

Analytical Method for Melengestrol Acetate (Targeted to Agricultural, Animal and Fishery Products)

The target compound to be determined is melengestrol acetate.

1. Instrument

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

2. 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 substance that may interfere with the analysis of the target compound.

n-Hexane: Use a reagent not containing any substance that may interfere with the analysis of the target compound.

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

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

Methanol: Use a reagent not containing any substance that may interfere with the analysis of the target compound.

3. Reference standard

Reference standard of melengestrol acetate: Contains not less than 98% of melengestrol acetate.

4. Procedure

a. Extraction

i. Muscle, fat, liver and kidney

Weigh 10.0 g of sample. Add 50 mL of acetonitrile saturated with *n*-hexane, 50 mL of *n*-hexane and 1 mL of acetic acid, and homogenize for 1 minute. Add 20 g of anhydrous sodium sulfate, and homogenize for 2 minutes. Centrifuge at 3,000 rpm for 5 minutes, discard the *n*-hexane layer, and collect the acetonitrile layer. Add 50 mL of acetonitrile to the residue, homogenize for 2 minutes, and centrifuge as described above. Collect the acetonitrile layer, combine the resulting acetonitrile

layers, and add acetonitrile to make exactly 100 mL. Take exactly 5 mL of the solution, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 1 mL of 0.1 vol% formic acid/methanol (1:4, v/v).

ii. Foods except those listed in i above

Extract according to the method described in “i. Muscle, fat, liver and kidney.”

b. Clean-up

Add 5 mL each of methanol and 0.1 vol% formic acid/methanol (1:4, v/v) to an octadecylsilylated silica gel cartridge (1,000 mg) sequentially, and discard the effluents. Transfer the solution obtained in “a. Extraction” to the cartridge and elute with 15 mL of 0.1 vol% formic acid/methanol (1:4, v/v). Collect the total eluate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile/0.1 vol% formic acid (1:3, v/v) to make exactly 1 mL, and use this solution as the test solution.

5. Measurement

a. Calibration curve

Prepare melengestrol acetate standard solutions (acetonitrile/0.1 vol% formic acid (1:3, v/v)) of several concentrations. Inject each standard solution to LC-MS/MS, and make a calibration curve by peak-height or peak-area method. When the test solution is prepared following the above procedure, the sample containing 0.0005 mg/kg of melengestrol acetate gives the test solution of 0.00025 mg/L in concentration.

b. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of melengestrol acetate from the calibration curve made in “a. Calibration curve”.

c. Confirmation

Confirm using LC-MS/MS.

d. Measurement conditions

Column: Octadecylsilylated silica gel, 3 mm in inside diameter, 150 mm in length, 3 µm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from 0.1 vol% formic acid/0.1 vol% formic acid-acetonitrile solution (1:3, v/v) to (1:9, v/v) in 5 min and hold at (1:99, v/v) for 5 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 397, product ion 337, 279

Injection volume: 5 μ L

Expected retention time: 4 min

6. Limit of quantification

0.0005 mg/kg