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# Determination of Vitamin D in Vitamin D Powder

## 1. Principle

The microencapsulated vitamin D powder, after being diluted and broken, is ultrasonically extracted with absolute ethanol and then determined by high performance liquid chromatography.

The vitamin D reference standard is used as a control and the external standard method is used for quantification.

## 2. Reagent

2.1 0.01 mol/L hydrochloric acid solution: Draw 0.86 ml of hydrochloric acid solution(AR), and add water to 1000 ml.

2.2 Anhydrous ethanol: Analytical reagent (deacetalized)

2.3 Methanol: HPLC grade

2.4 Ultra-pure water

2.5 Standard stock solution：

Accurately weigh 0.02g (accurate to 0.0001g) of the reference standard by purity, equivalent to 40,000,000IU/g vitamin D.

Dissolve in absolute ethanol and dilute to a 50-ml brown volumetric flask and shake well.

2.6 Standard solution：

Accurately draw standard stock solution 5.00ml in a 250-ml brown volumetric flask, dilute with absolute ethanol to volume.

Shake well, pass the solution through an organic syringe filter of 0.22-μm pore size, and take the filtrate for injection test. (Available at the time of use).

## 3. Sample processing

Accurately weigh 0.4g (accurate to 0.0001g) sample in a 250 ml brown volumetric flask and add 10ml of 0.01mol/L hydrochloric acid solution, treated in an ultrasonic bath at 60 ° C for 15 min to completely disintegrate, and then add anhydrous ethanol to the volume.

Shake well, place it in an Ultrasonic oscillator for 10 min, remove, cool to room

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temperature, dilute with absolute ethanol to volume.

Shake well, pass successively the solution through organic syringe filter of 0.45μm and 0.22-μm pore size, and take the filtrate for injection test.

## 4. Chromatographic conditions

Column：C18 column, 250mm × 4.6mm, particle size: 5μm

Mobile phase: methanol

Flow rate: 1 ml/min

Detection wavelength: 254 nm

Injection volume: 20μL

## 5. Determination of previtamin D correction factor

Accurately weigh the standard stock solution 5ml to 100ml saponification flask, add 3 BHT and appropriate amount of absolute ethanol, and reflux in a water bath at 90 ° C for 45 minutes, then cool down. Transfer to a 50-ml brown volumetric flask with an appropriate amount of absolute ethanol, add absolute ethanol to volume and shake well.

Pass through an Organic filter membrane of 0.22- μ m pore size, and use high-performance liquid chromatograph as the above-mentioned chromatographic conditions for injection detection to obtain the the peak area of vitamin D A' and the peak area of pre-vitamin D A'pre.

The standard solution is also passed through an Organic filter membrane of 0.22- μ m pore size, and use high-performance liquid chromatograph as the above-mentioned chromatographic conditions for injection detection to obtain the peak area of vitamin D A and the peak area of pre-vitamin D Apre .

Calculation formula:

Calculate the Pre-vitamin D correction factor (F):

$$F=(A-A')/(A'_{pre}-A_{pre})$$

## 6. Calculation formula：

$$X\ (IU/g)=\frac{(A_0\times F+A_1)\times M_2}{A_2\times M_1\times 10}\times 40000000$$

X——the content(IU/g) of Vitamin D in the sample

A 0 ——the peak area of Pre-vitamin D in the sample

A 1 ——the peak area of Vitamin D in the sample

A 2 ——the peak area of Vitamin D in the standard

M 1 ——Sample weight

M 2 ——Standard weight

**7. Precision**

The absolute difference between two independent determinations obtained under repeatability conditions shall not exceed 5% of the arithmetic mean.