Vitamin D3 content Assay kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4884

Sizes： 50T/48S

Product Description：

Vitamin D3 is a fat-soluble vitamin. The biological function of vitamin D3 is closely related to its metabolite caltriol, the main functions include improving the body's absorption of calcium and phosphorus, promoting the formation and calcification of new bone, and regulating the synthesis of calcium-binding proteins in the liver.

Vitamin D3 has ultraviolet absorption at 265 nm, and its content can be determined by ultraviolet detector.

Reagents and Equipment Required but Not Provided：

High-efficiency liquid chromatograph (C18 column (4.6×250 mm), ultraviolet detector (VWD)), desktop centrifuge, adjustable pipette, mortar/ homogenizer, EP tube, syringe filters (organic), syringe, suction filter, filter membrane (organic, water), brown injection bottle, carbinol (chromatographically pure), ultrapure water.

Product Composition:

Extract solution: 60 mL×1. Storage at 2-8℃.

Standard： Powder×1. Store at -20℃. Before use, 1 mL carbinol was added to prepare 5 mg/mL vitamin D3 standard solution, which was sealed and stored at-20℃, away from direct sunlight.

Preparations before the experiment:

1. Methanol (chromatography-pure) was extracted by organic filter membrane as mobile phase.
2. Ultrasound the filtered mobile phase for 20 min to remove bubbles.
3. Preparation of standard products: 5 mg/mL Vitamin D3 standard solution is diluted with distilled water into 1000μg/mL、500 μg/mL、100 μg/mL、20 μg/mL、4μg/mL、0.8μg/mL Vitamin D3 standard solution. (The standard concentration is for reference only and can be adjusted according to the actual sample concentration). Store (sealed) at -20℃ away from light, filter into brown sample bottle with water needle filter before test, to be tested.

Procedure

I. Vitamin D3 extraction:

By organizational quality (g): Extract solution volume (mL) 1:5~10 ratio for extraction, it is recommended to weigh about 0.2g sample, add 1 mL extract liquid, ice bath homogenate, sealed at room temperature and violently shake in dark swirl for 30min, centrifuge at 10000rpm for 10min, take the supernatant, filter it into brown sample bottle before testing using organic needle filter. To be tested.

II. Determination procedure：

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1. Turn on the computer, turn on the switch buttons of each module of the HPLC, install the chromatographic column, open the software, and set the injection volume in the method group to 10 μL, column temperature: 30°C, flow rate 1 mL/min, The UV detector has a wavelength of 265 nm. The sampling time of a single sample is 15min, and the preservation method group is set.
2. Use the corresponding mobile phase to clean the column, balance the column with mobile phase A, and start adding samples after the baseline is stable.
3. Test the standard solution to be measured, the sample size is 10 μL, Vitamin D3 can be separated within 15 min, and the retention time of Vitamin D3 is about 10 min (the retention time is different with the system, column, mobile phase pH, temperature, etc., and is only for reference).
4. Test the sample solution to be measured, the injection volume is 10 μL, and test the peak area of Vitamin D3 at the corresponding retention time.

Note: After the completion of the determination of a single sample, pay attention to whether there is any residue of the sample material, and if necessary, the column can be cleaned by extending the running time accordingly.

III. Calculations：

The standard curve y=kx+b was drawn with the standard concentration (μg/mL) as the horizontal coordinate x and the peak area as the vertical coordinate y. The peak area of the sample was substituted into the standard curve to calculate the concentration x (μg/mL) of Vitamin D3 in the Extraction solution.

Vitamin D3 content (μg/mL)＝$x \times V_E \div W \times F = x \div W \times F$

V extraction: Add the total volume of Extraction solution, 1 mL; W: Sample quality, g; F: dilution ratio, The samples tested after dilution need to be multiplied by the corresponding dilution multiple when calculating.

Note:

Precautions:

1. After the test is completed, it is necessary to flush the column with a high concentration of ultra-pure water phase (about 20-30 column volumes) to prevent blocking the column, and then flush the column with a high concentration of organic phase, and finally flush according to the type of column to prevent damage to the column.
2. The dilution of the standard is determined according to the concentration of vitamin D3 in the sample. The concentration of vitamin D3 in the sample must be within the concentration range of the standard solution. The dilution of the standard is only a reference. If the concentration of vitamin A in the sample is too high, it is recommended to dilute it before testing.

3. If the sample size is too large, it is recommended to test the standard solution once a day (one standard solution can be used) to determine the corresponding retention time, and the solution to be tested must be placed at room temperature before testing.
4. In order to exclude the influence of solvents, a blank control test can be carried out.

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