Original: Japanese

Provisional Translation

Analytical Method for Dinotefuran (Animal and Fishery 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.

Porous diatomaceous earth cartridge (to hold 5 mL of solution): A polyethylene tube of about 20 mm in inside diameter packed with granular porous diatomaceous earth prepared for column chromatography (to hold 5 mL of solution), or a cartridge equivalent to the specified one in separation capability.

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 muscle, fat, liver, kidney, other edible parts and milk, weigh 5.00 g of sample. Add 50 mL of acetonitrile, 50 mL of n-hexane saturated with acetonitrile and 10 g of anhydrous sodium sulfate to the sample, homogenize, and centrifuge at 3,000 rpm for 5 minutes. Collect the acetonitrile layer, add 50 mL of acetonitrile to *n*-hexane layer and the residue, and treat as described above. Combine the second collected acetonitrile layer with the first collected acetonitrile layer, and add acetonitrile to make exactly 200 mL. Concentrate 100 mL of the solution at below 40ºC, and remove the solvent.

2) Clean-up

i) Porous diatomaceous earth column chromatography:

Dissolve the residue obtained in 1) in 5 mL of water, transfer the solution to a porous diatomaceous earth cartridge (to hold 5 mL of solution), and let stand for 10 minutes. Add 50 mL of ethyl acetate to the cartridge, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of ethyl acetate.

ii) 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 i) to the cartridge, add 10 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, and 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

10. Explanatory note

1) Outline of analytical method

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

2) Notes

For the samples including many interfering substances, in HPLC analysis, after eluting dinotefuran, flow the mobile phase sufficiently, elute the remaining contaminants in the

column, and perform the following analysis.

11. References

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

12. Type

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