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 μm 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