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Method of Test for Theanine in Foods

1. Scope

This method is applicable to the determination of theanine in tea beverage, foods in capsule or tablet form.

2. Method

After extraction, analytes are determined by liquid chromatography/tandem mass spectrometer (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer

2.1.1.1. Ion source: positive ion electrospray ionization (ESI^+).

2.1.1.2. Column: Poroshell 120 EC-C18, 2.7 μm, 3.0 mm i.d. × 15 cm, or an equivalent product.

2.1.2. Homogenizer.

2.1.3. Vortex mixer

2.1.4. Ultrasonicator

2.1.5. Centrifuge: centrifuge force > 3000 ×g.

2.2. Chemicals

Methanol, HPLC grade;

Glacial acetic acid, reagent grade;

Deionized water, resistivity ≥ 18 MΩ • cm (at 25°C);

Theanine, reference standard;

Theanine-d_5, internal standard.

2.3. Apparatus and materials

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Volumetric flask: 5 mL, 50 mL, and 100 mL.

2.3.3. Membrane filter: 0.22 μm, CA (cellulose acetate).

2.4. Mobile phase

2.4.1. Solvent A

Dilute 1 mL of glacial acetic acid with deionized water to 1000 mL. Filter with a membrane filter. Collect the filtrate as the solvent A.

2.4.2. Solvent B: methanol.

2.5. Internal standard solution preparation

Transfer about 10 mg of internal standard theanine-d_5 accurately weighed into a 10-mL volumetric flask. Dissolve and dilute with deionized water to 10 mL as the internal standard stock solution. Store in the refrigerator and keep in

dark place. When to use, dilute appropriate amount of the internal standard stock solution with deionized water to 100 ng/mL as the internal standard solution.

2.6. Standard solution preparation

Transfer about 10 mg of reference standard theanine accurately weighed into a 10-mL volumetric flask. Dissolve and dilute with deionized water to 10 mL as the standard stock solution. Store in the refrigerator and keep in dark place. When to use, mix appropriate volume of the standard stock solution and internal standard solution, and dilute with deionized water to 1~100 ng/mL (containing 20 ng/mL of internal standard) as the standard solution.

2.7. Sample solution preparation

After homogenization, transfer about 1 g of sample accurately weighed into a 50-mL centrifuge tube. Add 30 mL of deionized water. Mix thoroughly and sonicate for 30 min at the room temperature. Add deionized water to volume. Dilute properly. Transfer 1 mL of the above solution and 1 mL of internal standard solution into a 5 mL volumetric flask and add deionized water to volume. Centrifuge at 3000 ×g for 10 minutes. Collect the supernatant and filter with a membrane filter as the sample solution.

2.8. Standard curve preparation

Accurately inject 5 μL of the standard solutions into LC-MS/MS separately and operate according to the following conditions. Establish the standard curve of theanine by the ratios of peak area of theanine to that of the internal standard vs. the concentrations of theanine.

LC-MS/MS operating condition (Note):

Column: Poroshell 120 EC-C18, 2.7 μm, 3.0 mm i.d. × 15 cm.

Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	A (%)	B (%)
0.0 → 4.0	99 → 99	1 → 1
4.0 → 4.1	99 → 10	1 → 90
4.1 → 6.0	10 → 10	90 → 90
6.0 → 6.1	10 → 99	90 → 1
6.1 → 12.0	99 → 99	1 → 1

Flow rate: 0.3 mL/min.

Injection volume: 5 μL.
Ionization mode: ESI+.
Capillary voltage: 4.5 kV.
Ion source temperature: 100°C.
Desolvation temperature: 500°C.
Curtain Gas: 25 psi.
Collision Gas: 12 psi.
Nebulizer gas, GS1: 60 psi.
Heated gas, GS2: 70 psi.
Detection mode: multiple reaction monitoring (MRM). Detection ion pair, declustering potential cone voltage and collision energy are as follows.

Analyte	Ion pair	Cone voltage (V)	Collision energy (eV)
	Precursor ion (*m/z*) ＞Product ion (*m/z*)		
Theanine	175 > 158*	46	16
	175 > 84	46	28
Theanine-d_5	180 > 163*	50	17

*quantitative ion

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.9. Identification and quantification:

Accurately inject 5 μL of the sample solution and the standard solutions into LC-MS/MS separately and operate according to the conditions in section 2.8. Identify theanine based on the retention time and the relative ion intensities [(note)]. Calculate the amount of theanine (g/kg) in the sample by the following formula:

$$\text{The amount of theanine in the sample (g/kg)} = \frac{C \times V \times F}{M \times 10^6}$$

C: the concentration of theanine in the sample solution calculated by the standard curve (ng/mL)
V：the final make-up volume of the sample (mL)
M：the weight of the sample (g)

F：the dilution factor

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤100%). Maximum permitted tolerances for relative ion intensities are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20～50	± 25
> 10～20	± 30
≤ 10	± 50

Remark

1. Limit of quantification (LOQ) of this method is 0.001 g/kg.
2. Further validation should be performed when interference compounds appear in samples.

References

Bedner, M., Sander, L. C. and Sharpness, K. E. 2010. An LC-ESI/MS method for determining theanine in green tea dietary supplements. Anal. Bioanal. Chem. 397: 1773–1777.