Original: Japanese

Provisional Translation

Analytical Method for Glycyrrhizic Acid (Animal and Fishery Products)

1. Analyte

Glycyrrhizic acid

2. Instrument

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

3. Reagents

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

Ammonia solution: Add 270 mL water to 10 mL of ammonia water and mix.

Ammonia water-methanol solution: Add 270 mL methanol to 10 mL of ammonia water and mix.

Reference standard of glycyrrhizic acid: Contains not less than 99% of glycyrrhizic acid.

4. Procedure

1) Extraction

i) Muscle, fat, liver, kidney, fish, shellfish, milk and honey

Add 10 mL of ammonia solution to 10.0 g of sample and homogenize. Add 40 mL of ammonia water-methanol solution and 50 mL of *n*-hexane, homogenize, and centrifuge at 3,500 rpm for 5 minutes. Remove the *n*-hexane layer and collect the ammonia water-methanol solution layer. Add 30 mL of ammonia water-methanol solution and 30 mL of *n*-hexane to the residue, homogenize, and centrifuge as described above. Remove the *n*-hexane layer, combine the ammonia water-methanol solution layers, and filter with suction. Add ammonia water-methanol solution to the filtrate to make exactly 100 mL.

ii) Egg

Add 10 mL of ammonia solution to 10.0 g of sample and homogenize. Add 40 mL of ammonia water-methanol solution and 50 mL of *n*-hexane, homogenize, and centrifuge at 3,500 rpm for 5 minutes. Remove the *n*-hexane layer, add 50 mL of ammonia water-methanol solution, shake gently, centrifuge as described above, and collect the ammonia water-methanol solution layer. Add 30 mL of ammonia water-methanol solution and 30 mL of *n*-hexane to the residue, homogenize, and centrifuge as described above. Remove the *n*-hexane layer, combine the ammonia water-methanol solution layers, and filter with suction. Add ammonia water-methanol solution to the filtrate to make exactly 200 mL.

2) Clean-up

Add 10 mL of methanol to a neutral alumina cartridge (1,710 mg) and discard the effluent. Transfer 10 mL (20 mL for egg) of the extract obtained in 1) to the cartridge, add 10 mL of ammonia water-methanol solution, and discard the effluent. Elute with 30 mL of water, add 20 mL of 1-propanol to the eluate, and remove 1-propanol and water at below 40 ºC. Dissolve the residue in water to make exactly 2 mL, and use this solution as the test solution.

5. Calibration curve

Dissolve reference standard of glycyrrhizic acid in water/methanol (1:1, v/v) and prepare stock standard solution (1 mg/mL). Dilute the stock standard solution with water to prepare 0.005–0.1 mg/mL standard solutions. Inject 10 µL of each standard solution to LC-MS/MS and make a calibration curve by peak-height or peak-area method.

6. Quantification

Inject 10 µL of the test solution to LC-MS/MS, and calculate the concentration of glycyrrhizic acid from the calibration curve made in **5**.

7. Confirmation

Confirm using LC-MS/MS.

8. Measurement conditions

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 150 mm in length and 2 µm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/formic acid/water (25:1:475, v/v/v) to (400:1:100, v/v/v) in 15 min and hold for 1 min.

Ionization mode: ESI (-)

Major monitoring ions (*m/z*): Precursor ion 821, product ion 351, 113

Expected retention time: 11 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of glycyrrhizic acid from sample with ammonia solution, ammonia water-methanol solution and *n*-hexane. After removing of *n*-hexane, clean-up with a neutral alumina cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) Liquid samples such as milk and honey can be extracted by shaking instead of by homogenization.

ii) In the case of egg, the ammonia water-methanol solution layer after the first centrifugation becomes gelatinous. This gelation, however, can be reverted by shaking with additional ammonia water-methanol solution and centrifuging after *n*-hexane has been removed.

iii) Filtration with suction should be conducted using filter paper, covered with a 2–3-mm-thick layer of diatomaceous earth.

iv) Because glycyrrhizic acid is absorbed inside the measurement instrument, especially by the needle of the autosampler, the use of the autosampler equipped with a needle-washing mechanism is recommended to prevent carry-over.

11. References

MHLW ed. "Shokuhineisei Kensashishin. (Standard Methods of Analysis in Food Safety Regulation.) Shokuhin Tenkabutu Hen. (Chapter of Food Additives.)". p.228-232, Japan Food Hygiene Association (2003)

12. Type

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