**Notification (draft)**

**Analytical Method for Nitrofurazone**

*(Targeted to Agricultural, Animal and Fishery Products)*

The target compounds to be determined is nitrofurazone.

1. **Instrument**
   Liquid chromatograph-mass spectrometer (LC-MS)

2. **Reagents**
   Use the reagents listed in Section C *Reagent/Test Solution, Etc.,* Part II *Food Additives,* except the following.
   - Acetonitrile: Acetonitrile prepared for liquid chromatography.
   - Water: Water prepared for liquid chromatography.

3. **Reference standard**
   Reference standard of nitrofurazone: Contains not less than 99% of nitrofurazone
   Melting point of the standard is 238–240°C.

4. **Procedure**
   1) **Honey**
      Homogenize, and then weigh 5.00 of the sample. Dissolve the sample in 10 mL of 0.1 mol/L of hydrochloric acid. Add 20 mL of acetonitrile and 5 g of sodium chloride, shake vigorously for 10 minutes with a shaker, let stand, and transfer the acetonitrile layer to a vacuum rotary evaporator flask. Add 20 mL of acetonitrile to the residue and aqueous layer, treat as described above, and combine the acetonitrile layers in the vacuum rotary evaporator flask. Add 10 mL of n-propanol, and remove acetonitrile and n-propanol at below 40°C. Dissolve 1.0 mL of methanol containing 0.1 vol% formic acid to the residue, and use this solution as he test solution.

   2) **Foods except those listed in 1)**
      Homogenize the sample, and then weigh 5.00 g of the sample. For muscle, remove the fat layer as possible before homogenizing. Add 30 mL of acetonitrile, 20 mL of n-hexane saturated with acetonitrile and 10 g of anhydrous sodium sulfate to the sample, homogenize, centrifuge at 3,000 rpm for 5 minutes, and transfer the acetonitrile layer to a vacuum rotary evaporator flask. Add 20 mL of acetonitrile to the n-hexane saturated with acetonitrile layer and residue, shake vigorously for 10 minutes with a shaker, centrifuge at 3,000 rpm for 5
minutes, and transfer the acetonitrile layer to a vacuum rotary evaporator flask. Add 10 mL of \(n\)-propanol, and remove acetonitrile and \(n\)-propanol at below 40°C. Dissolve 1.0 mL of methanol containing 0.1 vol% formic acid to the residue, and use this solution as the test solution.

5. Measurement

1) Qualification
   Perform the test under the measurement conditions described below. The results shall agree with those obtained using the reference standards.
   Measurement conditions
   - Column packing: Octadecylsilanized silica gel (2-5 \(\mu\)m in particle diameter).
   - Column: A stainless tube of 2.0-6.0 mm in inner diameter, 100-150 mm in length.
   - Column temperature: 40°C
   - Mobile phase: Linear gradient from acetonitrile/10 mmol/L ammonium acetate (1:99, v/v) to (1:0, v/v) in 35 minutes. Adjust the flow rate to elute nitrofurazone at about 20 minutes.

2) Quantification
   Quantify using peak-height or peak-area method, on the basis of the result obtained using the measurement conditions described in 1)