Original: Japanese

Provisional translation

Analytical Method for Prednisolone (Animal Products)

1. Analyte

Prednisolone

2. Applicable food

Animal products

3. Instrument

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

4. Reagents

Use reagents listed in Section 3 of the General Rules, except the following.

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

5. Procedure

1) Extraction

Add 50 mL of *n*-hexane to 10.0 g of the sample, homogenize, add 50 mL of acetonitrile saturated with *n*-hexane, and homogenize again. Centrifuge at 3,000 rpm for 10 min and collect the acetonitrile layer. Add 25 mL of acetonitrile to the residue and the *n*-hexane layer, homogenize, and centrifuge as described above. Combine the resulting acetonitrile layers and add acetonitrile to make exactly 100 mL. Take exactly a 10 mL aliquot of the solution, concentrate at below 40℃, and remove the solvent. Dissolve the residue in 5 mL of acetonitrile and water (1:9, v/v).

2) Clean-up

Inject 10 mL each of acetonitrile and a mixture of acetonitrile and water (1:9, v/v) sequentially into an octadecylsilanized silica gel cartridge (500 mg) and discard the effluent. Inject 10 mL of acetonitrile into an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) and discard the effluent. Transfer the solution obtained in 1) to the octadecylsilanized silica gel cartridge, add a 10 mL mixture of acetonitrile and water (1:9, v/v), and discard the effluent. Connect the ethylenediamine-*N*-propylsilanized silica gel cartridge to the bottom of the octadecylsilanized silica gel cartridge, add 10 mL of acetonitrile, concentrate the eluate at below 40℃, and remove the solvent. Dissolve the residue in water and methanol (9:1, v/v) to make exactly 1 mL and use this solution as the test solution.

6. Calibration curve

Prepare prednisolone standard solutions (water and methanol [9:1, v/v]) of several concentrations, inject each solution into LC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of prednisolone in the test solution corresponding to 0.0005 mg/kg in the sample results in 0.0005 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of prednisolone from the calibration curve made in 6.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.1 mm inside diameter, 150 mm in length, and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from 0.1 vol% formic acid and 0.1 vol% formic acid-methanol solution (3:2, v/v) to (2:3, v/v) in 20 min, followed by a linear gradient to (1:9, v/v) in 2 min, and hold for 8 min.

Ionization mode: ESI (−)

Major monitoring ion (*m/z*): Precursor ion 405, product ions 329, 295

Injection volume: 10 μL

Expected retention time: 17 min

10. Limit of quantification

0.0005 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of prednisolone from the sample with *n*-hexane using acetonitrile, clean-up with an octadecylsilanized silica gel cartridge and an ethylenediamine-*N*-propylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) When the analytical methods for prednisolone using LC-MS/MS were developed, the following monitoring ions were used:

for quantitative ions *(m/z)*: precursor ion 405, product ion 329

for qualitative ions (*m/z*): precursor ion 405, product ion 295

ii) Food items used to develop the analytical method: pig muscle, pig fat, pig liver, and cow's milk

12. References

None

13. Type

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