national standards of People's Republic of China

GB ××××—××××

National food safety standards

Food nutrition enhancer cholecalciferol (vitamin D 3)

(draft for comments)

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

National food safety standards

Food nutrition enhancer cholecalciferol (vitamin D_3)

1 Scope

This standard is applicable to the process of chemically synthesizing 7-dehydrocholesterol by lanolin cholesterol as raw material, followed by ultra
The obtained food nutrition enhancer cholecalciferol (vitamin D_3) crystals.

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

2.1 Chemical Name

(5Z,7E)-9,10-open-ring cholester-5,7,10(19)-triene-3β-ol

2.2 Structure

2.3 Molecular formula

$C_{27}H_{44}O$

2.4 Relative molecular mass

384.64 (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	Colorless or white		Place an appropriate amount of sample in a clean, dry white porcelain dish and observe it under natura
odor	Odorless		Color and state, smelling its smell
status	Needle crystal or crystalline powder		

3.2 Physical and chemical indicators

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

Table 2 Physical and chemical indicators

Project		Indicator	Testing method
Vitamin D_3 content, w /%		97.0~103.0	Appendix A, A.4
Specific optical rotation, $[\alpha]_D^{20}$ / (°·m²·kg⁻¹)		+105.0～+112.0	Appendix A, A.5
Absorption coefficient, $E_{1cm}^{1\%}$		465-495	Appendix A, A.6
Related substances: Precursor vitamin D_3, trans vitamin D_3, previtamin D_3, Sterol D_3, 7-dehydrocholesterol		Except for the previtamin D_3 peak, the single impurity peak area is not More than 0.5% of the main peak area of the control solution; The sum of the mass peak areas shall not be greater than the main peak surface of the control solution 1.0% of the product	Appendix A, A.7
Lead (Pb) / (mg / kg)	≤	2.0	GB5009.75

Total arsenic (as As) / (mg/kg)	≤	2.0	GB5009.76

Note: Commercialized cholecalciferol (vitamin D_3) products should be based on cholecalciferol (vitamin D_3) crystals in accordance with this standard.
It is made from edible vegetable oil, starch, dextrin, sucrose and other auxiliary materials required by relevant food quality specifications.

Appendix A

Testing method

A.1 General provisions

The reagents and water used in this standard refer to the analytical pure reagent or the above specifications and the tertiary water specified in GB/T
The standard solution used in the test, the standard solution for the determination of impurities, preparations and products shall be in accordance with G
Prepared in accordance with the provisions of GB/T 603.
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 Coloration reaction of acetic anhydride concentrated sulfuric acid

A.2.1.1 Reagents and materials

A.2.1.1.1 Trichloromethane.

A.2.1.1.2 Acetic anhydride.

A.2.1.1.3 Sulfuric acid.

A.2.1.2 Identification method

Weigh 0.5 mg of the sample, add 5 mL of chloroform to dissolve, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, shake, and initially y
Red, quickly turns purple, blue-green, and finally turns green.

A.2.2 Infrared spectroscopy

A.2.2.1 Reagents and materials

Potassium bromide.

A.2.2.2 Instruments and equipment

infrared spectrometer.

A.2.2.3 Analysis steps

The potassium bromide tableting method was carried out in accordance with GB/T 6040, and the infrared spectrum of the sample should be consis

Vitamin D

The standard infrared spectrum is shown in Figure B.1 in Appendix B.

A.3 Determination of vitamin D ₃ content

A.3.1 Reagents and materials

A.3.1.1 n-hexane.

A.3.1.2 n-pentanol.

A.3.1.3 isooctane.

A.3.1.4 Vitamin D ₃ standard: mass fraction not less than 98%.

A.3.1.5 Water: Level 1 water in accordance with GB/T 6682.

A.3.2 Instruments and equipment

High performance liquid chromatography: equipped with a UV detector. Or other equivalent detector.

A.3.3 Chromatographic reference conditions

Recommended columns and typical chromatographic operating conditions are shown in Table A.1. Other columns and chromatographic condition

Table A.1 Columns and Typical Chromatographic Operating Conditions

Column	Column length 250 mm, column inner diameter 4.6 mm, silica gel column
Mobile phase	N-hexane: n-pentanol = 997:3

Flow rate	2 mL/min
Injection volume	100 μL
Detector detection wavelength	254 nm

A.3.4 Analysis steps

A.3.4.1 Preparation of standard solution

Weigh the vitamin D ₃ standard 25 mg (accurate to 0.0001 g), place a 100 mL brown volumetric flask, add isooctane 80 mL, avoid heating,

Sonicate for 1 min to completely dissolve, dilute to 100 mL with isooctane, shake well, and use as stock solution I.

Measure the above solution 5.0 mL, set

In a 50 mL brown volumetric flask, dilute to the mark with isooctane and shake well to serve as stock solution II.

A.3.4.2 System suitability test

Take 15.0 mL of the stock solution, place it in a stoppered glass bottle, seal it with nitrogen, and heat it in a water bath at 90 °C for 1 h. Take it out

5.0 mL of hexane, shake well, set in a 1 cm quartz cell with absorption buffer, in 2 UV lamps with 8W main wavelengths of 254 nm and 365 nm respec

Next, the quartz absorption cell is placed obliquely at 45°, and 5 to 6 cm from the tube, and irradiated for 5 minutes to make the solution contain pre-vit

, and trans-vitamin

D ₃ , vitamin D ₃ and fast sterol D ₃ . The solution was weighed into a liquid chromatograph, and the chromatographic conditions were referred to Table

A.1. Inject 5 times and record the peak area

Calculate the relative standard deviation of vitamin D ₃ peak area is not more than 2.0%; pre-vitamin D ₃ peak and trans vitamin D ₃ peak and vitamin

D ₃ peak and speed

The resolution of the sterol D ₃ peak should be greater than 1.0. Pre-vitamin D ₃ , trans-vitamin D ₃ , fast-acting D ₃ and vitamin D ₃ relative retention

time

Don't be about 0.5, 0.6, 1.1. Analyzed under the chromatographic conditions of A.4.3, the chromatogram is shown in Figure C.1.

A.3.4.3 Preparation of sample solution

Weigh 25 mg of vitamin D ₃ (accurate to 0.0001 g), place a 100 mL brown volumetric flask, add 80 mL of isooctane to avoid heating.

Sonicate for 1 min to dissolve completely, dilute to the mark with isooctane, and shake well.

Precisely measure 5.0 mL of the above solution and set a brown volume of 50 mL

In a bottle, dilute to the mark with isooctane, shake well, and use this solution as a sample solution.

A.3.4.4 Determination

Analyze under A.4.3 reference chromatographic conditions.

Under standard A.3.3 reference chromatographic conditions, standard solution II (A.3.4.1) and sample solution (A.3.4.3) were chromatographed.

Recording standard

The peak areas of vitamin D ₃ in the chromatogram of the solution and the sample solution , and the corresponding values are denoted as A_1 and A_2, respectively

Calculate the dimension in the sample according to formula (A.1)

The content of the biotin D ₃ .

A.3.5 Calculation of results

The mass fraction w of the vitamin D ₃ content in the sample , the value is expressed in %, calculated according to formula (A.1):

$$w = \frac{m_1 A_2}{A_1 m_2} \times 100\% \quad \text{.....(A.1)}$$

In the formula:

m_1 - the amount of vitamin D ₃ injected in the standard solution, in micrograms (μg);

m_2 - the amount of vitamin D ₃ injected in the sample solution, in micrograms (μg);

A_1 - the peak area value of vitamin D ₃ in the chromatogram of the standard solution;

A_2 - the peak area value of vitamin D ₃ in the chromatogram of the sample solution;

The calculation of the results is based on the arithmetic mean of the results of the two parallel measurements.

Absolute determination of two independent measurements obtained under the same experimental conditions

The difference is no more than 2% of the arithmetic mean.

A.4 Determination of specific optical rotation

A.4.1 Reagents and materials

Anhydrous ethanol.

A.4.2 Instruments and equipment

Polarimeter.

A.4.3 Determination

Weigh 0.5 g of sample (accurate to 0.0001 g). Add 100 ml of absolute ethanol and make up to 100 mL to make 5.0 mg per 1.0 mL.
A solution of the sample. Others are carried out in accordance with the method specified in GB/T 613.

Note: The measurement is completed within 30 minutes after the solution is prepared.

A.4.4 Calculation of results

Specific optical rotation value $\alpha_m(20,℃D)$, the value is expressed as (°)•dm ^{2} •kg $^{-1}$ and is calculated according to formula (A.2):

$$\alpha_m(20,℃D) = \frac{\alpha}{l \times \rho_\alpha} \quad \text{.....(A.2)}$$

In the formula:

α - the measured optical rotation in degrees (°);

l - measure the length of the tube in decimeters (dm);

Pα - the mass concentration of vitamin D $_{3}$ in the solution, in grams per milliliter (g/mL).

The specific optical rotation value of the sample is based on the arithmetic mean of the results of two parallel determinations.
Two independent tests obtained under the same experimental conditions
The absolute difference of the results is not more than 3% of the arithmetic mean.

A.5 Determination of absorption coefficient

A.5.1 Reagents and materials

Anhydrous ethanol.

A.5.2 Instruments and equipment

UV-visible spectrophotometer.

A.5.3 Analysis steps

Weigh 0.01 g (accurate to 0.0001 g) of the sample, dissolve it in absolute ethanol, dilute to 100 mL, and shake well as a stock solution. Take again
Dilute to 10 mL of 10 mL stock solution to make a solution containing approximately 10 μg of sample per 1.0 mL.
Take this sample solution in a 1 cm cuvette in
Absolute ethanol was used as a blank control, and the absorbance was measured by a spectrophotometer at a wavelength of 265 nm.

A.5.4 Calculation of results

Absorption coefficient $E_{1cm}^{1\%}(265\,Nm)$, calculated according to formula (A.3):

$$E_{1cm}^{1\%}(265\,Nm) = \frac{A}{c} \quad \text{.....(A.3)}$$

In the formula:

A - the absorbance value of the sample solution;

c ——The mass fraction of the sample solution, in %.

The absorbance value of the sample solution is based on the arithmetic mean of the results of two parallel determinations.
Two independent determinations obtained under the same experimental conditions
The absolute difference of the results is not more than 2% of the arithmetic mean.

A.6 related substances

A.6.1 Reagents and materials

Same as A.3.1.

A.6.2 Instruments and equipment

Same as A.3.2.

A.6.3 Chromatographic reference conditions

Same as A.3.3.

A.6.4 Analysis steps

A.6.4.1 Preparation of standard solution

Same as A.3.4.1.

A.6.4.2 System suitability test

Same as A.3.4.2.

A.6.4.3 Preparation of sample solution

Same as A.3.4.3.

A.6.4.4 Determination

Under standard A.3.3 reference chromatographic conditions, standard solution II (A.3.4.1) and sample solution (A.3.4.3) were chromatographed. Recording standard

The peak area of the chromatogram of the solution and the sample solution, and the high performance liquid chromatogram is shown in Figure C.1.

A.6.4.5 Results

If there is an impurity peak in the chromatogram of the sample solution, the area of the single impurity peak shall not be larger than the main peak area of the standard solution except for the peak of the former vitamin D_3 0.5%, the sum of the peak areas of each impurity shall not be greater than 1.0% of the main peak area of the control solution.

Appendix B

Standard infrared spectrum of vitamin D_3

The standard infrared spectrum of vitamin D_3 is shown in Figure B.1.

Figure B.1 Standard infrared spectrum of vitamin D_3

Appendix C

Standard chromatogram of vitamin D_3 and related substances

The standard chromatogram of vitamin D_3 and related substances is shown in Figure C.1.

Figure C.1 Standard chromatogram of vitamin D_3 and related substances

Table C.1 Names of components corresponding to each peak

Peak sequence	Component name
1	Previtamin D_3

2	Trans vitamin D_3
3	Vitamin D_3
4	Fast sterol D_3

8