Cinnamic Acid Content Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4684

Size: 50T/48S

Product Description:

Cinnamic acid, also known as β - phenylacrylic acid or 3-phenyl-2-acrylic acid, is an organic acid isolated from cinnamon or benzoin. Phenylpropanoic acid produced by deamination of phenylalanine in plants. Widely used in daily chemical, food additives, pharmaceutical industry, pesticides and other fields.

Cinnamic acid has an absorption peak at 270 nm and its content can be determined by high-performance liquid chromatography.

Required reagents and equipment:

High performance liquid chromatography (C18 column (4.6 × 250 mm), UV detector (VWD)), desktop centrifuge, adjustable pipette, mortar/homogenizer, EP tube, needle filter (50, organic system, 0.45 μm), syringe, suction filter, filter membrane (organic system, water system), brown injection bottle (50, 1.5 mL), acetonitrile (chromatographically pure, 500 mL), ultrapure water, phosphoric acid (analytical pure), methanol (analytical pure).

Product Composition:

1. Extract solution：Liquid 80 mL×1, stored at 2-8 °C.
2. Reagent I：Liquid 3 mL×1, stored at 2-8 °C.
3. Reagent II：Powder×2, stored at 2-8 °C.
4. Standard： Powder×1, stored in the dark at 2-8 ℃. Before use, add 2.5 mL of methanol to prepare a 2 mg/mL standard solution of cinnamic acid, seal and store at 2-8 ℃, and avoid direct sunlight.

Preparation before the experiment:

1. Dissolve a bottle of Reagent II in 1000 mL of ultrapure water, then add 1.2 mL of Reagent I and mix well to obtain mobile phase A.
2. Filter 500 mL of chromatographically pure acetonitrile (mobile phase B) and 1000 mL of prepared mobile phase A through a filter membrane. (Acetonitrile was filtered using a 0.45 μm organic filter membrane, while the prepared mobile phase A was filtered using a 0.22 μm aqueous filter membrane.)
3. Filter the mobile phase A and B-ultrasound for 20 minutes to remove any bubbles.
4. Preparation of standards: Dilute 2 mg/mL of cinnamic acid standard solution with methanol to 500 μg/mL、50 μg/mL、10 μg/mL、1 μg/mL、0.5 μg/mL of cinnamic acid standard solution. (The concentration of the prepared standard sample is for reference only and can be adjusted

according to

the actual sample concentration.) Store at 4℃ (sealed), filter with an organic needle filter into a brown injection bottle before testing.

Operation steps:

I. Extraction of cinnamic acid:

According to the ratio of weight (g): Extract solution volume (mL) 1:5-10, it is recommended to weigh 0.15 g of sample, grind and crush it thoroughly, add 1 mL of Extract solution, seal it, mix evenly, and then place it in an ultrasonic cleaning machine at room temperature (25-35 ℃) for ultrasonic extraction for 30 minutes. Centrifuge at 12000 rpm for 10 minutes, take the supernatant, add 0.5 mL of Extract solution to the filter residue, shake and mix well, and extract by ultrasound at room temperature (25-35 ℃) for 15 minutes. Mix the extracted liquids from two extractions, centrifuge at 12000 rpm for 10 minutes, take the supernatant, and filter it into a brown injection bottle using an organic needle filter before testing (if the supernatant color is too dark or the concentration is too high, it can be diluted and filtered again for testing).

II. Measurement steps:

1. Turn on the computer, open the switch buttons of each module of the liquid chromatograph, install the chromatography column, open the software, set the injection volume to 10 μL, column temperature to 30 ℃, flow rate to 1 mL/min, wavelength to 270 nm. The elution procedure is shown in the table below, with a deformation time of 50 minutes. After setting, save the method group.
2. Elution Procedure Table:

Time	Mobile phase	
	Mobile phase B	Mobile phase A
0 min	30%	70%
25 min	65%	35%
25.1 min	70%	30%
35 min	70%	30%
35.1 min	30%	70%
50 min	30%	70%

3. Use the corresponding mobile phase to clean the column, and use a mobile phase equilibrium column with a ratio of mobile phase B: mobile phase A=30:70. Start adding samples after the baseline is stable.
4. Test the standard solution to be tested, with an injection volume of 10 μL. Cinnamic acid can be separated within 25 minutes, and its retention time is about 21.4 minutes (the retention time varies depending on the system, column, mobile phase pH, temperature, etc., and is only for reference).

5. Detect the sample solution to be tested, with an injection volume of 10 μL, and measure the peak area of cinnamic acid at the corresponding retention time.

III. Calculation of cinnamic acid content

Plot the standard curve of cinnamic acid using the standard concentration (μg/mL) as the horizontal axis and peak area as the vertical axis. Substitute the peak area of the sample into the standard curve to calculate the concentration of cinnamic acid in the sample x (μg/mL).

The content of cinnamic acid (μg/g)=x × Ve ÷ W=1.5x ÷ W

Ve: volume of Extract solution, 1.5 mL;

W: Sample weight, g.

Note: The sample tested after dilution needs to be multiplied by the corresponding dilution factor before calculation.

Note:

1. After the test is completed, it is necessary to rinse the chromatography column with high concentration ultrapure water (about 20-30 column volumes) to prevent blockage of the chromatography column. Then, rinse the chromatography column with high concentration organic phase, and finally rinse according to the specifications of the column type to prevent damage to the chromatography column.
2. The dilution factor of the standard should be determined based on the concentration of cinnamic acid in the sample. The peak area of cinnamic acid in the sample must be within the peak area of the standard solution at different concentrations. The dilution factor of the standard is only a reference. If the concentration of cinnamic acid 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 is sufficient) to determine the corresponding retention time. All test solutions must be left at room temperature before testing.