national standards of People's Republic of China

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National food safety standards

Food nutrition enhancer cyanocobalamin

(draft for comments)

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

National Health and Wellness Committee of the People's Republic

release

State Market Supervisory Administration

National food safety standards

Food nutrition enhancer cyanocobalamin

1 Scope

This standard is applicable to the food nutrition enhancer cyanocobalamin which is obtained by fermentation and crystallization of a fermentation l

2 Molecular formula, structural formula and relative molecular mass

2.1 Molecular formula

Cyanocobalamin: $C_{63}H_{88}CoN_{14}O_{14}P$

2.2 Structure

2.3 Relative molecular mass

Cyanocobalamin: 1355.38 (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

item Head	Want begging	Testing method
Color	dark red	Take an appropriate amount of sample in a clean, dry white porcelain dish, in nature
status	Crystalline particles or powder	Under light, observe its color and state.

3.2 Physical and chemical indicators

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

Table 2 Physical and chemical indicators

Project	Means	Standard	Testing method
Cyanocobalamin content (on dry basis), *w* /%		96.0~102.0	Appendix A, A.4
Dry reduction, *w* /%	≤	12	Appendix A, A.5
Related substances, *w* /%	≤	2.0	Appendix A, A.6
Acetone/mg/kg	≤	5000	Appendix A, A.7
Lead (Pb) /mg/kg	≤	1.0	GB 5009.12 First Act
Total arsenic (as As) /mg/kg	≤	1.0	GB 5009.11 The first second law
Total aerobic bacteria, *w* / (cfu / g)	≤	1000	Appendix A, A.8
The total number of molds and yeasts, *w* / (cfu / g)	≤	100	Appendix A, A.9

Note: Commercialized cyanocobalamin (vitamin B_{12}) products should be based on cyanocobalamin in accordance with this standard and can be added for processing, storage, standardization.

Food materials and food additives such as maltodextrin, sodium citrate, citric acid, mannitol, and calcium hydrogen phosphate for dissolution purp

Appendix A

Testing method

A.1 Safety Tips (or Alerts)

Some of the reagents used in the test methods of this standard are toxic or corrosive, and appropriate safety and protective measures should be taken

A.2 General provisions

Unless otherwise specified in this standard, the purity of the reagents used shall be analytically pure, the standard titration solution used, the standa
And products, should be prepared in accordance with the provisions of GB/T 601, GB/T 602, GB/T 603, the experimental water should meet the requir
The solution used in the test refers to an aqueous solution when it is not indicated which solvent is used.

A.3 Identification test

A.3.1 Reagents and materials

A.3.1.1 Acetone.
A.3.1.2 chloroform.
A.3.1.3 Ether.
A.3.1.4 Hydrochloric acid.
A.3.1.5 sodium acetate.
A.3.1.6 Potassium bromide.
A.3.1.7 Sodium fluoride.
A.3.1.8 Potassium hydrogen sulfate.

A.3.1.9 hypophosphorous acid.

A.3.1.10 Phenolphthalein indicator.

A.3.1.11 acetic acid (1 mol/L).

A.3.1.12 Sodium hydroxide solution (100 g / L): Weigh 10 g of sodium hydroxide, add water and dissolve to a volume of 100 mL.

A.3.1.13 Sodium hydroxide solution (20 g / L): Weigh 1 g of sodium hydroxide, dissolve with water and dilute to 50 mL.

A.3.1.14 1-Nitroso-2-naphthol-3,6-disulfonic acid sodium solution (2 g/L): Weigh 0.1 g of 1-nitroso-2-naphthol-3,6 - sodium disulfonate, slowly added Dissolve in water and bring up to 50 mL.

A.3.1.15 Sulfuric acid solution (1+7): Take 70 mL of water, slowly inject 10 mL of sulfuric acid, and mix.

A.3.1.16 Ammonium sulphate saturated solution: Weigh 42 g of ammonium ferrous sulfate hexahydrate, dissolve with water and dilute to 100 mL.

A.3.2 Instruments and equipment

A.3.2.1 坩埚.

A.3.2.2 Distillation unit.

A.3.2.3 UV-visible spectrophotometer.

A.3.3 Identification method

A.3.3.1 Solubility

Slightly soluble in water and ethanol, insoluble in acetone, chloroform or ether.

A.3.3.2 UV identification

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Accurately weigh 30 mg sample in 1000 mL volumetric flask, dilute to volume with water, measure with UV-visible spectrophotometer, absorb The largest peaks should appear on the spectrum at 278 ± 1 nm, 361 ± 1 nm, and 550 ± 2 nm. Ratio of absorbance at 361 nm to absorbance at 278 nm The value should be 1.70 to 1.90. The ratio of the absorbance at 361 nm to the absorbance at 550 nm should be 3.15 to 3.40.

A.3.3.3 Identification of cobalt atoms

Take about 1 mg of sample, add about 50 mg of potassium hydrogen sulfate, place in a crucible, burn to melt, let cool, mash with a glass rod, add 3 Boiling to dissolve, add 1 drop of phenolphthalein indicator, mix well, add sodium hydroxide solution (A.3.1.12) to reddish color, add 500 mg sodium a Acetic acid (A.3.1.11) and 0.5 mL of 1-nitroso-2-naphthol-3,6-disulfonic acid sodium solution (A.3.1.14), immediately appear red or orange-red, add sa Acid 0.5mL, boil for 1 minute, the color should not disappear.

A.3.3.4 Cyanide ion identification

Pipette about 5 mg of cyanocobalamin in a 50 mL distillation flask containing 5 mL of water. The distillation flask was connected to a short, water The coagulation device, the outlet end of the condensing device was immersed in a test tube containing 1 mL of sodium hydroxide solution (A.3.1.13). After the sample is dissolved in the distillation flask. Add 2.5 mL of hypophosphorous acid, connect the condensing device, slowly heat and boil for 10 min, and collect 1 mL of distillate in the test tube. Add 4 to the test tube Dilute cold, ammonium ferrous sulfate saturated solution (A.3.1.16), gently shake, add 30 mg of sodium fluoride, heat to boiling, immediately add a few (A.3.1.15) until the solution becomes clear, add 3~5 drops of sulfuric acid solution, and the solution turns blue to blue-green within a few minutes.

A.3.3.5 Infrared identification

Comparing the spectrum of the potassium bromide tablet sample with the cyanocobalamin standard spectrum (see B.1 in Appendix B), the two sho

A.4 Cyanocobalamin content

A.4.1 Instruments and equipment

Spectrophotometer.

A.4.2 Analysis steps

Accurately weigh approximately 0.025 g (accurate to 0.1 mg) of the dried sample in a 1000 mL volumetric flask, dissolve in water and bring up to A sample solution was obtained. Determined by an ultraviolet-visible spectrophotometer. Using water as a blank, use a 1 cm cuvette to measure the sample solution at 361 nm. Photometric value.

A.4.3 Calculation of results

The mass fraction $w_{1\ \text{of the}}$ cyanocobalamin content (on a dry basis) is calculated according to the formula (A.1).

$$w_1 = \frac{A}{207} \times \frac{V}{m} \times 100\% \quad \text{.....(A.1)}$$

In the formula:

A - the ultraviolet absorbance of cyanocobalamin;

207 - standard percent absorption coefficient of cyanocobalamin ($E^{1\%}_{1cm}$);

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

m - the amount of sample (on a dry basis) in grams (g);

The experimental results are based on the arithmetic mean of the parallel determination results, and one decimal place is reserved.

The absolute difference between the two independent determinations obtained under repeatability conditions is no more than 2.0% of the arithmeti

A.5 Dry reduction

A.5.1 Principle of the method

Dry reduction refers to the ratio of the mass of the lost water and other volatile substances to the mass of the sample after drying at 105 ° C. The score is expressed.

A.5.2 Instruments and equipment

A.5.2.1 Balance: The sensitivity is 0.0001 g.

A.5.2.2 Weighing bottle.

A.5.2.3 Vacuum drying oven.

A.5.3 Analysis steps

Weigh 50 mg (accurate to 0.0001 g) and place in a weighed bottle of dry constant weight (105 ° C, 30 min).

Place the sample in the weighing bottle.

Spread evenly in the middle. Dry to constant weight at 105 ° C and vacuum pressure below 2.67 KPa.

Place the weighing bottle in a desiccator and cool to room temperature.

the amount.

A.5.4 Calculation of results

The mass fraction w_2 of the sample drying reduction is calculated according to the formula (A.2):

$$w_2 = \frac{m_1 - m_2}{m_1 - m_0} \times 100\% \quad \text{.....(A.2)}$$

In the formula:

m_1 - the mass of the weighing bottle with the sample before drying, in grams (g);

m_2 - the mass of the weighing bottle with the sample after drying, in grams (g);

m_0 ——The mass of the weighing bottle after drying, in grams (g).

The calculation result retains 2 significant digits.

The arithmetic mean of the parallel determination results is the measurement result, and the absolute difference between the two parallel determina

A.6 related substances

A.6.1 Principle of the method

After the sample was dissolved in the mobile phase, it was detected by liquid chromatography, and the peak area comparison method was used for

A.6.2 Reagents and materials

A.6.2.1 Chloramine T.

A.6.2.2 Methanol.

A.6.2.3 Disodium hydrogen phosphate.

A.6.2.4 Phosphoric acid.

A.6.2.5 Hydrochloric acid.

A.6.2.6 Disodium hydrogen phosphate solution: 0.028 mol/L.

Weigh 10.03 g of disodium hydrogen phosphate dodecahydrate, dissolve with water and dilute to 1000 mL.

A.6.2.7 Hydrochloric acid solution: 0.05 mol/L. Take 4.5 mL of hydrochloric acid, add water to 1000 mL, and mix.

A.6.2.8 Chloramine T solution: 0.1%. Measure 0.1 g of chloramine T, dissolve in water and dilute to 100 mL.

A.6.3 Instruments and equipment

High performance liquid chromatography

A.6.4 Analysis steps

A.6.4.1 Sample solution: Weigh 10 mg of the sample into a 10 mL volumetric flask, dissolve it with mobile phase and dilute to volume, and mix.

A.6.4.2 Control solution: Accurately measure 1 mL of sample solution in a 100 mL volumetric flask, dilute to the mark with mobile phase, and mix.

A.6.4.3 System suitability solution: Weigh sample 25 mg in 25 mL volumetric flask, add 10 mL water to dissolve the sample, add 5 mL 0.1% chloramii

The solution, 0.5 mL of 0.05 mol/L hydrochloric acid solution, was diluted with water to the mark, mixed, and allowed to stand for 5 minutes.

Accurately measure 1 mL of the above solution in 10 mL

in the measuring flask, dilute to the mark with the mobile phase and mix.

A.6.4.4 Sensitivity solution: Accurately measure 1 mL of the control solution in a 10 mL volumetric flask, dilute to the mark with the mobile phase, and

A.6.5 Reference chromatographic conditions

Column: C_{18} column (column length 250 mm, internal diameter 4.6 mm, particle size 5 μm), or other equivalent column.

Mobile phase: Mix 260 mL of methanol and 740 mL of disodium hydrogen phosphate solution (A.6.2.6) and adjust the pH to 3.5 with phosphoric

Column temperature: 25 ° C.

Detector wavelength: 361 nm.

Injection volume: 10 μ L.

Run time: 3 times the retention time of cyanocobalamin main peak

A.6.6 System suitability test

Use freshly prepared solutions and avoid strong light exposure.

According to the operating procedures of the liquid chromatograph, take the system suitability solution (A.6.4.3) and

The sensitivity solution (A.6.4.4) was separately injected into the liquid chromatograph to observe the chromatogram.

The cyanocobalamin peak and a degradation product peak (relative retention time of about 1.4) should appear in the system suitability solution, an

2.5, the signal to noise ratio of the main peak in the sensitivity solution should be greater than 3.

A.6.7 Determination

After the system suitability is passed, the sample is tested.

The sample solution (A.6.4.1) and the control solution (A.6.4.2) were sequentially injected for measurement.

A.6.8 Calculation of results

The content of the relevant substance in the sample w_3 (%) is calculated according to the formula (A.3):

$$w_3 = \frac{A_1}{A_2} \times 100\% \quad \text{.....(A.3)}$$

Where: A_1 - the sum of all impurity peak areas of the sample solution except the main peak.

A_2 - main peak area of the control solution.

A.6.9 Precision

The absolute difference between the two independent determinations obtained under repeatability conditions does not exceed 10% of its arithmeti

A.7 Acetone

A.7.1 Reagents and materials

Acetone: chromatographically pure.

A.7.2 Instruments and equipment

Gas Chromatograph: equipped with a hydrogen flame ionization detector (FID) and a headspace sampler.

A.7.3 Reference chromatographic conditions

A.7.3.1 Column: Capillary column (column length 60 m, inner diameter 0.32 mm, film thickness 1.6 μm, fixing solution 6% cyanopropylphenyl-94% d

Polysiloxane), or other equivalent column.

A.7.3.2 Carrier gas: nitrogen (purity greater than 99.99%).

A.7.3.3 Carrier gas flow rate: 1.5 mL/min.

A.7.3.4 Column temperature: 40 ° C after 10 °C/min, to 120 °C/min was raised to 240 ° C for 2 min.

A.7.3.5 Inlet temperature: 140 °C.

A.7.3.6 Detector temperature: 250 °C.

A.7.3.7 Detector gas flow: air: 400 mL/min; hydrogen: 60 mL/min; makeup gas: 25 mL/min.

A.7.3.8 Injection volume: 1.0 mL.

A.7.3.9 Split ratio: 1:50.

A.7.4 Reference headspace conditions

A.7.4.1 Headspace bottle equilibrium temperature: 80 °C.

A.7.4.2 Equilibrium time: 45 min.

A.7.4.3 Quantitative ring temperature: 90 °C.

A.7.4.4 Transmission line temperature: 100 °C.

A.7.5 Analysis steps

A.7.5.1 Preparation of blank solution

Pipette 5.0 mL of water and place in a headspace bottle to quickly compress the cap.

A.7.5.2 Standard solution preparation

Weigh 0.1 g of acetone (accurate to 0.001 g) in a 100 mL volumetric flask with 80 mL of water pre-added, dilute to volume with water, shake well

A standard stock solution of acetone in mg/L.

Draw an appropriate amount of standard stock solution of acetone in a 10 mL volumetric flask and prepare 10 mg/L and 20 mg/L with water.

50 mg / L, 100 mg / L, 200 mg / L of acetone standard use solution, take 5.0 mL of each of the above series of solution solutions, respectively, placed ir

Quickly compress the cap.

A.7.5.3 Preparation of sample solution

Weigh 0.1 g (accurate to 0.001 g) of the sample in a 10 mL volumetric flask, dissolve in water, dilute to volume, and shake.
Pipette the solution 5.0 mL
Place in a headspace bottle and quickly compress the cap for later use.

A.7.5.4 Determination

Under the reference conditions (A.7.3 and A.7.4), the blank solution, the standard series solution and the sample solution were respectively measu
Peak area value. See Figure C.1 in Appendix C for the standard spectrum.
The peak area of acetone in the standard series solution chromatogram is the Y-axis to the corresponding solvent
The concentration (mg/L) is plotted on the X-axis, and a standard curve is drawn to obtain an acetone standard curve.
According to the peak area value of acetone in the chromatogram of the sample solution, from the standard
The curve determines the concentration of acetone (mg/L) in the sample solution.

A.7.6 Calculation of results

The content of acetone in the sample w_4 (mg/kg) is calculated according to formula (A.4):

$$w_4 = \frac{c \times V}{m} \times \frac{1000}{1000} \quad \text{.....(A.4)}$$

In the formula:

c —— The concentration of acetone in the sample solution obtained from the standard curve in milligrams per liter (mg/L).

V——The volume of the sample solution in milliliters (mL).

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

A.7.7 Precision

The absolute difference between the two independent determinations obtained under repeatability conditions does not exceed 10% of its arithmeti

A.8 Total number of aerobic bacteria

A.8.1 Equipment and materials

In addition to routine sterilization and culture equipment in microbiology laboratories, other equipment and materials are as follows:

A.8.1.1 Constant temperature incubator: 30 ~ 35 ° C.

A.8.1.2 Constant temperature water bath: 45 ±1 ℃.

A.8.1.3 Balance: The sensitivity is 0.1 g, 0.01 g.

A.8.1.4 Homogenizer or oscillator.

A.8.1.5 Sterile pipette: 1 mL (with 0.01 mL scale), 10 mL (with 0.1 mL scale) or micropipette and tip.

A.8.1.6 Sterile culture dish: 90 mm in diameter.

A.8.1.7 pH meter or pH colorimetric tube or precision pH test paper.

A.8.1.8 Magnifier or / and colony counter.

A.8.2 Media and reagents

A.8.2.1 Pancreatic soy meal agar medium: 15.0 g of pancreatic casein, 5.0 g of soybean papain hydrolysate, 5.0 g of sodium chloride, agar 15.0 g, distilled water 1000 mL.
In addition to agar, the above ingredients were taken, mixed, dissolved at a slight temperature, and the pH was adjusted so that the pH at 25 ° C after ste
Add agar, heat to dissolve, shake well, dispense, autoclave at 121 ° C for 15 min.

A.8.2.2 pH7.0 sterile sodium chloride-peptone buffer: take 3.56 g of potassium dihydrogen phosphate, 5.77 g of anhydrous sodium phosphate, 4.30 g o
Protein 胨 1.00 g, add distilled water 1000 mL, dissolve at a slight temperature, filter, dispense, autoclave at 121 °C for 15 min.

A.8.2.3 pH7.2 phosphate buffer: stock solution: Weigh 34.0 g of potassium dihydrogen phosphate dissolved in 500 mL of distilled water, using approxi
The pH of the mol/L sodium hydroxide solution was adjusted to 7.2, diluted to 1000 mL with distilled water, and stored in a refrigerator.
Diluent: Take 1.25 mL of stock solution and steam
The distillate was diluted to 1000 mL, dispensed in a suitable container, and autoclaved at 121 °C for 15 min.

A.8.3 Operation steps

A.8.3.1 Preparation and dilution of samples

Generally, no less than 2 samples of the smallest package should be randomly selected. Under aseptic operation, mix and take 10 g for inspection.
With pH 7.0
Dilute the sodium chloride-peptone buffer or pH 7.2 phosphate buffer to make a 1:10 sample homogenate.
Adjust the pH of the diluent to 6~ if necessary
8. The sample was aliquoted further 10 times serially with the same dilution based on the estimated contamination level of the sample.
Take 1 mL of sample homogenate and place in a sterile plate with a diameter of 90 mm. Make two plates for each dilution.
At the same time, draw 1 mL separately
Blank dilutions were added to two sterile dishes as blank controls. Timely pouring 15-20 mL of pancreatic soy agar agar culture cooled to 45 °C
Base (can be placed at 45 ± 1 ℃ constant temperature water bath insulation), mix, to be solidified.

A.8.3.2 Cultivation and counting

The pancreatic soybean meal agar medium plate was inverted and cultured at 30 to 35 ° C for 3 to 5 days to observe the colony growth.
Point gauge growing on the plate
Count all colonies, count and report. Plates in which colonies spread and grow into tablets should not be counted.
Calculate the average colony of a dilution after counting the number of colonies
The number is reported according to the number of bacteria report rules:
If the average number of colonies of the two plates in the same dilution is not less than 15, the number of colonies of the two plates cannot be different.
1 time or more.

A.8.4 Results and reports

The determination of the total number of aerobic bacteria should be based on the dilution of the average number of colonies less than 300 cfu, as th
Take the highest average colony
Count the number of colonies contained in the 1 g sample and report the two significant figures.

The plate of the plates had colony growth, but when the average number of colonies is less than 1, the number of bacteria is reported by multiplying <1
If there is no colony growth, the test results are invalid.

A.9 Total number of molds and yeasts

A.9.1 Equipment and materials

In addition to routine sterilization and culture equipment in microbiology laboratories, other equipment and materials are as follows:

A.9.1.1 Constant temperature incubator: 20 ~ 25 °C.

A.9.1.2 Constant temperature water bath: 45 ±1 ℃.

A.9.1.3 Balance: The sensitivity is 0.1 g, 0.01 g.

A.9.1.4 Homogenizer or oscillator.

A.9.1.5 Sterile pipette: 1 mL (with 0.01 mL scale), 10 mL (with 0.1 mL scale) or micropipette and tip.

A.9.1.6 Sterile culture dish: 90 mm in diameter.

A.9.1.7 pH meter or pH colorimetric tube or precision pH test strip.

A.9.1.8 Magnifier or / and colony counter.

A.9.2 Media and reagents

A.9.2.1 Sabouraud dextrose agar medium: animal tissue pepsin hydrolysate and trypticase equal mixture 10.0 g, glucose 40.0 g, 15.0 g of agar and 1000 mL of distilled water.

In addition to glucose and agar, take the above ingredients, mix, dissolve at a slight temperature, adjust the pH to make the sterilized at 25 ° C.
The pH value was 5.6 ± 0.2. After adding agar, heating and melting, glucose was added, shaken, and dispensed, and autoclaved at 121 °C for 15 min.

A.9.2.2 pH7.0 sterile sodium chloride-peptone buffer: take 3.56 g of potassium dihydrogen phosphate, 5.77 g of anhydrous sodium phosphate, 4.30 g o
Protein 胨 1.00 g, add distilled water 1000 mL, dissolve at a slight temperature, filter, dispense, autoclave at 121 °C for 15 min.

A.9.2.3 pH7.2 phosphate buffer: stock solution: weigh 34.0 g of potassium dihydrogen phosphate dissolved in 500 mL of distilled water, using about 17
The pH was adjusted to 7.2 with a 1 mol/L sodium hydroxide solution, diluted to 1000 mL with distilled water, and stored in a refrigerator.
Dilute the distilled water to 1000 mL, dispense in a suitable container, and autoclave at 121 °C for 15 min.
Diluent: Take 1.25 mL of stock solution, use

A.9.3 Operation steps

A.9.3.1 Preparation and dilution of samples

Generally, no less than 2 samples of the smallest package should be randomly selected. Under aseptic operation, mix and take 10 g for inspection.
With pH 7.0
Dilute the sodium chloride-peptone buffer or pH 7.2 phosphate buffer to make a 1:10 sample homogenate.
If you need to adjust the pH of the diluent to 6 ~
8. The sample was aliquoted further 10 times serially with the same dilution based on the estimated contamination level of the sample.

Take 1 mL of sample homogenate and place in a sterile plate with a diameter of 90 mm. Make two plates for each dilution.
At the same time, draw 1 mL separately
Blank dilutions were added to two sterile dishes as blank controls. Timely pouring 15-20 mL of Sabouraud glucose agar culture cooled to 45 °C
Base (can be placed at 42 ~ ℃ constant temperature water bath insulation), mix, to be solidified.

A.9.3.2 Cultivation and counting

The Sabouraud dextrose agar medium plate was inverted at 20 to 25 ° C for 5 to 7 days to observe the colony growth.
Point gauge growing on the plate
Count all colonies, count and report. Plates in which colonies spread and grow into tablets should not be counted.
Calculate the average colony of a dilution after counting the number of colonies
The number is reported according to the number of bacteria report rules.
If the average number of colonies of the two plates in the same dilution is not less than 15, the number of colonies of the two plates cannot be different.
1 time or more.

A.9.4 Results and reports

Number of bacteria reporting rules.
The total number of molds and yeasts should be determined by selecting the dilution of the average number of colonies less than 100 cfu.
according to:
Take the highest average number of colonies, calculate the number of colonies contained in the 1g sample, and report the two significant figures.
Colony growth, or only the lowest dilution plate has colony growth, but when the average number of colonies is less than 1, the value of <1 multiplied b
If there is no plate for each dilution
The number of scams. If there is colony growth on the blank control, the test result is invalid.

Appendix B

Infrared spectrum of cyanocobalamin standard

The infrared spectrum of the cyanocobalamin standard is shown in Figure B.1.

Figure B.1 Infrared spectrum of cyanocobalamin standard

Appendix C

Acetone gas chromatogram

The reference gas chromatogram of acetone is shown in Figure C.1.

Figure C.1 Acetone gas chromatogram