Vitamin C content Assay kit

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

Operation Equipment: High performance liquid chromatography

Catalog Number: BC1234

Sizes： 50T/48S

Product Description：

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin found mainly in fresh vegetables and fruits. Vitamin C has strong reducibility and is easily oxidized. It is beneficial to tissue wound healing, promote collagen synthesis and other physiological functions.

Vitamin C has ultraviolet absorption at 210 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 (1.5mL), syringe filters (water), syringe, suction filter, filter membrane (organic, water), brown injection bottle (2mL), carbinol (chromatographically pure), ultrapure water.

Product Composition:

Extract solution: 50 mL×1. Storage at 2-8℃.

Reagent I: 1.5 mL×1. Storage at 2-8℃.

Reagent II: Powder×2. Storage at 2-8℃.

Standard： Powder×1. Store at 2-8℃ away from light. Before use, add 1 mL distilled water to prepare 5 mg/mL Vitamin C standard solution. Sealed at 4℃, it can be stored for two weeks to avoid direct sunlight.

Preparations before the experiment:

1. Dissolve 1 bottle of Reagent II into 1000 mL of ultra-pure water, then add 0.55 mL of Reagent I and mix well to obtain mobile phase A.
2. Filter 1000 mL of prepared mobile phase A and methanol (chromatographically pure) with filter membrane. (The prepared mobile phase A was filtered by 0.22μm aqueous filter membrane, and methanol was filtered by 0.45μm organic filter membrane).
3. Ultrasound the filtered mobile phase for 20 min to remove bubbles.
4. Preparation of standard products: 5 mg/mL vitamin C standard solution is diluted with distilled water into 2000 μg/mL, 500 μg/mL, 100 μg/mL, 20 μg/mL, 4 μg/mL, 0.8 μg/mL vitamin C standard solution. (The standard concentration is for reference only and can be adjusted according to the actual sample concentration). Store (sealed) at 4°C away from light, filter into brown sample bottle with water needle filter before test, to be tested.

Procedure

I. Vitamin C extraction:

By sample quality (g): Extraction solution volume (mL) 1:5~10 ratio for extraction, it is recommended to weigh about 0.2g fresh sample, add 1 mL of Extraction solution, ice bath homogenate,

centrifuge at 10000 rpm for 10 min at 4℃, take supernatant (if there is still turbidity, can be centrifuged again), and filter it into brown sample bottle with water needle filter before testing. To be tested (if the color of the supernatant is too deep or the concentration is too high, it can be diluted with distilled water and filtered again to be tested).

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 0.4 mL/min, and the ultraviolet detector wavelength to 210 nm. The sampling time of a single sample is 15 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 C can be separated within 15 min, and the retention time of vitamin C is about 11.3 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 C at the corresponding retention time.
5. Complete sequence sampling table: (including the cleaning and rebalancing process of the column after the determination of a single sample is completed)

Time (t)	Carbinol (%)	Mobile phase A (%)	flow rate (mL/min)
0 min	0	100	0.4
1 min	0	100	0.4
1.1 min	3	97	0.4
15 min	3	97	0.4
15.1 min	60	40	1
25 min	60	40	1
25.1 min	0	100	1
35 min	0	100	1

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 C in the Extraction solution.

Vitamin C content (μg/g) = x× V extraction ÷W×F= x×F÷W

V extraction: Add the total volume of extraction liquid, 1 mL; W: Sample quality, g; F: dilution ratio, the sample tested after dilution, the calculation needs to be multiplied by the corresponding dilution ratio. **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 specification to prevent damage to the column.
2. The dilution of the standard product should be determined according to the concentration of vitamin C in the sample, and the peak area of vitamin C in the sample must be within the peak area of the standard solution of different concentrations, and the dilution of the standard product is only a reference. If the concentration of vitamin C 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 gradient elution baseline drift, a blank detection can be performed.