

## **Analytical Method for Chlorpromazine (Target to Animal and Fishery Products)**

The target compound to be determined is chlorpromazine.

### **1. Instrument**

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

### **2. Reagents**

Use the reagents listed in Section C *Reagents/Test Solutions, Etc.*, Part II *Food Additives*, except the following.

Acetone: Use a reagent not containing any substance that may interfere with the analysis of the target compound.

Sulfonate-modified methacrylate copolymer cartridge (1,000 mg): A polyethylene tube of 20-21 mm in inside diameter packed with 1,000 mg of sulfonate-modified methacrylate copolymer, or a cartridge equivalent to the specified one in separation capability.

Methanol: Use a reagent not containing any substance that may interfere with the analysis of the target compound.

Water: Use water suitable for chemical analysis, including distilled water, purified water, or pure water. If it contains any substance that may interfere with the analysis of the target compound, wash with a solvent such as *n*-hexane before use.

### **3. Reference standard**

Reference standard of chlorpromazine hydrochloride: Contains not less than 98% of chlorpromazine hydrochloride.

### **4. Procedure**

#### **a. Extraction**

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

For honey, weigh 10.0 g of sample and dissolve the sample with 10 g of water.

Add 50 mL of acetone to the sample, homogenize, centrifuge at 3,000 rpm for 5 minutes, and collect the supernatant. Add 30 mL of acetone to the residue, homogenize, and centrifuge as described above. Collect the supernatant, combine the resulting supernatants, and add acetone to make exactly 100 mL. Take exactly a 10 mL aliquot of the solution, add 3 mL of water and 130  $\mu$ L of formic acid for except fat, 1 mL of water and 110  $\mu$ L of formic acid for fat.

b. Clean-up

Add 10 mL each of methanol and acetone/formic acid/water (10:0.13:3, v/v/v) for except fat, add 10 mL each of methanol and acetone/formic acid/water (10:0.11:1, v/v/v) for fat, to a sulfonate-modified methacrylate copolymer cartridge (1,000 mg) sequentially, and discard the effluents. Transfer the solution obtained in "a. Extraction" to the cartridge, and discard the effluent. Add 20 mL each of formic acid/methanol (1:99, v/v), methanol and acetone to the cartridge sequentially, and discard the effluent. Elute with 15 mL of acetone/ammonia solution (19:1, v/v), concentrate the eluate to about 1 mL at below 40°C. Add 0.1 vol% formic acid/0.1 vol% formic acid-acetonitrile solution (3:2, v/v) to make exactly 5 mL, and use this solution as the test solution.

## 5. Measurement

a. Calibration curve

Prepare chlorpromazine standard solutions (0.1 vol% formic acid/0.1 vol% formic acid-acetonitrile solution (3:2, v/v)) of several concentrations. Inject 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 "4. Procedure", the sample containing 0.0001 mg/kg of chlorpromazine gives the test solution of 0.00002 mg/L in concentration.

b. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of chlorpromazine from the calibration curve made in "a. Calibration curve".

c. Confirmation

Confirm using LC-MS/MS.

d. Measurement conditions

(Example)

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

Column temperature: 40°C

Mobile phase: 0.1 vol% formic acid/0.1 vol% formic acid-acetonitrile solution (3:2, v/v)

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 319, product ion 86, 58

Injection volume: 5 µL

Expected retention time: 4 min

## 6. Limit of Quantification

0.0001 mg/kg