

Analytical Method for Dichlobenil (Fishery Products)

1. Analyte

Dichlobenil

2. Application

Fishery products

3. Instruments

Gas chromatograph-mass spectrometer (GC-MS) or gas chromatograph-tandem mass spectrometer (GC-MS/MS)

4. Reagents

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

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

5. Procedure

1) Extraction

Weigh 10.0 g of sample, add 25 mL of *n*-hexane and 50 mL of acetonitrile saturated with *n*-hexane, homogenize, centrifuge at 3,000 rpm for 5 min, and collect the acetonitrile layer. Add 25 mL of acetonitrile saturated with *n*-hexane to the residue and the *n*-hexane layer, homogenize, and centrifuge at 3,000 rpm for 5 min. Combine the acetonitrile layer with the previous acetonitrile layer to make exactly 100 mL.

2) Clean-up

i) Octadecylsilanized silica gel column chromatography

Inject 10 mL of acetonitrile into an octadecylsilanized silica gel cartridge (1,000 mg) and discard the effluent. Take exactly a 25 mL aliquot of the solution obtained in '1) Extraction', transfer to the cartridge, add another 5 mL of acetonitrile, collect the total eluate, and concentrate to less than 4 mL at below 40°C. Add 40 mL of 10 w/v% sodium chloride solution, extract with shaking twice with 40 mL and 20 mL of *n*-hexane. Combine the extracts, dehydrate with anhydrous sodium sulfate, filter out anhydrous sodium sulfate, and concentrate to approximately 2 mL at below 40°C.

ii) Ethylenediamine-*N*-propylsilanized silica gel column chromatography

Inject 10 mL of *n*-hexane into an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) and discard the effluent. Transfer the solution obtained in i) to the cartridge, add another 8 mL of *n*-hexane, collect the total eluate, concentrate to approximately 2 mL at below 40°C, add *n*-hexane to make exactly 5 mL, and use this solution as the test solution.

6. Calibration curve

Prepare dichlobenil standard solutions (*n*-hexane) of several concentrations, inject each standard solution into GC-MS or GC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of dichlobenil in the test solution corresponding to 0.005 mg/kg in the sample is 0.0025 mg/L.

7. Quantification

Inject the test solution into GC-MS or GC-MS/MS, and calculate the concentration of dichlobenil from the calibration curves prepared in 6.

8. Confirmation

Confirm using GC-MS or GC-MS/MS.

9. Measurement conditions

(Example)

Column: 5% phenyl-methyl silicone, 0.25 mm inside diameter, 30 m in length and 0.25 µm in film thickness

Column temperature: 50°C (1 min) → 25°C/min → 125°C (0 min) → 10°C/min → 300°C (10 min)

Inlet temperature: 250°C

Carrier gas: Helium

Ionization mode (Ionized energy): EI (70 eV)

Major monitoring ions (*m/z*)

GC-MS: 173, 171

GC-MS/MS: Precursor ion 171, product ions 136, 100

Injection volume: 1 µL

Expected retention time: 8 min

10. Limit of quantification

0.005 mg/kg

11. Outline of analytical method

The method consists of extracting dichlobenil from sample with acetonitrile in the presence of *n*-hexane, cleaning up with an octadecylsilanized silica gel cartridge, transferring it into *n*-hexane for re-dissolution, cleaning up with an ethylenediamine-*N*-propylsilanized silica gel cartridge, quantifying and confirming using GC-MS or GC-MS/MS.

12. Note

- 1) When the analytical method for dichlobenil using GC-MS was developed, the following monitoring ions were used:
 - for quantitative ion (*m/z*): 171
 - for qualitative ion (*m/z*): 173
- 2) When the analytical method for dichlobenil using GC-MS/MS was developed, the following monitoring ions were used:
 - for quantitative ions (*m/z*): precursor ion 171, product ion 100
 - for qualitative ions (*m/z*): precursor ion 171, product ion 136

- 3) If residues from the sample are observed when collecting the acetonitrile extract after centrifugation, it is recommended to filter the acetonitrile extract through filter paper.
- 4) Emulsions may form when operating liquid-liquid extraction into *n*-hexane depending on the food. In that case, it is recommended to centrifuge at 3,000 rpm for 5 min and collect the *n*-hexane layer.
- 5) Since dichlobenil has a high vapor pressure and is easily volatilized, concentrate gently and be careful not to dry out.
- 6) Food items used to develop the analytical method: eel and *Corbicula* (freshwater clam).

13. Reference

None

14. Type

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