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GB 20XX—XXXX

Replace GB/T 19422-2003

Feed additives Part 2: Vitamins and vitamins Cyanocobalamin (Vitamin B12)

Feed additives —Part 2: Vitamins,provitamins and chemically well-defined substances having similar effect—Cyanocobalamin cobione (Vitamin B_{12})

(Draft for comments)

20××-××-×× release 20××-××-×× implementation

State Administration for Market Regulation
National Standardization Management Committee release

before Speak

GB 7300 "Feed Additives" is divided into several parts according to products:

This document is part 205 of GB 7300.

This document was drafted in accordance with the rules given in GB/T 1.1-2020.

This document replaces GB/T 9841-2006 "Feed Additive Vitamin B_{12} (Cyanocobalamin) Powder".

Compared with GB/T 9841-2006, the main technical differences between this document and GB/T 9841-2006 are as follows:

——Increase the relevant indicators and detection methods of vitamin B_{12} crystal products.

——Powder products in this standard no longer include products with a labelled amount of 5% (see 1, 2006 1).

——The content of vitamin B12 in powder products of this standard is determined as 96% ~ 120% of the labeled amount (on a dry basis) (see 4.3, 2006 3.2).

——Revised the limit of arsenic (see 4.4, 3.2 in 2006) and added the Arbitration Law (see 6.6.2).

——Increase the index requirements for cadmium (see 4.4, 3.2 in 2006).

——Modify inspection rules, labels and signs, packaging, transportation and storage (see 5, 6, 7 2006, 5).

——Increase the identification test and particle size detection method (see 6.3 and 6.10).

This document was proposed and managed by the Ministry of Agriculture and Rural Affairs of the People's Republic of China.

This document was drafted by: Institute of Quality Standards and Testing Technology, Chinese Academy of Agricultural Sciences [National Feed Quality Supervision and Inspec Beijing)],

The main drafters of this document: Suo Decheng, Zhao Xiaoyang, Li Lan, Zhang Su, Tian Jing, Jiao Yufeng, Ma Wenge.

Feed additives Part **2** : Vitamins and vitamins

Cyanocobalamin (Vitamin **B12**)

1 Scope

This part of GB 7300 specifies the technology of feed additive vitamin B_{12} (cyanocobalamin) crystal products, liquid products and powder products

Requirements, test methods, inspection rules and labels, packaging, transportation, storage and shelf life.

This document is applicable to the fermentation broth containing cobalamin produced by microbial fermentation, and the cyanocobalamin crystal product is obtained after purificati

This document is applicable to specifications made with cyanocobalamin (vitamin B_{12}) produced by fermentation as the main material and auxiliary materials such as calcium carb

It is a 0.1% to 2% powder product. Modified here to be consistent with the modification sheet

2 Normative references

The contents of the following documents constitute indispensable clauses of this document through normative references in the text. Among them, dated reference documents,

Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable to this document

file.

GB/T 601 Preparation of standard solutions for chemical reagent titration analysis (volume analysis)

GB / T 602 Preparation of Standard Solution for Impurity Determination of Chemical Reagents

GB / T 603 Preparation of preparations and products used in chemical reagent test methods

GB/T 6435 Determination of moisture in feed

GB/T 6682 Analytical laboratory water specifications and test methods

GB/T 10648 Feed label

GB/T 13079 Determination of total arsenic in feed

GB/T 13080 Determination of lead in feed Atomic absorption spectrometry

GB/T 13082 Method for determination of cadmium in feed

GB/T 14699.1 Feed sampling

3 Chemical name, molecular formula and relative molecular weight

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Chemical name: Vitamin B_{12} (Cyanocobalamin, Cobalamin)

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

Relative molecular mass: 1355.38 (1999 international relative atomic mass)

4 Technical requirements

4.1 Appearance and traits

The crystal product is dark red, crystalline particles or powder.

This product is light red to brown fine powder with hygroscopicity.

4.2 Identification

It should meet the maximum absorption peak of ultraviolet wavelength or the retention time requirement of liquid chromatography.

4.3 Physical and chemical indicators

The technical indicators should meet the requirements of Table 1.

Table 1 Physical and chemical indicators

project	index	
	Crystals	powder
Content (calculated as vitamin B_{12} /%)	(On dry basis) 96 ~ 102	(On a dry basis, accounting for the marked amount) 96 ~ 120
relative substance,% ≤	2	-

		Use corn starch as diluent ≤	12
Loss on drying,/% ≤	12		
		Use calcium carbonate as diluent ≤	5
granularity/%	-	All pass through 0.28mm aperture standard sieve (60 mesh)	

4.4 Sanitary indicators

Table 2 Health indicators

project		index
Total arsenic/(mg/kg)	≤	2.0
Lead/(mg/kg)	≤	5.0
Cadmium/(mg/kg)	≤	2.0

5 sampling

According to the provisions of GB/T 14699.1.

6 Test method

Except for special instructions, all reagents used are analytically pure and meet the third-grade water specified in GB/T 6682, and the water used for chromatographic analysis meets

According to GB/T 6682, the preparation of reagents and solutions should meet the requirements of GB/T 601, GB/T602 and GB/T603.

6.1 Sensory inspection

Crystal and powder products: Take an appropriate amount of sample and place it in a clean and dry white porcelain dish, and observe its color under natural light.

6.2 Identification test

6.2.1 Reagents or materials

6.2.1.1 Acetonitrile: chromatographically pure.

6.2.1.2 Methanol: chromatographically pure.

6.2.1.3 Glacial acetic acid: top grade pure.

6.2.1.4 Ethanol: analytically pure.

6.2.1.5 Sodium Hexane Sulfonate: Chromatographic grade.

6.2.1.6 Vitamin B_{12} standard product: Vitamin B_{12} content ≥ 96.0%.

6.2.1.7 Vitamin B_{12} standard stock solution: accurately weigh 0.1g (accurate to 0.0001g) of vitamin B_{12} standard product (5.1.6) and place it in 100mL

Add a suitable amount of ethanol (5.1.4) to the brown volumetric flask to dissolve it, dilute it to the mark, and shake it well. The standard stock solution vitamin B12

The content is 1mg/mL. Store at -18°C, valid for one year.

6.2.1.8 Vitamin B_{12} standard working solution: accurately draw 1 mL of vitamin B_{12} standard stock solution (5.1.8) into a 100 mL brown volumetric flask,

Dilute to volume with water and shake well.

6.2.2 Instruments

6.2.2.1 Ultrasonic water bath.

6.2.2.2 Ultrapure water device.

6.2.2.3 UV spectrophotometer (or diode matrix detector).

6.2.2.4 Quartz cuvette (1cm).

6.2.2.5 High performance liquid chromatograph with UV adjustable wavelength detector (or diode matrix detector).

6.2.2.6 Balance: Sensitivity 0.001g; Sensitivity 0.0001g

6.2.3 Identification method

Note: The following operations must be carried out under dark conditions.

6.2.3.1 Take the sample solution and measure it with a spectrophotometer. Use a 1cm quartz cuvette to determine the sample solution in the wavelength range of 300nm to 600nm.

The absorption spectrum of the liquid should have a maximum absorption peak at a wavelength of 361 nm ± 1 nm and 550 nm ± 2 nm.

6.2.3.2 Weigh about 0.1g ~ 1g of the sample (accurate to 0.0001g), place it in a 100mL brown volumetric flask, add about 60mL of water,

Ultrasonic extraction in a water bath for 15 minutes, cool to room temperature, dilute to volume with water, mix, and filter, and pass the filtrate through a 0.45μm filter membrane. Press h

The manual of the gas chromatograph adjusts the operating parameters of the instrument, and injects the vitamin B 12 standard working solution and sample solution into the chromatograp

The retention time of the chromatographic peak of the quasi-solution and the sample solution should be within 5%.

6.3 Determination of vitamin B 12 content in powder products

6.3.1 Principle

After the sample is extracted or diluted with water, it is injected into the chromatographic column, separated by mobile phase elution, and the content of vitamin B 12 is calculated by

6.3.2 Reagents or materials

6.3.2.1 Acetonitrile: chromatographically pure.

6.3.2.2 Methanol: chromatographically pure.

6.3.2.3 Glacial acetic acid: top grade pure.

6.3.2.4 Ethanol: analytically pure.

6.3.2.5 Sodium Hexane Sulfonate: Chromatographic grade.

6.3.2.6 Vitamin B 12 standard product: Vitamin B 12 content ≥ 98.0%.

6.3.2.7 Mobile phase: 300mL methanol, 1.1g sodium hexanesulfonate (5.1.5) and 10mL glacial acetic acid (5.1.3) per liter of aqueous solution.

Filter, ultrasonic degassing.

6.3.2.8 Vitamin B 12 standard stock solution: accurately weigh 0.1g (accurate to 0.0001g) of vitamin B 12 standard product (5.1.6) and place it in 100mL

Add a suitable amount of ethanol (5.1.4) to the brown volumetric flask to dissolve it, dilute it to the mark, and shake it well. The standard stock solution vitamin B 12

The content is 1mg/mL. Store at -18°C, valid for one year.

6.3.2..9 Vitamin B 12 standard working solution: accurately draw 1 mL of vitamin B 12 standard stock solution (5.1.8) into a 100 mL brown volumetric flask,

Dilute to volume with water and shake well.

The concentration of vitamin B 12 standard working solution is determined and calculated according to the following method:

Using water as the blank solution, use an ultraviolet spectrophotometer to determine the absorbance of the vitamin B 12 standard working solution (5.1.9) at 361nm. dimension

The concentration c of Shengsu B 12 standard working solution is expressed in micrograms per milliliter (μg/mL), calculated according to formula (1):

$$c = \frac{A \times 10000}{207} \quad \text{..... (1)}$$

Where:

A—— The absorbance value of vitamin B 12 standard working solution measured at 361nm wavelength;

207-Vitamin B 12 standard percent absorbance coefficient ($E^{1\%}_{1cm}$=207);

10000—— Conversion coefficient of vitamin B 12 standard working solution concentration unit.

The standard working solution of vitamin B 12 with corresponding concentration can also be configured according to experimental needs .

6.3.3 Instruments

6.3.3.1 Ultrasonic water bath.

6.3.3.2 Ultrapure water device.

6.3.3.3 Ultraviolet spectrophotometer (or diode matrix detector).

6.3.3.4 Quartz cuvette (1cm).

6.3.3.5 High performance liquid chromatograph with UV adjustable wavelength detector (or diode matrix detector).

6.3.3.6 Balance: Sensitivity 0.001g; Sensitivity 0.0001g

6.3.4 experiment procedure

6.3.4.1 Preparation of test solution

Weigh an appropriate amount of sample (refer to Appendix B, accurate to 0.0001g), put it in a brown volumetric flask of the corresponding volume, add about two-thirds of water, Ultrasonic extraction in an ultrasonic water bath for 15 minutes, cool to room temperature, dilute to volume with water, mix, and filter. The filtrate is passed through a 0.45μm filter memb For analysis by high performance liquid chromatography.

6.3.4.2 Chromatographic conditions

a) Amino column

Chromatographic column: amino column, length 250mm, inner diameter 4mm, particle size 5μm (or equivalent analytical column with similar performance);

Mobile phase: Acetonitrile + 1% phosphoric acid water (25+75);

Flow rate: 1.0mL/min;

Temperature: room temperature;

Detection wavelength: 361nm.

b) C18 column

Chromatographic column: C18 type column, length 150mm, inner diameter 4.6mm, particle size 5μm (or equivalent analytical column with similar performance);

Mobile phase: methanol + sodium hexanesulfonate solution (30+70);

Flow rate: 1.0 mL/min;

Temperature: room temperature;

Detection wavelength: 361nm.

6.3.4.3 Quantitative determination

Adjust the operating parameters of the instrument according to the instructions of the high performance liquid chromatograph, and inject the vitamin B_{12} standard working solution Solution, obtain the chromatographic peak area response value, and quantify it with an external standard method.

6.3.5 Test data processing

The content of vitamin B_{12} in the sample ω_1 is expressed in mass fraction (%) and calculated according to formula (2):

$$\omega_1 = \frac{PC_X \times 100}{P_{st} m_X \times (1 - x)} \times 10^{-4} \quad \text{……(2)}$$

Where:

P—peak area of sample test solution (4.4.2.1);

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C— Concentration of vitamin B_{12} standard working solution (4.1.18), in micrograms per milliliter (μg/mL);

P_{st} —The peak area of vitamin B_{12} standard working solution (4.1.18);

m — sample mass, in grams (g);

x -Loss on drying, the unit is% (%)

Parallel determination results are expressed by the arithmetic mean, and three significant figures are retained.

The mass fraction (%) of the labeled amount of vitamin B_{12} in the sample is expressed as ω_2 and calculated according to formula (3):

$$\omega_2 = \omega_1 \times 100 \quad \text{……(3)}$$

K

Where:

K— labeled amount of vitamin B 12 in the product ;

6.3.6 Precision

The difference between the two independent measurement results obtained under repeatability conditions and its arithmetic mean is not greater than the arithmetic mean of thes

5% of the value. .

6.4 Determination of vitamin B 12 content in crystal products

6.4.1 Instruments and equipment

Spectrophotometer.

6.4.2 Analysis steps

Weigh approximately 0.025g (accurate to 0.1mg) of the sample in a 1000mL volumetric flask, dissolve it with water and dilute to the mark, and mix well

Sample solution. Measured with UV-Vis spectrophotometer. Using water as a blank, use a 1cm cuvette to measure the absorbance of the sample solution at 361nm

Degree value.

6.4.3 Result calculation

The mass fraction ω 3 of vitamin B 12 content (on a dry basis) is calculated according to formula (4).

$$\omega_3 = \frac{A}{207} \times \frac{V}{m} \times 100\% \quad \text{.....(4)}$$

Where:

A ——Cyanocobalamin UV absorption value;

207——1% colorimetric light absorption coefficient of cyanocobalamin;

V ——The constant volume of the sample, the unit is milliliters (mL);

m——sample amount (calculated on a dry basis), in grams (g);

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

6.4.4 Precision

The absolute difference between two independent determination results obtained under repeatability conditions is not more than 2.0%.

6.5 Determination of related substances

6.5.1 Reagents or materials

6.5.1.1 Chloramine T.

6.5.1 .2 Methanol: Chromatographically pure.

6.5.1 .3 Disodium hydrogen phosphate solution : 0.028 mol/L. Weigh 10.03g disodium hydrogen phosphate dodecahydrate and add 1000mL water to dissolve it.

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

6.5.1.5 0.1% chloramine T solution: Measure 0.1 g of chloramine T and dissolve it with 100 mL of water.

6.5.2 Instruments

High performance liquid chromatography

6.5.3 Analysis steps

6.5.3.1 Preparation of sample solution:

Weigh 10 mg of the sample in a 10 mL volumetric flask, dissolve it with mobile phase and dilute to the mark, and mix.

6.5.3.2 Preparation of control solution:

Accurately measure 1 mL of sample solution (6.5.3.1) in a 100 mL measuring flask, dilute to the mark with mobile phase, and mix.

6.5. 3.3 Preparation of system suitability solution:

Weigh 25 mg of the sample into a 25 mL volumetric flask, add 10 mL of water to dissolve the sample, add 5 mL of 0.1% chloramine T solution and 0.05 mol/L hydrochloric acid 0.5 mL of the solution, dilute to the mark with water, mix well, and let stand for 5 minutes. Accurately measure 1 mL of the above solution in a 10 mL measuring flask, dilute with mobile Release to the mark and mix well.

6.5.3.4 Preparation of sensitivity solution:

Accurately measure 1 mL of the control solution (6.5.3.2) in a 10 mL volumetric flask, dilute to the mark with mobile phase, and mix.

6.5.4 Reference chromatographic conditions

Chromatographic column: High performan umn with octadecy is filler, or other equivalent chromatographic column.

Mobile phase: mix 260 mL methanol and 7 osphate solution (to 3.5 with phosphoric acid.

Column temperature: 25°C.

Detector: The wavelength is 361 nm.

Injection volume: 10 [mu] L.

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System suitability test

Under the reference chromatographic conditions, inject sample solution (6.5.3.1), control solution (6.5.3.2), system suitability solution (6.5.3.3) and The sensitivity solution (6.5.3.4) is measured. Record the chromatogram to 3 times the retention time of the main component peak.

There should be a cyanocobalamin peak and a degradation product peak (the relative retention time is about 1.4) in the system suitability solution (6.5.3.3). The resolution should be greater than 2.5, and the signal-to-noise ratio of the main peak in the sensitivity solution should be greater than 3.

6.5.5 Determination

The ratio of related substances in the sample ω_4 (%) is calculated according to formula (5):

$$\omega_4 = \frac{A_1}{A_2} \times 100 \quad \text{.....(5)}$$

In the formula: A_1 -the sum of peak areas of impurities in the sample solution

A_2 —The main peak area of the sample solution

6.6 Determination of arsenic

6.6.1 Arsenic spot method (quick method)

6.6.1.1 Principle

After the sample is digested, the high-valent arsenic is reduced to trivalent arsenic with potassium iodide and stannous chloride, and then the new ecological hydrogen generated by Arsenic hydrogen, and then mercury bromide test paper to generate yellow to orange stains, compared with standard arsenic spots quantitative.

6.6.1.2 Reagents

6.6.1.2.1 Hydrochloric acid (analytical grade).

6.6.1.2.2 Arsenic-free zinc particles.

6.6.1.2.3 Nitric acid-perchloric acid mixture (4+1): Measure 80ml nitric acid, add 20ml perchloric acid, and mix well.

6.6.1.2.4 Potassium iodide solution (150g/L): stored in a brown bottle.

6.6.1.2.5 Acidic stannous chloride solution: Weigh 40g stannous chloride ($SnCl_2 \cdot 2H_2O$), add hydrochloric acid to dissolve and dilute to 100ml, shake well, Immediately.

6.6.1.2.6 Lead acetate cotton: After soaking the absorbent cotton with a lead acetate solution (100g/L), remove the excess solution and make it loose.

After drying, store in a glass bottle.

6.6.12.7 Arsenic standard solution (1.0μg/ml): draw 1.0ml of commercially available arsenic standard stock solution (100μg/ml) and place it in a 100ml volumetric flask.

Add 1ml of dilute sulfuric acid, dilute with water to the mark, shake well, and get it.

6.6.1.2.8 Mercury bromide-ethanol solution (50g/L): Weigh 25g of mercury bromide and dissolve it with a small amount of ethanol, and then dilute to 500ml.

6.6.1.2.9 Mercury bromide test paper: Cut into a circular filter paper sheet with a diameter of 2cm and immerse it in a mercury bromide ethanol solution (50g/L) for more than 1 hour.

Store in the refrigerator, take out before use and dry in the dark for later use.

6.6.1.3 Test procedure

6.6.1.3.1 Sample solution preparation

Weigh 1.00g of the sample and place it in a 150 ml Erlenmeyer flask, first add 3ml of water to moisten, add 15ml of nitric acid-perchloric acid mixture (4+1) and 4 ~ 5 glass beads, put aside for a while, put it on the electric hot plate and slowly heat it on a small fire, when the reaction is subdued, increase the heating temperature to digest to produce After birth, the sample disappears again. When the sample is almost dry, stop heating and let it cool. Add 10ml of mixed acid and continue to digest until nearly dry, repeat this 4 times, st Let it cool. Add 10ml of water, heat the acid on a hot plate until it is nearly dry, and let it cool.

6.6.1.3.2 Preparation of arsenic standard solution

Measure 2.00ml of arsenic standard solution (1μg/ml) and place it in a 150ml Erlenmeyer flask. The following operations are the same as "5.7.4.1 Sample Solution Preparation".

6.6.1.3..3 Analysis steps

Transfer the arsenic standard solution and sample solution prepared above to the arsenic measuring flask, and wash the conical flask with water 3 to 4 times (the total water consump 25ml), the lotion is respectively incorporated into the corresponding arsenic measuring bottle, each add 5ml potassium iodide solution (150g/L), 5 drops of acidic stannous chloride solutic hydrochloric acid. Add 3g zinc granules to the arsenic test bottle containing the sample solution and arsenic standard solution, and immediately stopper with lead acetate cotton and mercu The paper arsenic test tube is placed at room temperature for 1 hour, and the mercury bromide test paper of the sample is compared with the standard arsenic spot. The mercury bromide tc The color should not be deeper than the standard.

6.6.2 Arbitration Law

Accurately weigh 1.00 g of the sample (accurate to 0.001 g), in accordance with the provisions of GB/T 13079.

6.7 Determination of lead

Accurately weigh 1.00 g of the sample (accurate to 0.001 g), treat it according to 7.1.1 dry ashing method in GB/T 13080, and then measure it according to 7.3. .

6.8 Determination of cadmium

Accurately weigh 1.00 g of the sample (accurate to 0.001 g), in accordance with the provisions of GB/T 13082.

6.9 Determination of loss on drying

Accurately weigh 1.00 g of the sample (accurate to 0.001 g) and determine it in accordance with GB/T 6435 in 8.1.

6.10 Granularity

Weigh 50 g of the sample (accurate to 0.01 g) and place it in a 0.28mm aperture standard sieve for sieving. All samples should pass through the analytical sieve within 5 minutes.

7 Inspection rules

7.1 Batch

Products produced with the same material, the same production process, continuous production or the same shift shall be a batch, but each batch of products shall not exceed 40 ton

7.2 Factory inspection:

The factory inspection items are:

Crystal products: appearance and properties, vitamin B_{12} content, and related substances.

Powder products: appearance and properties, vitamin B_{12} content, loss on drying.

7.3 Type inspection

Type inspection items are all the requirements of Chapter 3. When the product is in normal production, type inspection shall be carried out at least once every six months, but there are t

In one case, type inspection should be carried out:

a) When a new product is put into production;

b) When there are major changes in raw materials, formulas, equipment, and production processes;

c) When the product has been stopped for more than three months and resumed production;

d) When there is a big difference between the factory inspection result and the last type inspection result;

e) When the feed management department proposes the type inspection request.

7.4 Judgment rules

7.4.1 Test Result seized are indicators of this standard an actuator judged to be acceptable products.

7.4.2 test results, if Yi indicators does not meet this standard, re-sampling of the items do not meet the re-examination of the original batch, if

If the re-inspection result still does not meet the requirements of this standard, the batch of products shall be deemed unqualified.

8 Labeling, packaging, transportation, storage and shelf life

8.1 Label

According to the provisions of GB 10648.

8.2 Packaging

The packaging materials should be clean, hygienic, non-toxic, non-polluting, and have the properties of preventing light, moisture, and leakage.

8.3 Transportation

The means of transportation should be clean and sanitary, protected from exposure to sunlight, rain, and moisture, and should not be mixed or transported with harmful and toxic sul

damage.

8.4 Storage

Store in the sun, ventilated, dry place, prevent insects and rodents, and should not be mixed with toxic and harmful substances.

8.5 Shelf life

The shelf life of crystal products is 36 months under the specified transportation and storage conditions for unopened products . Liquid product shelf life is 6

Months. The powder product shelf life is 36 months.

Appendix A
(Informative appendix)
Vitamin B_{12} standard chromatogram

The standard chromatogram of Vitamin B_{12} (amino column) is shown in Figure A.1; the standard chromatogram of Vitamin B_{12} (C18 column) is shown in Figure A.2.

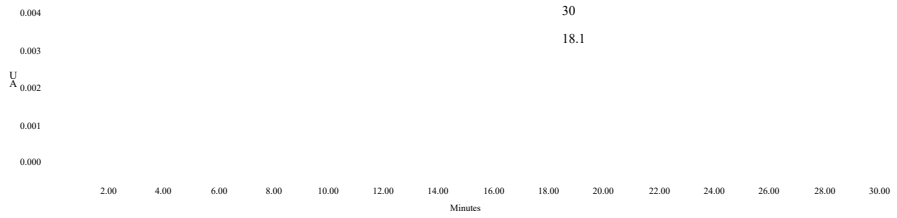

Figure A.1 10μg/mL Vitamin B 12 standard solution chromatogram (amino column)

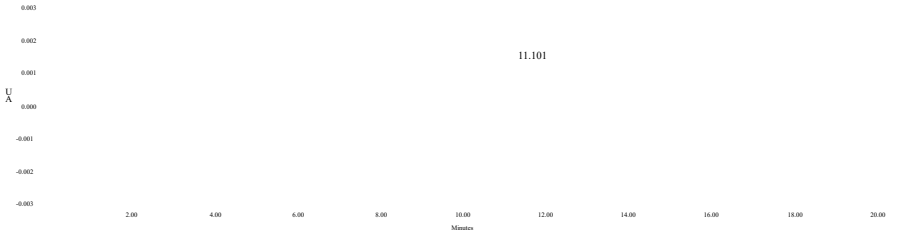

Figure A.2 Chromatogram of 10μg/mL vitamin B 12 standard solution (C18 column)

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Figure A.3 Chromatogram of related substances in vitamin B 12 standard solution

Appendix B

(Normative appendix)

Reference example of the labeled amount of vitamin B 12 in the product , the sample amount and the dilution volume of the extract

Table B.1 Examples of labeled volume, weighed sample volume, and dilution volume of extract

Sample type	Labeling quantity/%	Weighing sample quantity/g (mL)	Extraction solution dilution volume/mL	Extraction solution vitamin B_concentr
Powder products	0.1	1.00	100	10
	0.5	0.20	100	10
	1.0	0.10	100	10
	2.0	0.10	200	10

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