, weigh 20.0 g of sample. For tea leaves, add 20 mL of water to 5.00 g of sample, and let stand for 2 hours. Add 100 mL of acetonitrile to the sample, 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. Concentrate 50 mL of the solution (For tea leaves, 10mL) to about 5 mL at below 40ºC.

2) Clean-up

i) Porous diatomaceous earth column chromatography: Add 10 mL of water to the solution obtained in 1), transfer the solution to a porous diatomaceous earth cartridge (to hold 20 mL of solution), and let stand for 10 minutes. Add 100 mL of *n*-hexane to the cartridge, and discard the effluent. Elute with 200 mL of ethyl acetate, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of ethyl acetate.

ii) Graphitized carbon black column chromatography: Add 5 mL of ethyl acetate to agraphitized carbon black cartridge (500 mg), and discard the effluent. Transfer the extract obtained in i) to the cartridge, and add 15 mL of ethyl acetate. Collect the total eluate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of ethyl acetate.

iii) Neutral alumina column chromatography: Add 5 mL of ethyl acetate to a neutral alumina cartridge (1,710 mg), and discard the effluent. Transfer the extract obtained in ii) to the cartridge, add 15 mL of ethyl acetate, and discard the effluent. Elute with 20 mL of acetone, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the

residue in water to make exactly 1 mL for grains, legumes, nuts, seeds, and to make exactly 2 mL for fruits, vegetables, herbs. Use this solution as the test solution.

5. Calibration curve

Prepare 0.025-0.5 mg/L dinotefuran standard solutions of several concentrations, inject 40 µL of each standard solution to HPLC, and make a calibration curve by peak-height or peak-area method.

6. Quantification

Inject 40 µL of the test solution to HPLC, and calculate the concentration of dinotefuran from the calibration curve made in 5.

7. Confirmation

Confirm using LC-MS.

8. Measurement conditions

1) HPLC

Detector: UV (270 nm in wavelength)

Column: Octadecylsilanized silica gel, 4.6 mm in inside diameter, 150-250 mm in length and 3-5 µm in particle diameter

Column temperature: 40ºC

Mobile phase: acetonitrile/water (1:9, v/v)

Expected retention time: 8 min

2) LC-MS

Column: Octadecylsilanized silica gel, 2-2.1 mm in inside diameter, 150 mm in length and 3-5 µm in particle diameter

Column temperature: 40ºC

Mobile phase: acetonitrile/2 mmol/L ammonium acetate (1:9, v/v)

Ionization mode: ESI (+)

Major monitoring ions (m/z): 203

Injection volume: 2 µL

Expected retention time: 5 min

9. Limit of quantification

0.01 mg/kg (0.1 mg/kg for tea)

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of dinotefuran from sample with acetonitrile, clean-up with a graphitized carbon black cartridge and a neutral alumina cartridge, quantification using HPLC-UV, and confirmation using LC-MS.

2) Notes

i) If beans and other samples are not sufficiently dispersed in the extraction steps, add diatomaceous earth to the sample swollen with water, add acetonitrile to the sample, and

homogenize the solution to improve extraction efficiency.

ii) For samples with many interfering components, in HPLC analysis, after eluting dinotefuran, and flowing the mobile phase sufficiently, to elute remaining contaminants in the column, perform the next analysis.

11. References

MOE Notification No. 35, Analytical Method for Dinotefuran (April 24, 2002)

12. Type

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