NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National food safety standard - Determination of niacin and niacinamide in foods

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1 Scope

This Standard specifies the methods for the determination of niacin and niacinamide in foods.

In this Standard, Method 1 is applicable to the determination of niacin and niacinamide in prepared milk powder, special dietary foods (excluding partially hydrolyzed milk protein formulas, deeply hydrolyzed milk protein formulas or amino acid formulas, and infant formulas for special medical purposes for amino acid metabolism disorders) and special-purpose beverages. Method 2 is applicable to the determination of niacin (or niacinamide) in foods.

Method I - High Performance Liquid Chromatography

2 Principle

After pre-treatment, such as. enzymatic hydrolysis and protein precipitation, the sample is extracted through ultrasonic oscillation in a weakly acidic environment, separated by C18 chromatographic column and detected by a UV detector. In accordance with the retention time of the chromatographic peak, conduct qualitative determination, and adopt the external standard method for quantitative determination. Calculate the content of niacin and niacinamide in the specimen.

3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 water specified in GB/T 6682.

3.2.1 Hydrochloric acid solution (5.0 mol/L). measure-take 415 mL of hydrochloric acid and

add water to reach a constant volume of 1,000 mL.

3.2.2 Hydrochloric acid solution (0.1 mol/L). draw-take 8.3 mL of hydrochloric acid and add

water to reach a constant volume of 1,000 mL.

3.3.1 Niacin (C6H5NO2, CAS. 59-67-6). purity ? 98%, or a standard substance certified by the

state and awarded a reference material certificate.

3.4.2 Niacin and niacinamide standard mixed intermediate solution (100.0 ?g/mL). respectively

and accurately draw-take 10.0 mL of niacin and niacinamide standard stock solution into a 100 mL volumetric flask, add water to reach a constant volume to the scale, and evenly mix it. It can be stored for 1 month when refrigerated at 2 ?C ~ 8 ?C.

3.4.3 Niacin and niacinamide standard mixed working solutions. respectively and accurately

draw-take 1.0 mL, 2.0 mL, 5.0 mL, 10.0 mL and 20.0 mL of the standard mixed intermediate solution into 100 mL volumetric flasks, add water to reach a constant volume to the scale and evenly mix them. Thus, standard mixed working solutions with a mass concentration of 1.0 ?g/mL, 2.0 ?g/mL, 5.0 ?g/mL, 10.0 ?g/mL and 20.0 ?g/mL are obtained. Prepare them right before use.

5.1.1 Specimen preparation

Sample pre-treatment. take at least 200 g of representative sample. For lumpy or granular samples, use a pulverizer to pulverize them; for powdery, pasty or liquid samples, thoroughly mix them and place them in a closed container.

5.3 Drawing of Standard Curve

In accordance with the chromatographic conditions that have been established, successively determine the niacin and niacinamide mixed standard series of determination solutions based on the above recommended chromatographic conditions (see Figure A.1 in Appendix A for the standard sample chromatogram). Record the chromatographic peak area of each component, take the mass concentration of the standard determination solutions as the x-coordinate, and the peak area as the y-coordinate to draw a standard curve.

5.4 Determination of Specimen Solution

Inject the specimen determination solution for determination in accordance with the above recommended chromatographic conditions.

6.1 Calculation of Niacin or Niacinamide Content in the Specimen

The content of niacin or niacinamide in the specimen is calculated in accordance with Formula (1).

6.2 Calculation of Total Niacin and Niacinamide Content in the Specimen

The total content (calculated by niacin) of niacin and niacinamide in the specimen is calculated in accordance with Formula (2).

7 Precision

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

8 Others

For solid samples. when the sampling size is 5 g, the detection limit of niacin is 30 ?g/100 g, and the quantitation limit is 100 ?g/100 g; the detection limit of niacinamide is 40 ?g/100 g, and the quantitation limit is 120 ?g/100 g.

9 Principle

Niacin and niacinamide are essential nutrients for the growth of Lactiplantibacillus

plantarum.

In the niacin determination culture medium, the growth of Lactiplantibacillus plantarum is correlated with the niacin (or niacinamide) content.

10 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 or Grade-2 water specified in GB/T 6682.

10.1 Strain

Lactiplantibacillus plantarum (the former Lactobacillus plantarum) ATCC 8014, or equivalent strain.

10.4.1 Ethanol solution (with a volume fraction of 25%). measure-take 250 mL of absolute

ethanol, add water to reach a constant volume of 1,000 mL.

10.4.2 Sulfuric acid solution A (10 mol/L). measure-take 560 mL of sulfuric acid, add it to water

and dilute it to 1,000 mL.

10.4.3 Sulfuric acid solution B (0.5 mol/L). measure-take 50 mL of sulfuric acid A (10 mol/L), add it to water and dilute it to 1,000 mL.

10.4.4 Sodium hydroxide solution A (10 mol/L). weigh-take 400 g of sodium hydroxide, add

water to dissolve it and dilute to 1,000 mL.

10.5.1 Niacin (C6H5NO2, CAS. 59-67-6). purity ? 98%, or a standard substance certified by the

state and awarded a reference material certificate.

10.5.2 Niacinamide (C6H6N2O, CAS. 98-92-0). purity ? 98%, or a standard substance certified

by the state and awarded a reference material certificate.

10.6.1 Niacin (or niacinamide) standard stock solution (50 ?g/mL). place niacin (or