GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National Food Safety Standard - Food Nutritional Fortification Substance - Nicotinamide

食品安全国家标准 食品营养强化剂 烟酰胺

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Table of Contents

National Food Safety Standard - Food Nutritional Fortification Substance - Nicotinamide

1 Scope

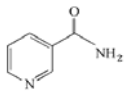
This Standard is applicable to the food nutritional fortification substance of nicotinamide obtained and produced through corresponding chemical synthetic process, and take methyl nicotinate (or ethyl nicotinate, or 3-methylpyridine, or 3-cyanopyridine, or 5-pentanediamine, 2-methyl-1) as the raw material.

2 Chemical Name, Structural Formula, Molecular Formula and Relative Molecular Mass

2.1 Chemical name

3-pyridine-carboxamide

2.2 Structural formula



2.3 Molecular formula

$C_6H_6N_2O$

2.4 Relative molecular mass

122.13 (according to 2018 international relative atomic mass)

3 Technical Requirements

3.1 Sensory requirements

The sensory requirements shall comply with the provisions of Table 1.

Appendix A

Inspection Methods

A.1 General rules

When other requirements are not specified, the reagents and water that are used in this Standard refer to analytically pure reagents and the Class-III water specified in GB/T 6682. When other requirements are not specified, the standard solutions, preparations, and products that are used in the test shall be prepared according to the provisions of GB/T 601, GB/T 602, GB/T 603. When the solutions that are used in the test are not specified by which solvent to prepare, they all indicate the aqueous solution.

A.2 Identification test

A.2.1 Color reaction

A.2.1.1 Reagents and materials

A.2.1.1.1 Sodium hydroxide solution: Take 4.3g of sodium hydroxide; add 100mL of water; stir to dissolve and mix evenly.

A.2.1.1.2 Phenolphthalein indicator solution.

A.2.1.1.3 Sulfuric acid solution: Take 57mL of sulfuric acid; slowly add it to water; and dilute to 1000mL by water and mix evenly.

A.2.1.1.4 Copper sulfate solution: Take 12.5g of copper sulfate pentahydrate; add 100mL of water; and stir to dissolve and mix evenly.

A.2.1.2 Analysis procedures

Take 0.1g of specimen (accurate to 0.01 g); add 5mL of water to dissolve; add 5mL of sodium hydroxide solution; and slowly heat it to generate ammonia gas and make the wet red litmus paper turn blue (the difference from niacin). Continue heating until the ammonia odor is completely removed; let it cool; add 1 ~ 2 drops of phenolphthalein indicator solution; neutralize by sulfuric acid solution; and add 2mL of copper sulfate solution; a light blue precipitate shall slowly precipitate.

A.2.2 Infrared spectrum test

Use the potassium bromide pellet technique to test in accordance with GB/T 6040. The infrared spectrogram of the specimen shall be consistent with that of the nicotinamide standard substance (see Figure B.1)

detector.

A.3.2.2 Potentiometric titrator.

A.3.2.3 Electronic balance: the accuracy is 0.0001 g

A.3.2.4 pH meter: the accuracy is 0.01.

A.3.3 Analysis procedures

A.3.3.1 Perchloric acid titration method

A.3.3.1.1 Indicator titration method

A.3.3.1.1.1 Method summary

Use crystal violet as an indicator; titrate the specimen with perchloric acid standard solution; and calculate the nicotinamide content based on the amount of the consumed perchloric acid standard titrant.

A.3.3.1.1.2 Analysis procedures

Take 0.1g of specimen (accurate to 0.0001g); add 30mL of glacial acetic acid to dissolve it (if necessary, warm it up to dissolve it completely). Add 1 drop ~ 2 drops of crystal violet indicator solution; titrate with perchloric acid standard titration solution until the solution turns blue-green and does not fade within 30s, which is the end point of the titration. Do a blank test at the same time.

A.3.3.1.2 Potentiometric titration method

A.3.3.1.2.1 Method summary

Take calomel electrode as reference electrode, non-aqueous acid-base titration glass electrode as indicator electrode; and titrate the specimen with perchloric acid standard solution. According to the potential "jump", determine the titration end point. According to the amount of consumed perchloric acid standard titrant, calculate the nicotinamide content.

A.3.3.1.2.2 Analysis procedures

Take 0.1g of specimen (accurate to 0.0001 g); add 30mL of glacial acetic acid to dissolve it (if necessary, slightly warm it to dissolve it completely; if glacial acetic acid cannot flood the electrode, add glacial acetic acid appropriately). Use a potentiometric titrator to titrate by a perchloric acid standard titration; and do a blank test at the same time.

A.3.3.2 Liquid chromatography

A_s - the peak area of nicotinamide in the standard substance solution;

m – the mass of the specimen, in g;

w – mass fraction of the specimen drying loss, in %;

100 – coefficient, the results shall be converted into %;

1000 – conversion coefficient, convert mg into g.

The test results are based on the arithmetic mean of the parallel determination results. The absolute difference between two independent determination results obtained under repeatability conditions shall be no more than 0.5% of the arithmetic mean.

A.4 Determination of absorption coefficient $E_{1cm}^{1\%}$ (261nm)

A.4.1 Method principle

The purity of the specimen shall be expressed by measuring the absorption coefficient of the specimen solution at a specific wavelength.

A.4.2 Reagents and materials

Hydrochloric acid solution: 0.1 mol/L.

A.4.3 Apparatus

A.4.3.1 1 cm quartz cuvette.

A.4.3.2 UV spectrophotometer.

A.4.3.3 Electronic balance with an accuracy of 0.0001 g.

A.4.4 Analysis procedures

Accurately take 0.15g of specimen (accurate to 0.0001g); use the hydrochloric acid solution to make constant volume of 100 mL; and mix evenly. Then take another 1.00mL into a 100mL volumetric flask; add hydrochloric acid solution to make the constant volume to the mark; and mix evenly. That is the specimen solution. Pour the specimen solution into a 1cm quartz cuvette; use the hydrochloric acid solution as the reference solution; use a spectrophotometer to measure absorption coefficient ($E_{1cm}^{1\%}$) at a wavelength of 261nm.

A.4.5 Calculation of results

The absorption coefficient $E_{1cm}^{1\%}$ is calculated according to Formula (A.3):

A.8.1 Method summary

Dissolve the specimen in a sulfuric acid solution; and compare it with the control solution; its color shall not be darker.

A.8.2 Reagents and materials

A.8.2.1 Sulfuric acid.

A.8.2.2 Ammonia test solution: Take 40 mL of concentrated ammonia water; dilute to 100 mL with water; and mix evenly.

A.8.2.3 Hydrochloric acid: analytically pure.

A.8.2.4 Colorimetric cobalt chloride solution: Weigh 32.5g of cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) (accurate to 0.0001g); add an appropriate amount of hydrochloric acid solution (1 → 40) to dissolve it into 500mL. Then take 2.00mL; place it in a conical flask; add 200mL of water to mix evenly; add ammonia solution until the solution turns from light red to green. Add 10mL of acetic acid-sodium acetate buffer solution (pH 6.0); and heat to 60 °C. Add 5 drops of xylenol orange indicator solution; and titrate with edetate disodium titration solution (0.05 mol/L) until it appears yellow. Each 1 mL of edetate disodium titration solution (0.05 mol/L) is equivalent to 11.90mg of cobalt chloride hexahydrate. According to the above measurement results, add an appropriate amount of hydrochloric acid solution (1 → 40) to the remaining original solution; so that each 1 mL of the solution contains 59.5mg of cobalt chloride hexahydrate; then the solution is obtained.

A.8.2.5 Colorimetric potassium dichromate solution: Take 0.4g of reference potassium dichromate (accurate to 0.0001g) dried at 120 °C to constant weight; place it in a 500mL volumetric flask; and add appropriate amount of water to dissolve and dilute to the mark; shake well; then the solution is obtained. Each 1 mL of the solution contains 0.800mg of potassium dichromate ($K_2Cr_2O_7$).

A.8.2.6 Colorimetric copper sulfate solution: Take 32.5g of copper sulfate pentahydrate (accurate to 0.001 g); add an appropriate amount of hydrochloric acid solution (1 → 40) to dissolve it into 500mL. Then take 10.00 mL; and place it into the iodine flask; add 50mL of water, 4 mL of acetic acid (glacial acetic acid) and 2g of potassium iodide. Then titrate with sodium thiosulfate titrant (0.1 mol/L). When it is near the end point, add 2mL of starch indicator solution and continue titrate until the blue color disappears. Each 1 mL of sodium thiosulfate titrant (0.1 mol/L) is equivalent to 24.97mg of $CuSO_4 \cdot 5H_2O$. According to the above measurement results, add an appropriate amount of hydrochloric acid solution (1 → 40) to the remaining original solution; so that each 1 mL of the solution contains 62.4 mg of $CuSO_4 \cdot 5H_2O$; and the solution is obtained.

A.8.2.7 Control solution: Take 1.0 mL of colorimetric cobalt chloride solution, 2.5 mL of colorimetric potassium dichromate solution, and 1.0 mL of colorimetric copper sulfate

40mg); and take it as specimen solution A. Take 0.5mL of specimen solution A; add anhydrous ethanol and make constant volume to 100 mL, which is the control solution (I). Take 10 mL of control solution (I); dilute with ethanol and make constant volume to 20 mL, which is control solution (II).

A.9.3.1.2 Take 0.1g of niacin (accurate to 0.001g); add anhydrous ethanol to dissolve and make constant volume to 50mL; mix evenly; then obtain the niacin intermediate stock solution. Pipette 2.5mL of the niacin intermediate stock solution; dilute with anhydrous ethanol and make constant volume to 25 mL; mix evenly (each 1mL of the solution contains approximately 0.2mg of niacin); then obtain the reference solution.

A.9.3.1.3 Respectively, take 10mL of niacin intermediate stock solution (A.9.3.1.2), 2.5mL of specimen solution A; mix evenly; dilute with anhydrous ethanol and make constant volume to 100 mL; then obtain the control solution (III).

A.9.3.2 Analysis procedures

Respectively pipette 5μL of specimen solution A, control solution (I), control solution (II), control solution (III), and reference solution in A.9.3.1; and respectively place them on the same silica gel GF_{254} thin-layer board above; take chloroform-anhydrous ethanol-water (48:45:4) as developing agent; unfold; dry; and inspect under ultraviolet lamp (254 nm).

A.9.3.3 Judgment of results

The control solution (III) shall display two clearly separated spots; the control solution (II) shall display a clearly visible spot. If the specimen solution A displays impurity spots corresponding to the reference solution, its color shall be no darker than that of the main spots of the reference solution. If the specimen solution A displays other impurity spots, compared with the main spots of the control solution (I), when the color of impurity spots displayed by the specimen solution A is no darker than that of the main spots of control solution (I), it shall pass the test.

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