Analytical Method for Clorsulon
(targeted to animal products)

The target compound to be determined is clorsulon.

1. Instrument

A Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS)

2. Reagents and test solutions

In addition to the reagents and test solutions listed below, use those listed in Section C *Reagents/Test Solutions*, Etc., Part II *Food additives*. Reagents designated as "special grade" in this section must meet the requirements for "special grade" specified in the Japan Industrial Standards for the reagents.

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

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

Ethylenediamine-*N*-propylsilanized silica gel cartridge column (1,000 mg): A polyethylene column of 10–12 mm in inner diameter packed with 1,000 mg of ethylenediamine-*N*-propylsilanized silica gel or a column equivalent to the specified one in separation capability.

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

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

Sodium sulfate (anhydrous): Use a reagent not containing any substance that may interfere with 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 analysis of the target compound, wash with a solvent such as n-hexane before use.

3. Reference standard

Clorsulon Reference Standard: This product contains not less than 98% of clorsulon.

4. Preparation of sample solutions

a. Extraction

Weigh 10.0 g of the test sample for muscle, fat, liver, kidney, fish/shellfish, milk, egg, or honey. Dissolve it by adding 20 mL of water, homogenize the mixture with 100 mL of acetone, and centrifuge at 3,000 rpm for 5 minutes. Collect the supernatant. To the residue, add 50 mL of acetone to homogenize the mixture, and centrifuge at 3,000

rpm for 5 minutes. Combine the supernatants, and add acetone to make exactly 200 mL of solution. Measure 20 mL of this solution, add 100 mL of 10% (w/v) sodium chloride solution, and perform shaking-extraction twice with ethyl acetate (100 mL and then 50 mL). Combine the extracts obtained, and add anhydrous sodium sulfate to dehydrate. Remove the anhydrous sodium sulfate by filtration, and evaporate the filtrate at a temperature not more than 40°C to remove the solvent. Add 30 mL of *n*-hexane to the residue, and perform shaking-extraction twice with 30 mL of acetonitrile saturated with *n*-hexane each time. Combine the extracts obtained and evaporate at a temperature not more than 40°C to remove the solvent. Dissolve the residue by adding 2 mL of a mixture of acetone/*n*-hexane (1:1).

b. Clean-up

Pour 5 mL each of acetone and *n*-hexane in series into an ethylenediamine-*N*-propylsilanized silica gel (1,000 mg) and discard the effluent. Into the column, pour the solution obtained in a. "Extraction," pour 10 mL of a mixture of acetone/*n*-hexane (1:1), and discard the effluent. Then pour 15 mL of acetone and evaporate the eluate at a temperature not more than 40°C to remove the solvent. Dissolve the residue in a mixture of acetonitril/water (1:1) to make exactly 5 mL of solution.

5. Procedure

a. Calibration curve

Prepare several solutions of Clorsulon Reference Standard in a mixture of acetonitrile/water (1:1) with different concentrations. Inject them into LC-MS/MS to prepare a calibration curve using the peak height or peak area method. When the sample solution is prepared as directed in this method, the concentrate in the sample solution equivalent to 0.001 mg/kg in the sample is 0.0002 mg/L.

b. Quantitative tests

Inject the sample solutions in LC-MS/MS and determine the content of clorsulon using the calibration curve prepared.

c. Confirmation tests

Conduct confirmation tests using LC-MS/MS.

d. Testing conditions

Column: Octadecylsilanized silica gel (2.1 mm in inner diameter, 150 mm in length, 3μm in particle size)

Column temperature: 40°C

Mobile phase: Maintain a mixture of acetonitrile/40 mmol/L ammonium acetate at 1:9 for 1 minute and then create a concentrate gradient of 1:9 to 8:2 in 9 minutes.

Ionized mode: Electrospray ionization method (negative ion)

Main ions (m/z): Precursor ion 380 product ions 344
Precursor ion 378 product ions 342
Injection volume: 5 μL
Retention time: About 6 minutes

6. Limit of quantification

0.001 mg/kg

Notes

✧ Outline of the testing method

This method is designed to extract clorsulon in a sample with acetone, transfer it into ethyl acetate, then delipidated by an acetonitrile-hexane partition, clean up through ethylenediamine-*N*-propylsilanized silica gel cartridge column, and quantify by LC-MS/MS.

✧ Recommendation

If suspended matter is found in the supernatant after centrifugation, it should be filtrated out.

✧ Ions used in the development of LC/MS/MS determination method for clorsulon.

Quantification ions (*m/z*): Precursor ion 380 product ions 344
Qualification ions (m/z): Precursor ion 378 product ions 342