



新昌新和成维生素有限公司

Xinchang NHU Vitamins Co., Ltd.

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Test method for Vitamin D3 100 SD CWS/S (SP03-03)

1. Appearance

Take an appropriate amount of samples and use a visual method.

2. Odour

Take a proper amount of samples and smell them at room temperature.

3. Identification

According to USP Standard.

4. Sieve Analysis (US standard sieve)

Take the corresponding standard sieve and discharge it from top to bottom in turn. Put 50 g sample on the standard sieve and gently shake it left and right until no sample passes through the sieve. Weigh and record the sample quality respectively, and calculate the pass rate.

5. Loss on Drying

Take a sample of about 1 g (accurate to 0.0002 g), put it in a weighing bottle that has been dried to a constant weight, open the weighing bottle cap, put it in an electric thermostat at 105°C, and dry it for 4 hours to a constant weight.

5. 1 calculate and result

Loss on drying x (expressed in mass percentage) is calculated as follows:

$$X(\%) = \frac{m_1 - m_2}{m_1 - m_0} \times 100\%$$

In which:

m_1 ——the mass of weighing bottle to the sample before drying, g;

m_2 ——the mass of weighing bottle to the sample after drying, g;

m_0 ——the quality of weighing bottle, g.

6. Assay (HPLC)

6. 1 Preparation of reference solution

Reference stock solution: Weigh 60 mg VD3 CRS (accurate to 0. 1 mg) into a 100 mL brown volumetric flask, dissolve with n-hexane and dilute to the volume with n-hexane.

Reference solution: Accurately pipette 1.00 mL of the reference stock solution to a 50 mL brown volumetric flask, dissolve with n-hexane and dilute to the volume. Mix well.

6.2 Test solution

Accurately weigh an appropriate amount of sample (100,000 D3 weigh 0.5g, accurate to 0.0001g), put it in a 100mL volumetric flask, add 10mL of water to dissolve in 60°C water bath ultrasound, add an appropriate amount of ethanol ultrasound for 5 minutes, cool to room temperature, dilute with ethanol to volume, and use as stock soluti. Pipette 50mL of stock solution into a 250mL



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volumetric flask, add 34mL of 0.1 mol/L hydrochloric acid solution, then accurately transfer 50 mL of n-hexane, then put it on the shaker and shake for 15 minutes, take the upper solution at rest, and filter the sample solution to be injected in the liquid phase.

6.3 Chromatographic condition

Detection wavelength : 254nm

Chromatographic column: silica gel column 250mm× 4.6mm, 5μm;

Mobile phase: n-Hexane: n-amyl alcohol = 1000:6

Flow rate: 1.7ml/min

Injection: 200μl

6.4 Determination of pre-D3 correction factor F

Pipette 1 mL of the control reference solution into a 100 mL saponification bottle, add 10 mg BHT and 5 mL of n-hexane, and reflux in a water bath at 90 °C for 45 min in the dark, and cool. Transfer with n-hexane to a 50 mL brown volumetric flask, volume with n-hexane and shake well. After 0.45μm filter membrane, the sample was injected and analyzed according to the chromatographic conditions in 3) to obtain vitamin D3 peak area A and pre-vitamin D3 peak area A pre. Similarly, the reference solution was injected and analyzed to obtain the D3 peak area A. Pre-vitamin D3 correction factor (F) is calculated according to Equation (1):

$$F = \frac{A - A^*}{A^{*pre} - A^{pre}} \quad \text{-----} \quad (1)$$

In which:

A--Peak area of vitamin D3 in reference solution;

A*--The peak area of vitamin D3 after heating in a water bath at 90 °C for reference solution;

A*pre--The peak area of pre-vitamin D3 after heating in a water bath at 90 °C for reference solution;

Apre--Peak area of pre-vitamin D3 in the reference solution.

6.5 Sample determination

The above sample solution was injected and analyzed according to the above chromatographic conditions to obtain the D3 peak area AS and the pre-D3 peak area A pre in the sample. The result is calculated according to equation (2):

$$\text{D3 content in the sample (10000IU/g)} = \frac{(F \times A_{pre} + A_s) \times M_r \times C_r \times V_1}{A \times M_s \times V_2} \quad \text{-----} \quad (2)$$

In which:

F--Pre-D3 correction factor;

A Pre--Peak area of pre-D3 in the sample;

As--Peak area of D3 in the sample;

A--peak area of D3 in the control;

M r--Weigh the mass of the D3 control, g;

M s--Weigh the mass of the sample, g;

Cr --The content of D3 in the control substance, 10,000 IU/g;

V1--Diluted volume of sample, mL;

V2-- Diluted volume of the control, mL.

6. Heavy metal



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According to GB/T 5009.74

7. Arsenic Pb Cd

8. 1 Application of reagents

- 8. 1. 1 concentrated nitric acid (excellent grade pure);
- 8. 1.2 concentrated hydrochloric acid (excellent grade pure);
- 8. 1.3 concentrated hydrogen peroxide (analytically pure);
- 8. 1.4 2% dilute nitric acid (concentrated nitric acid: water = 1: 32);
- 8. 1.5 1000mg/kg arsenic, lead and cadmium standard stock solution (National Reference Material Research Center);
- 8. 1.6 Ultrapure water;
- 8. 1.7 Argon.

8.2 Application instruments

- 8.2. 1 Microwave Digester (with acid removal device);
- 8.2.2 German KNF LABOPORT diaphragm vacuum pump;
- 8.2.3 Spectrogenesis with coupled induced plasma excitation;
- 8.2.4 Ultra-pure water processor;
- 8.2.5 Electronic balance (1/10000);
- 8.2.6 Pipette gun;
- 8.2.7 Common glass instruments.

8.3 Working conditions of spectral determination

Plasma power is 1450 w;

Pump speed 2 [step];

The cooling gas flow rate is 11.0L/min; ;

Auxiliary gas flow rate is 0.6 l/min;

The atomization gas flow rate is 0.8 L/min.

8.4 Sample digestion

Accurately weigh 0.5 g of uniform sample into a microwave digestion tank, add 5 mL HNO₃, 1 mL H₂O₂ and 1 mL HCl in turn, gently shake to make the reaction full, and add acid to the blank tank without lofting. Tighten the flaring of the digestion tank cover, put it on the digestion tray according to the serial number (put 4 or 8 cans at a time), put on the tray cover and put it in the digestion furnace. Set the digestion program: (n tanks, power input n+2), raise the temperature to 220°C in 5 min, and keep the temperature for 5 min; After 10 min minutes, the temperature is raised to 260°C, and the temperature is kept for 15 minutes, and then the temperature is lowered and the pressure is reduced for 15 minutes. After digestion, take out the digestion tank for cooling, put arsenic in a fume hood to drive acid naturally, and connect vacuum pumps inside and outside the microwave oven to drive acid. After acid drive, take out the sample and move it to a 10 mL volumetric flask, and make a constant volume with high pure water to be measured, and do a blank test at the same time.

8.5 Preparation of Standard Solution

Use a pipette to remove 50ul 1000mg/kg of arsenic, lead and cadmium standard samples, put them in a 25 mL colorless volumetric flask, and use 2% dilute nitric acid to make a constant volume, and shake them evenly to make a stock solution. Absorb 5.00mL, 2.5mL, 1.0mL, 0.5mL and 0.25mL of the stock solution respectively, put them in another 10mL colorless volumetric flask, use 2% dilute nitric acid to make a constant volume, shake well, and make up a series of standard solutions of 1, 0.5, 0.2, 0.1 and



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0.05mg/kg respectively.

8.6 Test Steps:

8.6. 1 Operation method of the instrument:

Unscrew the argon switch, turn the main switch to ON, turn on the computer and circulating water, start the operating system and working software, after the instrument is stable, carry out argon flushing, plasma ignition, turn on the pump after the ignition is successful, and carry out distilled water flushing for about 30min.

8.6.2 Formulation of standard curve

Take 2% dilute nitric acid as blank solution, and measure it from low concentration to high concentration together with the standard solution prepared in 8.5. After the measurement, carry out background correction until the correction value reaches above 0.999.

8.6.3 Measurement of samples:

Measure the sample solution digested in 8.4, and calculate the arsenic content in the sample according to the detected concentration.

8.6.4 result

Heavy metal content in samples (mg/kg) = $C * V / m$

C---concentration detected by the instrument (mg/kg);

V---sample dilution volume (mL)

M---Weigh the sample mass (g)

9. Microbiological

items	Testing method
<i>Total Plate Count</i>	<i>GB4789.2</i>
<i>Mould & Yeast</i>	<i>GB4789.15</i>
<i>Coliforms</i>	<i>GB4789.3 Method I</i>
<i>Salmonella</i>	<i>GB4789.4</i>