Vitamin A content Assay kit

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

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4874

Sizes： 50T/48S

Product Description：

Vitamin A is a fat-soluble vitamin, which has important physiological and pharmacological effects, and is an important nutrient necessary for maintaining normal metabolism and function of the human body. Its main physiological functions include maintaining the integrity of epithelial tissues, maintaining the permeability and integrity of membrane structures in various cells and organelles, maintaining normal vision, and promoting the synthesis of mucopolysaccharides in connective tissues. When the body lacks vitamin A, it may cause epithelial tissue hyperplasia and keratinization.

Vitamin A has fluorescence characteristics under certain photoexcitation conditions, and its content can be determined by fluorescence detector.

Reagents and Equipment Required but Not Provided：

High-efficiency liquid chromatograph (C18 column (4.6×250 mm), Fluorescence Detector (FLD), desktop centrifuge, adjustable pipette, mortar/ homogenizer, EP tube, syringe filters (water), syringe, suction filter, filter membrane (organic, water), brown injection bottle, carbinol (chromatographically pure), Ether, methanol (analytically pure), anhydrous ethanol, ultrapure water.

Product Composition:

Reagent I: Powder×2. Storage at 2-8℃. Add 10 mL anhydrous ethanol to each bottle before use, dissolve fully, and store at 2-8℃.

Reagent II: 10 mL×1. Storage at 2-8℃.

Reagent III: Powder×1. Storage at 2-8℃.

Standard： Powder×1. Store at -20℃. Before use, 1 mL anhydrous ethanol was added to prepare 5 mg/mL vitamin A 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 A standard solution is diluted with distilled water into 200μg/mL、40 μg/mL、8 μg/mL、1.6 μg/mL、0.32μg/mL VitaminA standard solution. (The standard concentration is for reference only and can be adjusted according to the actual sample concentration). Store (sealed) at -20°C away from light, filter into brown sample bottle with water needle filter before test, to be tested.

Procedure

I. Vitamin A extraction:

1. Weigh 0.2g sample, add 0.4mL of Reagent I, then add 0.2mL of Reagent II, mix well, seal, place in

   80℃ water bath away from light for 30 min, and cool on ice.

2. Add 1 mL of ether into the fume hood, shake and mix well for about 2 min, stand for stratification, take the upper ether phase, add 1 mL of ether into the lower water phase, shake and mix well for about 2 min, stand for stratification, take the upper ether phase (if the ether phase is still colored, you can add ether again for extraction), and combine the ether phase.
3. Add 1 mL distilled water to the ether phase, shock wash, and stand to remove the lower water phase by layers (wash until the water phase pH7 (about 3 to 4 times)). Finally, a small amount of reagent is added to the ether phase to remove the very small amount of residual water in the ether phase.
4. Take out the ether phase and put it in a water bath at 40℃ in the fume hood until it is nearly dry (note: volatilization is fast and cannot be completely dried), add methanol at a constant volume to 1 mL, dissolve it under shock, centrifuge at 10000 rpm for 10 min, and take the supernatant, and filter it into a brown sample bottle with organic pinhead filter before testing.

II. Determination procedure：

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 0.6 mL/min, Fluorescence detector: Ex=330 mn, Em=480 nm. The sampling time of a single sample is 12 minutes, 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 A can be separated within 12 min, and the retention time of Vitamin A is about 7 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 A 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 A in the Extraction solution.

Vitamin A content (μg/mL)＝x ×V_E÷W×F=x÷W×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 A in the sample. The concentration of vitamin A 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.
5. If the delamination limit is not obvious during the extraction and washing process, the standing time should be extended to fully delaminate.