

## **Analytical Method for Dexamethasone (Targeted to Agricultural, Animal and Fishery Products)**

The target compound to be determined is Dexamethasone.

### **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.

Octadecylsilane-bonded silica gel cartridge column (360 mg): A polyethylene column of 8-9 mm in inner diameter packed with 360 mg of octadecylsilane-bonded silica gel, or a column equivalent to the specified one in separation capability.

Synthetic magnesium silicate (Florisil) for column chromatography:

Heat florisil (150-250  $\mu$ m in particle size) at 130°C for 12 hours or longer.

Cool down to room temperature in a desiccator.

Water: Water produced for liquid chromatography.

Methanol: Methanol produced for liquid chromatography.

Phosphate buffer solution (pH 5.0):

Solution 1: Dissolve 27.2 g of monopotassium dihydrogen mono phosphate in water to make a 1,000 ml of solution.

Solution 2: Dissolve 3.48 g of dipotassium hydrogen orthophosphate in water to make a 100 ml of solution.

Mix Solution 1 and Solution 2 and adjust the pH to 5.0.

### **3. Reference standard**

Dexamethasone: This product contains not less than 99% of dexamethasone, and its melting point is 262-264°C.

### **4. Procedure**

#### **a. Extraction**

Weigh 5.00 g of the test sample, previously ground, and homogenize it with 30 ml of 95% acetonitrile solution. Centrifuge the mixture at 2,500 rpm for five minutes and collect the acetonitrile layer.

Add 30ml of 95% acetonitrile solution to the residue and repeat the above procedure, and then collect the acetonitrile layer.

**b. Clean-up**

**i. Florisil for column chromatography**

Add 8 g of florisil for column chromatography suspended in acetonitrile into a chromatograph tube (15 mm in inner diameter, 300 mm in length).

Spill out the acetonitrile until only a small amount remains on the packing of the column and pour 100 ml of acetonitrile, and then discard the effluent.

Pour the solution obtained by the extraction described in 4-a into the column followed by 30 ml of acetonitrile and then collect the eluate into a 300-ml separating funnel.

Add 50 ml of n-hexane the funnel and shake it vigorously using a shaker for three minutes, and then leave it to stand.

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

Dissolve the residue in 4 ml of phosphate buffer solution (pH 5.0) and add 6 ml of water.

**ii. Octadecylsilane-bonded silica gel column chromatography**

Pour 10 ml of methanol, 10 ml of water and 2 ml of phosphate buffer solution (pH 5.0) into the octadecylsilane-bonded silica gel cartridge column (360 mg) in that order, and discard the effluent.

Pour the solution obtained by chromatography described in 4-b-i into the column followed by 5 ml of phosphate buffer solution (pH5.0) and 10 ml of 25% methanol solution, and then discard the effluent.

Pour 10 ml of 60% acetonitrile solution into the column and collect the eluate into a rotary vacuum evaporator, and then remove the acetonitrile and water at 40°C or lower.

Dissolve the residue in 0.5 ml of 10% acetonitrile solution, 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 (1,200:1:800).

Adjust the flow rate so that dexamethasone flows out in approximately 7-10 minutes.

b. Quantitative tests

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

**6. Limit of quantification**

0.00005 mg/kg