Analytical Method for Gentian violet
(Animal and fishery products)

1. Analyites

Gentian violet

Leucogentian violet.

2. Instrument

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

3. Reagents

Use the reagents listed in Section C ***Reagent/Test Solution, Etc.***, Part II ***Food Additives***, except the following. Reagents designated as "special grade" in this section must meet the requirements for "special grade" specified in the Japan Industrial Standards for the reagents.

Acetonitrile: Use a reagent not containing any substances that may interfere with the analysis of the target compounds.

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

Ethanol: Use a reagent not containing any substances that may interfere with the analysis of the target compounds.

Ammonium formate: Ammonium formate (special grade)

Citric acid (anhydrous): Use a reagent with a purity of 98% or higher.

Sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg): A polyethylene tube of 12–13 mm in inside diameter packed with 500 mg of sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer, or a cartridge equivalent to the specified one in separation capability.

Quaternary ammonium salt-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (150 mg): A polyethylene tube of 12–13 mm in inside diameter packed with 150 mg of quaternary ammonium salt-modified divinylbenzene-*N*-vinylpyrrolidone copolymer, or a cartridge equivalent to the specified one in separation capability.

50 mmol/L ammonium formate buffer (pH 3.5, v/v): Dissolve 3.15 g of ammonium formate in 990 mL of water. Adjust pH to 3.5 with formic acid, and add water to make exactly 1,000 mL.

4. Reference standard

Reference standard of gentian violet: Contains not less than 90% of gentian violet.

Reference standard of leucogentian violet: Contains not less than 98% of leucogentian violet.

5. Procedure

1) Extraction

Weigh sample accurately, and add half the amount in weight ratio at 15 w/w%

dibutylhydroxytoluene-ethanol solution and half the amount in weight ratio at 50 w/w% citric acid solution respectively. Homogenize, and take the sample equivalent to 10.0 g (5.00 g for fat). Add 100 mL of acetone, homogenize, and filter with suction using a glass fiber filter. Add 50 mL of acetone to the residue on the filter, homogenize, and filter as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take exactly a 1 mL (2 mL for fat) aliquot of the solution.

2) Clean-up

Add 5 mL each of acetonitrile and 2 vol% of formic acid to a sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (500 mg) sequentially and discard effluents. Add 5 mL of acetonitrile/ammonia water (9:1, v/v) to a quaternary ammonium salt-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (150 mm) and discard effluent. Add 4 mL of 2 vol% formic acid to the solution obtained **1)**, transfer the solution to the sulfonic-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, add 5 mL of acetonitrile, and discard the effluent. Connect the quaternary ammonium salt-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge to the bottom of the sulfonate-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, elute with 10 mL of acetonitrile/ammonia water (9:1, v/v), and take the eluate. Add acetonitrile/ammonia water (9:1, v/v) to make exactly 10 mL, and use this as the test solution.

6. Measurement

1) Calibration curve

Dissolve reference standards of gentian violet and leucogentian violet in methanol to make 100 mg/L respectively, and use these solutions as stock standard solutions. Mix each stock standard solution appropriately, dilute with acetonitrile/ammonia water (9:1, v/v), and prepare standard solutions of several concentrations. Inject each standard solution to LC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following **5**, the sample containing 0.002 mg/kg of gentian violet or leucogentian violet gives the test solution of 0.00001 mg/L in concentration.

2) Quantification

Inject the test solution to LC-MS/MS and quantify gentian violet and leucogentian violet from the calibration curves made in **1)**.

3) Confirmation

Confirm using LC-MS/MS.

4) Measurement conditions

(Example)

Column: Octadecylsilanized silica gel cartridge of 2.1 mm in inside diameter, 150 mm in length and 5 μm in particle diameter.

Column temperature: Maintain at 40℃

Mobile phase: Linear gradient from acetonitrile/50 mmol/L ammonium formate buffer (pH 3.5) (3:7, v/v) to (9:1, v/v) in 15 min and hold (9:1, v/v)for 10 min.

Ionization mode: Electrospray ionization method (positive ion mode)

Major monitoring ions (*m/z*):

Gentian violet: Precursor ion 372, product ions 356, 340

Leucogentian violet: Precursor ion 374, product ions 358, 238

Injection volume: 10 μL

Expected retention time: Gentian violet, 10 min

Leucogentian violet, 15 min