GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National food safety standard - Food nutritional fortification substance - Cholecalciferol (Vitamin D_3)

食品安全国家标准

食品营养强化剂 胆钙化醇（维生素 D_3）

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1 Scope

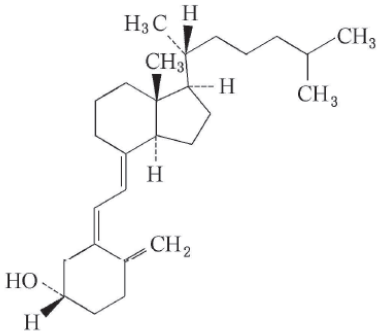
This Standard is applicable to food nutritional fortification substance - cholecalciferol (Vitamin D_3), which uses lanolin cholesterol as raw material, by chemical synthesis, to obtain 7-dehydrocholesterol, then is made through UV irradiation, refining and other processes.

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

2.1 Chemical name

(5Z,7E)-9,10-opening cholesteryl-5,7,10(19)-triene-3β-alcohol

2.2 Structural formula



2.3 Molecular formula

$C_{27}H_{44}O$

2.4 Relative molecular mass

384.64 (according to 2018 international relative atomic mass)

Annex A

Inspection methods

A.1 General provisions

All reagents and water used in this Standard, when other requirements are not specified, refer to analytically-pure reagents or of above specifications and grade 3 water specified in GB/T 6682. Standard solutions used in the test, standard solutions for impurity determination, preparations and products, when other requirements are not specified, are prepared in accordance with GB/T 601, GB/T 602 and GB/T 603. Solution used in the test, when the type of solvent is not specified, refers to aqueous solution.

A.2 Identification test

A.2.1 Color 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 0.5mg of sample. Add 5mL of trichloromethane to dissolve. Add 0.3mL of acetic anhydride and 0.1mL of sulfuric acid. Shake. Initially it is yellow. It gradually becomes red. Then it immediately changes to purple, blue-green. It finally turns green.

A.2.2 Infrared spectrum test

A.2.2.1 Reagents and materials

Potassium bromide.

A.2.2.2 Instruments and equipment

Infrared spectrometer.

A.2.2.3 Analysis steps

Use potassium bromide tablet method. Carry out the test according to GB/T 6040. The infrared spectrum of the sample shall be consistent with the standard

A.3.5 Analysis steps

A.3.5.1 Preparation of standard solution

Weigh 25mg of vitamin D_3 standard product (to the nearest of 0.0001g). Place in a 100mL brown volumetric flask. Add 80mL of isooctane. Avoid heating. Perform ultrasonic treatment for 1min to make it completely dissolved. Use isooctane to dilute to 100mL. Shake well. Use it as stock solution. Measure 5.0mL of the above solution. Place in a 50mL brown volumetric flask. Use isooctane to dilute to the scale. Shake well. Use it as standard solution.

A.3.5.2 System suitability test

Measure 5.0mL of vitamin D_3 stock solution (A.3.5.1) in a stoppered glass bottle. Access to nitrogen then stopper it. Place in 90°C water bath to heat 1h. Take it out to immediately cool. Add 5.0mL of n-ethane. Shake well. Place in a 1cm plugged quartz absorption cell. Under two 8W UV lamps with dominant wavelengths of 254nm and 365nm respectively. Place the quartz absorption cell at an angle of 45°, 5cm ~ 6cm away from the light tube. Irradiate for 5min. Make the solution contain vitamin D_3, pre-vitamin D_3, trans-vitamin D_3 and tachysterol D_3. Measure the solution into the liquid chromatograph. Refer to A.3.4 for chromatographic conditions. Conduct 5 sample injections. Record the peak area. Calculate the relative standard deviation of vitamin D_3 peak area not more than 2.0%. The resolution of the pre-vitamin D_3 peak and trans-vitamin D_3 peak, as well as the vitamin D_3 peak and tachysterol D_3 peak shall be greater than 1.0. The relative retention time of pre-vitamin D_3, trans-vitamin D_3, tachysterol D_3 and vitamin D_3 are about 0.5, 0.6, 1.1, respectively. Analyze under A.3.4 chromatographic conditions. The reference chromatogram is shown in Figure C.1.

Weigh 25mg of 7-dehydrocholesterol standard product. Prepare 7-dehydrocholesterol standard solution according to the preparation method of standard solution (A.3.5.1). Respectively measure 2mL of Vitamin D_3 standard solution (A.3.5.1) and 7-dehydrocholesterol standard solution to prepare mixed standard solution. Measure the solution into the liquid chromatograph. Refer to A.3.4 for chromatographic conditions. Conduct 5 sample injections. Record the peak area. Calculate the relative standard deviation of vitamin D_3 peak area not more than 2.0%. The resolution of the vitamin D_3 peak and the 7-dehydrocholesterol peak shall be greater than 1.0. The relative retention time of 7-dehydrocholesterol and vitamin D_3 is about 1.5. Analyze under A.3.4 chromatographic conditions. See Figure C.2 for reference chromatogram.

A.3.5.3 Preparation of sample solution

Weigh 25mg of vitamin D_3 sample (to the nearest of 0.0001g). Place in a 100mL brown volumetric flask. Add 80mL of isooctane. Avoid heating. Perform

A.5.4 Result calculation

The absorption coefficient, $E_{1\,cm}^{1\%}(265\ nm)$, is calculated according to formula (A.3):

$$E_{1\,cm}^{1\%}(265\ nm)=\frac{A}{c} \qquad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots(A.3)$$

Where,

A - The value of the absorbance of the sample solution;

c - The mass fraction of the sample solution.

The absorbance value of the sample solution is based on the arithmetic average of the two parallel determination results. The absolute difference between two independent determination results obtained under repeated conditions is not more than 2% of the arithmetic mean.

A.6 Relative substance

A.6.1 Reagents and materials

Same with A.3.2.

A.6.2 Instruments and equipment

Same with A.3.3.

A.6.3 Chromatographic reference conditions

Same with A.3.4.

A.6.4 Analysis steps

A.6.4.1 Preparation of sample solution

Weigh 25mg of vitamin D_3 sample (to the nearest of 0.0001g). Place in a 100mL brown volumetric flask. Add 80mL of isooctane. Avoid heating. Perform ultrasonic treatment for 1min to make it completely dissolve. Use isooctane to dilute to 100mL. Shake well and use it as the sample solution. Accurately measure 1mL of the above solution. Place in a 100mL brown volumetric flask. Use isooctane to dilute to the scale. Shake well and use it as the control solution.

A.6.4.2 System suitability test

Same with A.3.5.1, A.3.5.2.

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