Original: Japanese

Provisional Translation

Analytical Method for Dimethomorph (Animal and Fishery Products)

1. Analytes

(*E*)-Dimethomorph, (*Z*)-Dimethomorph

2. Instruments

Liquid chromatograph-mass spectrometer (LC-MS)

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

3. Reagents

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

Reference standard of dimethomorph ((*E*)-isomer and (*Z*)-isomer): Contains not less than 98% of dimethomorph ((*E*)-isomer and (*Z*)-isomer). Melting point of the standard is 127–148 °C.

4. Procedure

1) Extraction

For muscle, liver, kidney, fish, shellfish, milk, egg and honey, weigh 10.0 g of sample. For fat, weigh 5.00 g of sample.

Add 10 mL of 0.01 mol/L hydrochloric acid and homogenize. Add 50 mL of acetonitrile, 25 mL of *n*-hexane and 2 g of diatomaceous earth, homogenize, and filter with suction. Collect the acetonitrile layer from the filtrate. Add 5 mL of 0.01 mol/L hydrochloric acid and 25 mL of acetonitrile to the remaining *n*-hexane layer and the residue on the filter paper, homogenize, and filter with suction. Discard the *n*-hexane layer, combine the acetonitrile layers, and add acetonitrile to make exactly 100 mL.

Take a 20 mL aliquot of the extract, add 3 g of sodium chloride, and shake for 5 minutes. Let stand and discard the aqueous layer.

2) Clean-up

i) Octadecylsilanized silica gel column chromatography

Add 10 mL of acetonitrile to an octadecylsilanized silica gel cartridge (1,000 mg), and discard the effluent. Transfer the acetonitrile layer described above to the cartridge, elute with 2 mL of acetonitrile, collect the total eluate concentrate at below 40°C and remove the solvent. Dissolve the residue in 2 mL of acetone/*n*-hexane (1:1, v/v).

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

Add 5 mL of methanol and 10 mL of acetone/*n*-hexane (1:1, v/v) to an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) sequentially, and discard the effluents. Transfer the solution obtained in i) to the cartridge, elute with 20 mL of acetone/*n*-hexane (1:1, v/v), collect the total eluate, concentrate at below 40°C and remove the solvent. Dissolve the residue in methanol to make exactly 2 mL (1 mL for fat), and use this solution as the test solution.

5. Calibration curve

Prepare 0.01–0.2 mg/L dimethomorph standard solutions (methanol). Inject 3 µL of each standard solution to LC-MS or LC-MS/MS, and make a calibration curve using sum of the peak area (or peak height) of (*E*)-isomer and (*Z*)-isomer by peak-area (or peak-height) method.

6. Quantification

Inject 3 µL of the test solution to LC-MS or LC-MS/MS and calculate the concentration of dimethomorph from the calibration curve made in **5**.

7. Confirmation

Confirm using LC-MS or LC-MS/MS.

8. Measurement conditions

Column: Octadecylsilanized silica gel, 2.0–2.1 mm in inside diameter, 150 mm in length and 3–3.5 µm in particle diameter

Column temperature: 40°C

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Flow rate: 0.2 mL/min

Mobile phase A: 5 mmol/L ammonium acetate solution

Mobile phase B: 5 mmol/L ammonium acetate-methanol solution

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	85	15
1	60	40
3.5	60	40
6	50	50
8	45	55
17.5	5	95
30	5	95
30	85	15

Injection volume: 3 µL

Expected retention time: 15–17 min ((*E*)-isomer and (*Z*)-isomer are eluted in this order)

Ionization mode: ESI (+)

Major monitoring ions (*m/z*)

1) LC-MS: 388

2) LC-MS/MS: Precursor ion 388, product ion 301, 165

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of dimethomorph from sample with hydrochloric acid, acetonitrile and *n*-hexane, separation of acetonitrile layer, dehydration with salting out, clean-up with an octadecylsilanized silica gel cartridge and an ethylenediamine-*N*-propylsilanized silica gel cartridge, quantification and confirmation using LC-MS or LC-MS/MS.

2) Notes

i) When LC-MS/MS is used, use product ions (*m/z*) of 301 for quantification and 165 for confirmation.

ii) Regard the sum of concentration of (*E*)-dimethomorph and (*Z*)-dimethomorph as the analytical result of dimethomorph.

iii) Individual reference standards of (*E*)-dimethomorph and (*Z*)-dimethomorph can be used. Calibration curves can be constructed by the peak-area (or peak-height) method using the peak areas (or peak heights) of the (*E*)-isomer and (*Z*)-isomer individually.

iv) Homogenization can be performed without the addition of diatomaceous earth. In such a case, use diatomaceous earth during filtration as a filter aid. Instead of filtration, centrifugation or an alternative method can be used.

11. References

None

12. Type

C