national standards of People's Republic of China

GB ××××—201×

National food safety standards

Food nutrition enhancer niacinamide

(draft for comments)

201×-××-×× released 201×-××-×× implementation

National food safety standards

Food nutrition enhancer niacinamide

1 Scope

This standard applies to methyl nicotinate (or ethyl nicotinate, or 3-methylpyridine, or 3-cyanopyridine, or 2-methyl-1,5-pentanediamine) Raw material, the food nutrition enhancer nicotinamide obtained through the corresponding chemical synthesis process.

2 Chemical name, structural formula, molecular formula, relative molecular mass

2.1 Chemical Name

3-pyridinecarboxamide

2.2 Structure

2.3 Molecular formula

$C_6H_6N_2O$

2.4 Relative molecular mass

122.13 (according to 2016 international relative atomic mass)

3 Technical requirements

3.1 Sensory requirements

Sensory requirements should be in accordance with Table 1.

Table 1 Sensory requirements

project	Claim	Testing method
Color	white	Place an appropriate amount of sample on a clean, dry white porcelain dish or transparent bea In the natural light, observe its color and state, smell its smell
status	Crystalline granules or powder	
odor	Odorless or almost odorless	

3.2 Physical and chemical indicators

Physical and chemical indicators should meet the requirements of Table 2.

Table 2 Physical and chemical indicators

project		index	Testing method
Nicotinamide content (on dry basis), w/%	≥	99.0	Appendix A, A.3
Absorption coefficient, $A^{1\%}_{1cm}$ (261nm)		417~443	Appendix A, A.4
Absorbance ratio (245nm/261nm)		0.63-0.67	Appendix A, A.5
Melting point / °C		128~131	GB/T 617
pH value (100g/L)		5.5~7.5	GB/T 9724
Dry reduction, w/ %	≤	0.5	Appendix A, A.6
Burning residue, w /%	≤	0.1	GB/T 9741
Heavy metal (in Pb), (mg/kg)	≤	20	GB5009.74

Clarity and color of the solution	Pass the test	Appendix A, A.7
Easy char	Pass the test	Appendix A, A.8
relative substance	Pass the test	Appendix A, A.9

[a] 1.0~2.0g sample, burning temperature is 700 ~ 800 °C.

Appendix A

Testing method

A.1 General provisions

The reagents and water used in this standard refer to the analytical reagents and the tertiary water specified in GB/T 6682 when no other requireme
Used in the test
Standard solutions, standard solutions for determination of impurities, preparations and products are not included in other requirements, according to G
Preparation of the provisions. The solution used in the test refers to an aqueous solution when it is not indicated which solvent is used.

A.2 Identification test

A.2.1 Color reaction

A.2.1.1 Reagents and materials

A.2.1.1.1 Sodium hydroxide solution: Weigh 4.3g of sodium hydroxide, add 100mL of water, stir, dissolve and mix.

A.2.1.1.2 Phenolphthalein indicator solution.

A.2.1.1.3 Sulfuric acid solution: Measure 57 mL of sulfuric acid, slowly add to water, dilute to 1000 mL with water, and mix.

A.2.1.1.4 Copper sulfate solution: Weigh 12.5g of copper sulfate pentahydrate, add 100mL of water, stir, dissolve and mix.

A.2.1.2 Analysis steps

Weigh 0.lg sample (accurate to 0.01 g), add 5mL water to dissolve, add 5mL sodium hydroxide solution, slowly heat, should produce ammonia an
The wet red litmus paper turns blue (the difference from niacin).
Continue to heat until the ammonia odor is completely removed, let cool, add 1 or 2 drops of phenolphthalein indicator solution, use
Neutralize with sulfuric acid solution, add 2mL copper sulfate solution, and slowly precipitate a light blue precipitate.

A.2.2 Infrared spectroscopy

Using potassium bromide tableting method, according to GB/T 6040 test, the infrared absorption spectrum of the sample should be consistent with

See Appendix B).

A.2.3 UV absorption

A.2.3.1 Instruments and equipment

UV spectrophotometer.

A.2.3.2 Preparation of sample solution

Weigh the sample 0.1 g (accurate to 0.01 g), dissolve it in distilled water and dilute to 100 mL, shake well, pipette the solution 2.00 mL, and use di
Dilute with water and dilute to 100 mL, shake well, and use as a sample solution.

A.2.3.3 Analysis steps

The sample solution (A.2.3.2) was injected into a 1 cm quartz cuvette, and the absorbance at a wavelength of 261 nm and 245 nm was measured w
There should be maximum absorption at 261 nm and minimal absorption at 245 nm.

A.3 Determination of nicotinamide content (on a dry basis)

A.3.1 Reagents and materials

A.3.1.1 Perchloric acid ($HClO_4$): excellent grade pure.

A.3.1.2 Perchloric acid standard titration solution: $c(HClO_4) = 0.1$ mol / L.

A.3.1.3 Water: Primary water specified in GB/T 6682.

A.3.1.4 Crystal violet indicator solution: 5 g / L.

A.3.1.5 Glacial acetic acid.

A.3.1.6 Methanol (CH_3OH): chromatographically pure.

A.3.1.7 Isopropanol (C_3H_8O): chromatographically pure.

A.3.1.8 Sodium heptane sulfonate ($C_7H_{15}NaO_3S$): chromatographically pure.

A.3.1.9 Nicotinamide ($C_6H_6N_2O$) standard: a standard with a mass fraction greater than 99.0%, or a nationally certified and certified reference material
quality.

A.3.2 Instruments and equipment

A.3.2.1 High performance liquid chromatograph with UV detector.

A.3.2.2 Potentiometric titrator.

A.3.2.3 Electronic balance with an accuracy of 0.0001 g.

A.3.2.4 pH meter: The accuracy is 0.01.

A.3.3 Analysis steps

A.3.3.1 Perchloric acid titration

A.3.3.1.1 Method summary

Using crystal violet as an indicator, the sample was titrated with a perchloric acid standard solution, and the nicotinamide content was calculated ac

A.3.3.1.2 Potentiometric titration

Weigh 0.1 g of sample (accurate to 0.0001 g), add 30 mL of glacial acetic acid to dissolve (if necessary, slightly warm to dissolve completely).
Use electricity
The titrator is titrated with a perchloric acid standard titration solution while performing a blank test.

A.3.3.1.3 Indicator titration

Weigh 0.1 g of sample (accurate to 0.0001 g), add 30 mL of glacial acetic acid to dissolve (if necessary, slightly warm to dissolve completely).
Join
1 drop of crystal violet indicator solution, titrated with perchloric acid standard titration solution until the solution turns blue-green, does not fade within
At the same time
Blank test.

A.3.3.2 Liquid chromatography

A.3.3.2.1 Method summary

The sample is dissolved in water, separated by a C_{18} column, and the ultraviolet detector is detected at a wavelength of 261 nm, and is characterized according to the retention time of the chromatographic peak.
The external standard method is used to quantify the nicotinamide content in the sample.

A.3.3.2.2 Reference liquid chromatography conditions

Column: C_{18} (particle size 5 μm, 250 mm × 4.6 mm) or a column with equivalent properties.

UV detector: The detection wavelength is 261 nm.

Mobile phase: 70 mL of methanol, 20 mL of isopropanol, 1 g of sodium heptane sulfonate, dissolved in 910 mL of water and mixed, and adjusted
2.1±0.1, filtered through a 0.45 μm membrane;

Flow rate: 1.0 mL/min.

Injection volume: 20 μL.

A.3.3.2.3 Preparation of sample solution

Accurately weigh 0.1 g sample (accurate to 0.0001 g), place in a 100 mL volumetric flask, dissolve with mobile phase and dilute to volume, mix;
Accurately draw 10 mL into a 100 mL volumetric flask, add the mobile phase to volume, and mix as a sample solution.

A.3.3.2.4 Preparation of standard solution

Accurately weigh 0.1g (accurate to 0.0001g) nicotinamide standard, place it in a 100mL volumetric flask, dissolve it with mobile phase and dilute Mix well; then accurately draw 10mL to 100mL volumetric flask, dilute to the mark with mobile phase, mix, as a standard solution.

A.3.3.2.5 Determination

The nicotinamide standard solution and the sample solution were separately injected into a liquid chromatograph according to the established chro Recording chromatogram Peak area.

A.3.4 Calculation of results

A.3.4.1 Perchloric acid titration

The mass fraction w_1 of the nicotinamide content (on a dry basis) in the sample is calculated according to formula (A.1):

$$w_1 = \frac{c \times (V_1 - V_2) \times M}{1000 \times m \times (1 - w)} \times 100\% \quad \text{.....(A.1)}$$

In the formula:

c - the concentration of perchloric acid standard titration solution, in moles per liter (mol / L);

V_1 - the volume of the perchloric acid standard solution consumed by titrating the sample solution, in milliliters (mL);

V_2 - the volume of the perchloric acid standard solution consumed by titrating the blank solution, in milliliters (mL);

M - the molar mass of nicotinamide in grams per mole (g/mol) [M ($C_6H_6N_2O$) = 122.13];

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

w —— mass fraction of sample drying loss, %;

1000 - volume conversion factor.

The test results are based on the arithmetic mean of the parallel determination results.

The absolute difference between two independent determinations obtained under repetitive conditions is not Greater than the arithmetic mean of 0.5%.

A.3.4.2 Liquid chromatography

The mass fraction w_2 of the nicotinamide content (on a dry basis) in the sample is calculated according to the formula (A.2):

$$= \frac{\times \times}{\times \times (\quad)} \times \quad \text{.....(A.2)}$$

In the formula:

- the peak area of nicotinamide in the standard solution;

- the concentration of nicotinamide in the standard solution, in milligrams per milliliter (mg/mL);

V - constant volume, in milliliters (mL);

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

w —— the mass fraction of the sample drying reduction, the unit is %;

100 - the coefficient, the result is converted to %;

1000 - Conversion factor, which converts milligrams to grams.

The experimental results are based on the arithmetic mean of the parallel determination results.

The absolute difference between two independent determinations obtained under repetitive conditions is not Greater than 0.5% of the arithmetic mean.

A.4 Determination of absorption coefficient

A.4.1 Principle of the method

The purity of the sample is indicated by measuring the absorption coefficient of the sample solution at a specific wavelength.

A.4.2 Reagents and materials

Hydrochloric acid solution: 0.1 mol/L.

A.4.3 Instruments and equipment

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 steps

Accurately weigh 0.15 g (accurate to 0.0001 g), dilute to 100 mL with hydrochloric acid solution, mix; then measure 1.00 mL at 100 mL
In the volumetric flask, add hydrochloric acid solution to the scale and mix, which is the sample solution.
Inject the sample solution into a 1 cm quartz cuvette and dissolve it with hydrochloric acid.
The liquid is the reference solution, and the absorption coefficient is measured by a spectrophotometer at a wavelength of 261 nm ($E^{1\%}_{1cm}$).

A.4.5 Calculation of results

Absorption coefficient $E^{1\%}_{1cm}$ is calculated according to formula (A.3) :

$$E^{1\%}_{1cm} = \frac{A}{m} \times 100 \quad \text{.....(A.3)}$$

In the formula:

A - absorbance;

m - the mass of the sample in grams (g).

The test results are based on the arithmetic mean of the parallel determination results.
The absolute difference between two independent determinations obtained under repetitive conditions is not
It is greater than 2% of the arithmetic mean.

A.5 absorbance ratio

A.5.1 Instruments and equipment

UV spectrophotometer.

A.5.2 Analysis steps

The sample solution (A.2.3.2) was injected into a 1 cm quartz cuvette, and the absorbance at a wavelength of 245 nm and 261 nm was measured.
Note: The absorption peak wavelength should be within ±2nm of the specified wavelength, so that the peaks and troughs appear as the measuremer

A.5.3 Calculation of results

The UV absorbance ratio X *is* calculated according to formula (A.4):

$$X = \frac{A_{245}}{A_{261}} \quad \text{.....(A.4)}$$

In the formula:

A_{245} - the absorbance of the sample solution measured at 245 nm;

A_{261} - the absorbance of the sample solution measured at 261 nm;

The test results are expressed as the arithmetic mean of the parallel measurement results.
The absolute difference between two independent determinations obtained under repetitive conditions is not
Greater than 1% of the arithmetic mean.

A.6 Dry reduction

A.6.1 Instruments and equipment

A.6.1.1 Vacuum drying oven.

A.6.1.2 Electronic balance with an accuracy of 0.0001g.

A.6.1.3 Weighing bottles.

A.6.2 Analysis steps

Weigh 1 g of sample (accurate to 0.0001 g) and place it in a weighing bottle that has been dried to constant weight under the same conditions as th
Placed in a phosphorus pentoxide dryer, the bottle cap is obliquely supported at the edge of the bottle; then the dryer is placed in a vacuum drying oven
Take out the air in the vacuum drying oven (the pressure is kept below 2.67 kPa), close the piston on the vacuum pump, stop pumping, and make the va
The temperature was maintained at room temperature and the pressure was kept below 2.67 kPa.
After drying for 18h, open the piston and let the air slowly pass through the drying device into the vacuum drying oven.
Inside, wait until the pressure returns to normal and then turn it on again. Remove the dryer and weigh the weighing bottle in the dryer.

A.6.3 Calculation of results

The mass fraction $w_{3\ of\ the}$ drying reduction is calculated according to formula (A.5):

$$w_3 = \frac{m_1 - m_2}{m_1} \times 100\% \quad \text{(A.5)}$$

In the formula:

m_1 - the mass of the sample before drying, in grams (g).

m_2 - the mass of the sample after drying in grams (g).

A.7 Clarity and color

A.7.1 Instruments and equipment

The electronic balance has an accuracy of 0.01 g.

A.7.2 Analysis steps

Take 1 g of sample (accurate to 0.01 g), add 10 mL of water to dissolve, and the solution should be clear and colorless.

A.8 easy to char

A.8.1 Method summary

The sample was dissolved in a sulfuric acid solution and compared to the control solution, the color should not be deeper.

A.8.2 Reagents and materials

A.8.2.1 Sulfuric acid.

A.8.2.2 Ammonia test solution: Measure 40 mL of concentrated ammonia water, dilute to 100 mL with water, and mix.

A.8.2.3 Hydrochloric acid: Analytically pure.

A.8.2.4 Color Cobalt Chloride Solution: Weigh 32.5g of cobalt chloride hexahydrate (accurate to 0.0001 g), add appropriate amount of hydrochloric aci

500mL, weigh 2.00mL, set in a conical flask, add 200mL water to mix, add ammonia solution until the solution changes from light red to green, add ac

- 10 mL of sodium acetate buffer (pH 6.0), heated to 60 ° C, add 5 drops of xylenol orange indicator solution, titrate solution with disodium edetate

(0.05 mol/L) titrated to yellow.

Each lmL of ethylenediaminetetraacetic acid disodium titration solution (0.05mol / L) is equivalent to 11.90mg of cobalt chloride

($CoCl_2 \cdot 6H_2O$). According to the above measurement results, add an appropriate amount of hydrochloric acid solution (1→40) to the remaining original solution to make it contain 1 mL of solution.

59.5 mg of $CoCl \cdot 6H_2O$, that is.

A.8.2.5 Color comparison potassium dichromate solution: Weigh 0.4g of reference potassium dichromate (accurate to 0.0001 g) dried to constant weigh

In the volumetric flask, add appropriate amount of water to dissolve and dilute to the mark, shake well, that is. 0.800 mg of potassium dichromate ($K_2Cr_2O_7$) was contained per 1 mL of the solution.

A.8.2.6 Color solution copper sulfate solution: Weigh 32.5g of copper sulfate pentahydrate (accurate to 0.001 g), add appropriate amount of hydrochlor

500mL, precision measurement 10.00mL, placed in the iodine volumetric flask, add 50mL water, 4mL acetic acid (glacial acetic acid) and 2g potassium

The titration solution (0.1 mol/L) was titrated. To the near end point, 2 mL of the starch indicator solution was added, and the titration was continued ur

Titrate per lmL of sodium thiosulfate

The liquid (0.1 mol/L) corresponds to 24.97 mg of $CuSO4 \cdot 5H_2O$. According to the above measurement results, add an appropriate amount of hydrochloric acid solution to the remaining original solution

(1→40), so that 62.4 mg of $CuSO_4 \cdot 5H_2O$ per 1 mL of solution is obtained.

A.8.2.7 Control solution: Take 1.0mL colorimetric cobalt chloride solution, 2.5mL colorimetric potassium dichromate solution, 1.0mL colorimetric cop

Dilute to 50 mL with water.

Note: Commercialized colorimetric fluids can be used.

A.8.3 Analysis steps

Take two tube with the same inner diameter: add 5mL of control solution to the tube; add 5mL of sulfuric acid to the tube, and slowly add 0.2g to t

Indeed to 0.01g), shake to dissolve.

After standing for 15 minutes, the two tubes of A and B were placed in front of a white background and observed horizontally.

A.8.4 Result determination

The color displayed in the B tube shall not be deeper than the tube.

A.9 related substances

A.9.1 Reagents and materials

A.9.1.1 Anhydrous ethanol, analytically pure.

A.9.1.2 Niacin: A standard substance with a mass fraction greater than 99.0%, or a nationally certified and certified reference material.

A.9.1.3 Developer: chloroform-anhydrous ethanol-water (48:45:4).

A.9.2 Instruments and equipment

A.9.2.1 UV lamp (wavelength 254nm).

A.9.2.2 Electronic balance: accuracy is 0.0001 g.

A.9.2.3 Microinjector: 5 μL.

A.9.2.4 Expand the slot.

A.9.2.5 Hair dryer.

A.9.2.6 Thin layer board, GF_{254} .

A.9.3 Analysis steps

A.9.3.1 Sample preparation

A.9.3.1.1 Weigh 1.0 g sample (accurate to 0.001 g), dissolve it with absolute ethanol and dilute to 25 mL (about 40 mg solution per 1 mL).

As sample solution A; 0.5 mL of sample solution A was weighed, diluted with absolute ethanol and made up to 100 mL, which was the control solution

Measure 10 mL

Control solution (I), diluted with ethanol and made up to 20 mL, is the control solution (II).

A.9.3.1.2 Weigh 0.1g niacin (accurate to 0.001 g), dissolve it with absolute ethanol and dilute to 50 mL, mix well, ie dissolve in the middle of niacin

Liquid, draw 2.5 mL of niacin intermediate stock solution, dilute with absolute ethanol and dilute to 25 mL, mix (about 0.2 mg solution per l mL),

That is, the reference solution;

A.9.3.1.3 separately measure 10mL niacin intermediate stock solution (A.9.3.1.2), 2.5 mL sample solution A, mix, dilute with absolute ethanol and set

Capacitance to 100 mL is the control solution (III).

A.9.3.2 Analysis steps

Aspirate sample solution A, control solution (I), control solution (II), control solution (III), and reference solution in A.9.3.1, respectively.

5 μL of each solution was spotted on the same silica gel GF_{254} thin layer plate, and developed with chloroform-anhydrous ethanol-water (48:45:4) as a

developing solvent.

Dry and place it under UV light (254 nm).

A.9.3.3 Result determination

Control solution (III) should show two clearly separated spots; control solution (II) should show a clearly visible spot; sample solution A

If the impurity spots corresponding to the reference solution are not darker than the main spot of the reference solution; such as other impurity spots, an

The main spot of the solution (I) should not be deeper.

Appendix B

Infrared absorption spectrum of nicotinamide standard

Figure B.1 Infrared absorption of nicotinamide standards