Analytical Method for Clomeprop (Animal and Fishery Products)

1. Analytes

Clomeprop

Metabolite B [2-(2,4-dichloro-*m*-tolyloxy)propionic acid] (alias: clomeprop acid)

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.

Reference standard of clomeprop: Contains not less than 97% of clomeprop.

Reference standard of clomeprop acid: Contains not less than 99% of clomeprop acid.

4. Procedure

1) Extraction

Weigh 10.0 g of sample except for fat. For fat, weigh 5.00 g of sample.

To the sample (except for honey), add 100 mL of acetone/*n*-hexane (1:2, v/v), 6 mL of 1 mol/L hydrochloric acid and 8 g of sodium chloride, in this order. For honey, dissolve the sample in 6 mL of 1 mol/L hydrochloric acid, and then add 100 mL of acetone/*n*-hexane (1:2, v/v) and 8 g of sodium chloride, in this order. Homogenize, centrifuge for 5 minutes at 3,000 rpm, and collect the organic layer. Add 100 mL of acetone/*n*-hexane (1:2, v/v) to the residue and the aqueous layer, homogenize, and centrifuge for 5 minutes at 3,000 rpm. Combine the organic layers, and add acetone/*n*-hexane (1:2, v/v) to make exactly 200 mL solution. Take a 20 mL (40 mL for fat) aliquot of the extract, concentrate at below 40°C and remove the solvent. Add 20 mL of *n*-hexane to the residue and extract with shaking three times with 20 mL of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40°C and remove the solvent. Dissolve the residue in 3 mL of acetone/*n*-hexane (1:49, v/v).

2) Clean-up

Connect an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) at the bottom of a trimethylaminopropylsilanized silica gel cartridge (500 mg), and add 10 mL each of methanol, acetone and *n*-hexane sequentially, and discard the effluent. Transfer the extract obtained in 1) to the cartridge, add 10 mL of acetone/*n*-hexane (1:49, v/v), and discard the effluent. Then, elute with 15 mL of acetone/*n*-hexane (3:17, 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 2 mL, and use this solution as the test solution for clomeprop.

Remove the ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg), add 10 mL each of acetone and methanol to the trimethylaminopropylsilanized silica gel cartridge

(500 mg) sequentially, and discard the effluent. Elute with 10 mL of 0.4 vol% formic acid-methanol solution, concentrate the eluate at below 40°C and remove the solvent. Dissolve the residue in acetonitrile/water (1:1, v/v) to make exactly 2 mL, and use this solution as the test solution for clomeprop acid.

5. Calibration curve

Prepare clomeprop and clomeprop acid standard solutions (acetonitrile/water (1:1, v/v)) of several concentrations. Inject 10 μL of 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, clomeprop concentration (including clomeprop acid) in the test solution corresponding to the limit of quantification is 0.005 mg/L (as clomeprop).

6. Quantification

Inject 10 μL of the test solution to LC-MS/MS and calculate the concentrations of clomeprop and clomeprop acid from the calibration curves made in **5**. Use the following equation to calculate the sum of concentrations of clomeprop and clomeprop acid converted to clomeprop.

Concentration of clomeprop (including clomeprop acid) (ppm)= A + B × 1.302

A: Concentration of clomeprop (ppm)

B: Concentration of clomeprop acid (ppm)

7. Confirmation

Confirm using LC-MS/MS.

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 100 mm in length and 3.5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially 0.01 vol% acetic acid/0.01 vol% acetic acid-acetonitrile solution (1:1, v/v) for 0.5 min, followed by a linear gradient to (1:9, v/v) in 5.5 min and hold for 2 min.

Ionization mode: Clomeprop ESI (+); Clomeprop acid ESI (−)

Major monitoring ions (*m/z*):

Clomeprop	precursor ion: 326 and 324, product ion: 120
Clomeprop acid	precursor ion: 249, product ion: 177
	precursor ion: 247, product ion: 175

Expected retention time: 7 min for clomeprop, 5 min for clomeprop acid

9. Limit of quantification

Clomeprop	0.01 mg/kg

Clomeprop acid 0.01 mg/kg (as clomeprop)

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of clomeprop and its metabolite, clomeprop acid, from sample with mixture of acetone and *n*-hexane under the existence of hydrochloric acid and saturated sodium chloride aqueous solution, defatting with acetonitrile/hexane partitioning, clean-up with connected a trimethylaminopropylsilanized silica gel and ethylenediamine-*N*-propylsilanized silica gel columns, quantification and confirmation using LC-MS/MS.

2) Notes

i) When clomeprop acid is not tested, the procedure to prepare the test solution of clomeprop acid in **4** 2) can be omitted.

ii) When the analytical method for clomeprop and clomeprop acid using LC-MS/MS was developed, the following monitoring ions were used:

Clomeprop

for quantification (*m/z*): precursor ion 324, product ion 120

for confirmation (*m/z*): precursor ion 326, product ion 120

Clomeprop acid

for quantification (*m/z*): precursor ion 247, product ion 175

for confirmation (*m/z*): precursor ion 249, product ion 177

iii) In the testing of egg and milk, the solution may gelate in the extraction process if hydorochloric acid is added first. Therefore, add acetone/*n*-hexane (1:2, v/v), 1 mol/L hydrochloric acid, and sodium chloride, in this order.

iv) In the testing of honey, dissolve the sample in 1 mol/L hydrochloric acid, and then add acetone/*n*-hexane (1:2, v/v) and sodium chloride, in this order.

11. References

None

12. Type

C