Analytical Method for Chlorpromazine
(Targeted to Agricultural, Animal and Fishery Products)

The target compound to be determined is Chlorpromazine.

1. Instrument

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

2. Reagents

In addition to the reagents and test solutions listed below, use those listed in Section C ***Reagent/Test Solution, Etc.***, Part II ***Food Additives***.

Acetonitrile: Acetonitrile produced for liquid chromatography.

Strongly acidic cation exchanger cartridge column (500 mg): A polyethylene column of 8-9 mm in inner diameter packed with 500 mg of benzenesulfonyl propyl silane-bonded silica gel, or a column equivalent to the specified one in separation capability.

Water: Water produced for liquid chromatography.

Methanol: Methanol produced for liquid chromatography.

3. Reference standard

Chlorpromazine hydrochloride: This product contains not less than 98% of chlorpromazine hydrochloride, and its melting point is 194-196°C.

4. Procedure

a. Extraction

Weigh 5.00 g of the test sample, previously ground, and homogenize it with 25 ml of ethyl acetate and 1 ml of 4 mol/l potassium carbonate solution.

Centrifuge the mixture at 3,000 rpm for five minutes and transfer the ethyl acetate layer into a rotary vacuum evaporator.

Add 25 ml of ethyl acetate to the residue and repeat the above procedure and transfer the ethyl acetate layer into the rotary vacuum evaporator, and then remove the ethyl acetate at 40°C or lower.

Add 30 ml each of acetonitrile and acetonitrile-saturated n-hexane to the residue and shake the mixture vigorously for five minutes using a shaker and leave it to stand, and then transfer the acetonitrile layer to a 100-ml separating funnel.

Add 30 ml of acetonitrile-saturated n-hexane and repeat the above procedure.

Collect the acetonitrile layer into the rotary vacuum evaporator and remove the acetonitrile at 40°C or lower.

Dissolve the residue in 10 ml of methanol/1.2% metaphosphoric acid solution (2:3) and filter the mixture through a cotton plug.

b. Clean-up

Pour 3 ml of methanol followed by 3 ml of water into a strongly acidic cation exchanger cartridge column (500 mg) and discard the effluent.

Pour the solution obtained by the extraction described in 4-a into the column followed by 5 ml of water and discard the effluent.

Pour 15 ml of methanol/0.1 mol/l dipotassium hydrogen orthophosphate solution (9:1) to the column and collect the eluate into a rotary vacuum evaporator, and then remove the water and methanol at 40°C or lower.

Dissolve the residue in 1.0 ml of methanol, which is used as the sample solution.

5. Measurement

a. Qualitative tests

Perform qualitative tests under the following conditions. Test results obtained must be the same as those obtained for the reference standard.

Testing conditions

Column packing: Octadecylsilane-bonded silica gel (2-5 μm in particle size).

Column: A stainless tube (2.0-6.0 mm in inner diameter, 100-250 mm in length).

Column temperature: 40°C

Mobile phase: Use acetonitrile/formic acid/water (500:1:500). Adjust the flow rate so that chlorpromazine flows out in approximately 15 minutes.

b. Quantitative tests

Determine the quantity from the test results obtained under the same conditions described in 5-a using either the peak height or peak area method.

6. Limit of quantification

0.0001 mg/kg