NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.294-2023

National food safety standard - Determination of tryptophan in foods

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1 Scope

This standard specifies the determination method of tryptophan in food.

This standard is applicable to the determination of tryptophan in formula foods for infants and young children, formula foods for special medical purposes, and cereal supplementary foods for infants and young children.

Method A - Enzymatic hydrolysis-liquid chromatography

2 Principle

The sample is enzymatically hydrolyzed with protease, diluted with methanol-water, separated by using a reversed-phase liquid chromatography column, detected with a fluorescence detector or ultraviolet detector, and quantified with an external standard method.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade and the water is first-grade water specified in GB/T 6682.

3.1.4 Hydrochloric acid (HCl): 12 mol/L.

3.1.5 2-Amino-2-hydroxymethyl-1,3-propanediol (C4H11NO3, Tris base).

3.1.6 Streptomyces griseus protease (type XIV; derived from Streptomyces griseus):

The activity is >=3.5 U/mg.

3.2.1 Hydrochloric acid solution (1.0 mol/L): Measure 83 mL of hydrochloric acid, pour

it into 300 mL of water, mix well, and then add water to dilute to 1 L.

3.2.2 Tris buffer (0.1 mol/ L, pH=8.5): Weigh 6.06 g of Tris base into a 1000 mL beaker, add 500 mL of water to dissolve, and adjust the pH of the solution to 8.5+/-0.05 with 1.0 mol/L hydrochloric acid solution. Store it at room temperature, and the shelf life is 6 months.

3.2.3 Protease solution (17.5 U/mL): Weigh 0.05 g of Streptomyces griseus protease, dissolve it in Tris buffer, and make the volume up to 10 mL, so that the protease mass concentration is 17.5 U/mL. Prepare it fresh just before use.

3.2.4 Ammonium acetate solution (10 mmol/ L, pH=4.0): Weigh 0.77 g of ammonium

acetate, add 900 mL of water to dissolve, adjust the pH of the solution to 4.0+/-0.1 with acetic acid, add water to 1000 mL, and filter through a 0.22 um aqueous phase microporous filter membrane for later use.

3.3 Standards

L-Tryptophan standard (C11H12N2O2, CAS number: 73-22-3): purity of >=99%, or a reference material with national authentication and a Reference Material Certificate.

3.4.1 L-tryptophan standard stock solution (1.00 mg/mL): Accurately weigh 25 mg

(accurate to 0.0001 g) of L-tryptophan standard in a beaker, add 10mL of water to dissolve and transfer to a 25 mL volumetric flask, dilute to volume with water, and mix well. Transfer it to a brown glass container and store it in a 4 °C refrigerator. The shelf life is 6 months.

3.4.2 L-Tryptophan standard intermediate solution (100 ug/mL): Take 1.00 mL of L-

tryptophan standard stock solution into a 10 mL volumetric flask, add water to make the volume up to the mark, and mix well. Transfer it to a brown glass container and store it in a 4 °C refrigerator. The shelf life is 6 months.

3.4.3 L-tryptophan standard series working solution: Pipette 20.0 uL, 100 uL, 500 uL,

1.00 mL, 2.00 mL, and 5.00 mL of L-tryptophan standard intermediate solution into 10 mL volumetric flasks respectively, add 2.40 mL of methanol to each, add water to make the volume up to the mark, and mix well. The mass concentrations of L-tryptophan standard series working solutions are 0.200 ug/mL, 1.00 ug/mL, 5.00 ug/mL, 10.0 ug/mL, 20.0 ug/mL, and 50.0 ug/mL, respectively. Prepare the solutions fresh just before use.

4.1 High-performance liquid chromatograph: equipped with a fluorescence detector or

ultraviolet detector.

5.1.1 Sample preparation

Formula foods for infants and young children and formula foods for special medical purposes shall be mixed evenly. Cereal supplementary foods for infants and young children shall be crushed in advance and mixed.

5.1.2 Sample extraction

Accurately weigh 0.2 g of the sample (accurate to 0.001 g) into a 50 mL threaded centrifuge tube with a cap, add 0.5 mL of protease solution, 3.0 mL of Tris buffer, and 200 uL of methanol in sequence, vortex to mix, then dissolve with ultrasound, and keep it in an incubator at a constant temperature of 50 °C to enzymatic hydrolysis for 16 hours.

Take out the centrifuge tube, cool to room temperature, add 12 mL of methanol, transfer and dilute the sample to the mark with water in a 50 mL volumetric flask, and mix well;

filter through a 0.22 um organic phase microporous filter membrane, and use the filtrate for liquid chromatography measurement.

5.2 Liquid chromatography reference conditions

rho -- the tryptophan content in the sample solution obtained from the standard curve, in micrograms per milliliter (ug/mL);

rho0 -- the tryptophan content in the blank solution obtained from the standard curve, in micrograms per milliliter (ug/mL);

V -- the metered volume of the sample, in milliliters (mL);

100 -- conversion factor;

m -- the mass of the sample, in grams (g);

1000 -- conversion factor.

The calculation result is rounded to 3 significant figures.

7 Precision The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 10% of the arithmetic mean.

8 Others When the sample amount is 0.2 g, the detection limit of the method is 1.5 mg/100 g, and the quantitation limit is 5.0 mg/100 g.

Method B - Alkaline hydrolysis-liquid chromatography

9 Principle The sample is hydrolyzed under alkaline conditions, and the hydrolysate is adjusted to neutrality with hydrochloric acid, separated with a reversed-phase liquid chromatography column, detected with a fluorescence detector, and quantified with the internal standard method.

10 Reagents and materials Unless otherwise specified, all reagents are analytical grade and the water is first-grade water specified in GB/T 6682.

10.1 Reagents

10.1.1 Methanol (CH3OH): chromatographically pure.

10.1.2 Acetic acid (C2H4O2): chromatographically pure.

10.1.3 Ammonium acetate (C2H7NO2): chromatographically pure.

10.1.4 Lithium hydroxide (LiOH - H2O).

10.1.5 Hydrochloric acid (HCl): 12 mol/L.

10.2 Reagent preparation

10.2.1 Hydrochloric acid solution (3 mol/L): Measure 100 mL of hydrochloric acid, pour into 300 mL of water, and mix well.

10.2.2 Lithium hydroxide solution (4 mol/L): Weigh 167.8 g of lithium hydroxide, add an appropriate amount of water to dissolve, and make the volume up to 1000 mL with water.

10.2.3 Ammonium acetate solution (10 mmol/L, pH=4.0): Weigh 0.77 g of ammonium acetate, add 900 mL of water to dissolve, and adjust the pH of the solution to 4.0+/-0.1 with acetic acid; dilute to 1000 mL with water, shake well, and filter with a 0.22 um aqueous phase microporous filter membrane for later use.

10.3 Standards