Analytical Method for Dexamethasone and Betamethasone

1. Analytes

Dexamethasone

Betamethasone

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.

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

Ethylenediamine-*N*-propylsilanized silica gel cartridge (1,000 mg): A polyethylene tube of 12-13 mm in inside diameter packed with 1,000 mg of ethylenediamine-*N*-propylsilanized silica gel, or a cartridge equivalent to the specified one in separation capability.

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

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

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

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

4. Reference standard

Reference standard of dexamethasone: Contains not less than 98% of dexamethasone.

Reference standard of betamethasone: Contains not less than 98% of betamethasone.

5. Procedure

1) Extraction

Add 50 mL each of acetonitrile saturated with *n*-hexane and *n*-hexane to 10.0 g of sample, homogenize. Add 20 g of anhydrous sodium sulfate, homogenize. Centrifuge at 3,000 rpm for 5 minutes, discard the *n*-hexane layer and take the acetonitrile layer. Add 50 mL of acetonitrile to the residue, homogenize and centrifuge at 3,000 rpm for 5 minutes. Take the acetonitrile layer, combine with the previously obtained acetonitrile layer, and add acetonitrile to make exactly 100 mL. Take 50 mL of this solution accurately, concentrate at below 40℃ and remove the solvent. Add 20 mL of water to the residue, extract with shaking

twice with 20 mL each of ethyl acetate. Take the ethyl acetate layer, concentrate at below 40℃ and remove the solvent. Dissolve the residue in 1 mL of ethyl acetate.

2) Clean-up

Add 5 mL of ethyl acetate to an ethylenediamine-*N*-propylsilanized silica gel cartridge (1,000 mg) and discard effluent. Transfer the solution obtained in **1)** and 10 mL of ethyl acetate to the cartridge sequentially and discard effluent. Elute with 10 mL of ethyl acetate/methanol (9:1, v/v), take the eluate, concentrate at below 40℃ and remove the solvent. Dissolve the residue in a mixture of 0.1 vol% acetic acid and 0.1 vol% acetic acid/acetonitrile (3:1, v/v) to make exactly 1 mL, and use this solution as the test solution.

6. Measurement

1) Calibration curve

Dissolve dexamethasone and betamethasone in acetonitrile respectively and use these solutions as stock standard solutions. Mix each stock standard solution appropriately, dilute with a mixture of 0.1 vol% acetic acid and 0.1 vol% acetic acid/acetonitrile (3:1, 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 0.00005 mg/kg of dexamethasone or betamethasone gives the test solution of 0.00025 mg/L in concentration.

2) Quantification

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

3) Confirmation

Confirm using LC-MS/MS.

4) Measurement conditions

(Example)

Column: Adamantyl group chemical binding silica gel cartridge of 2.1 mm in inside diameter, 150 mm in length and 3 μm in particle diameter.

Column temperature: Maintaining at 40℃

Mobile phase: Linear gradient from a mixture of 0.1 vol% acetic acid and 0.1 vol% acetic acid/acetonitrile (3:1, v/v) to (7:3, v/v) in 5 min and from (7:3, v/v) to (1:1, v/v) in 5 min.

Ionization mode: Electrospray ionization method, negative ion mode

Major monitoring ions (*m/z*): Dexamethasone: Precursor ion 451, product ions 361, 307
Betamethasone: Precursor ion 451, product ions 361, 307

Injection volume: 5 μL

Expected retention time: Dexamethasone: 9 min
Betamethasone: 8 min