Chlorogenic acid Content Test Kit

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

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4474

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 100 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. Filter 500 mL of chromatographic-grade acetonitrile and 500 mL of 0.4% phosphoric acid aqueous solution through filter membranes to remove impurities in the solvents and prevent clogging of the chromatographic column. (Use a 0.45 μm organic filter membrane for acetonitrile and a 0.22 μm aqueous filter membrane for the 0.4% phosphoric acid aqueous solution.

2. Preparation of the mobile phase: A: One bottle of the filtered 0.4% phosphoric acid aqueous solution. B: One bottle of the filtered chromatographic-grade acetonitrile. Ultrasonicate the prepared mobile phase for 30 minutes to remove gases in the solvents and prevent pressure instability, which could affect the experimental results.

3. Preparation of standards: Before use, add 1 mL of extract solution to prepare a 1 mg/mL chlorogenic acid standard solution. 1 mg/mL chlorogenic acid standard solution was diluted into 100 μg/mL, 50 μg/mL, 10 μg/mL, 5 μg/mL, and 1 μg/mL chlorogenic acid standard solution with the extraction solution (the prepared standard concentration is for reference only and can be adjusted according to the actual sample concentration). Filter through a syringe filter before testing.

Product Description:

Chlorogenic acid, also known as coffee tannic acid, is a phenolic compound that is formed via the shikimate pathway during aerobic respiration in plants as a type of phenylpropanoid substance. Chlorogenic acid is widely found in plants such as Eucommia, honeysuckle, and Convolvulaceae, and it has biological activities including antibacterial, antiviral, hepatoprotective and choleretic, antitumor, antihypertensive, and free radical scavenging, which leads to its extensive application in the fields of food, medicine, health, and cosmetics.

Chlorogenic acid has an absorption peak at 327 nm, and its content can be determined using high-performance liquid chromatography (HPLC).

Reagents and Equipment Required but Not Provided:

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High Performance Liquid Chromatograph (C18 column (4.6×250 mm), ultraviolet detector (VWD)), benchtop centrifuge, ultrasonic cleaner, blast drying oven, adjustable pipette, mortar/homogenizer, brown EP tube, syringe filter (50, organic, 0.45 μm), syringe, suction filter, membrane (one organic and one aqueous), brown sample vials (50, 2 mL), acetonitrile (chromatographically grade, 500 mL), phosphoric acid (analytically grade, 500 mL), and ultrapure water.

Operation procedure

I. Extraction of chlorogenic acid:

The test plant samples were dried in a blast oven at 60°C, ground into powder, and passed through a 20~40 mesh sieve. According to the mass (g): the volume of the extracted liquid (mL) is 1:10~20 (it is recommended to weigh 0.15 g to dry the sample and add 1.5 mL of the extract), and then extract in a water bath at 60°C for 20 min. Centrifuge at 10000 rpm for 10 minutes (25°C) and take the supernatant (if solids are still present in the solution, centrifuge again). The obtained supernatant was filtered through a syringe filter into a brown vial for testing.

II. Determination procedure:

1. Turn on the computer, turn on the switch buttons of each module of the liquid chromatograph, install the chromatographic column, open the software, set the injection volume to 10 μL, the column temperature: room temperature (about 27°C), the flow rate is 1 mL/min, the wavelength is 327 nm, acetonitrile: 0.4% phosphoric acid aqueous solution = 13:87 is used as the mobile phase, the sampling time is 15 min, and the method set is stored after setting.
2. Wash the column with a mobile phase equilibration of acetonitrile:0.4% phosphoric acid in water = 13:87 and begin injection after the baseline is stable.
3. The injection volume of the prepared standard solution is 10 μL, and the chlorogenic acid can be separated within 15 min, and the retention time of chlorogenic acid is about 10 min (the retention time varies depending on the system and column, etc., and is only used as a reference). After testing one sample, repeat step 2 to wash the equilibrium column and add another sample after the baseline is stable. Calculate the peak area of the different concentrations of chlorogenic acid standards.
4. Inject 10 μL of the sample solution and measure the peak area of chlorogenic acid at the corresponding retention time. After testing one sample, repeat step 2 to wash the equilibrium column and add another sample after the baseline is stable.

III. Calculation:

Plot a standard curve for chlorogenic acid with the concentration of the standard solution (μg/mL) as the abscissa and the peak area as the ordinate. Substitute the peak area of the sample into the standard curve to calculate the concentration x (μg/mL) of chlorogenic acid in the extraction solution.

Content of chlorogenic acid in the sample (μg/g) = x×V÷W=1.5x÷W

V: The volume of extract solution, 1.5 mL;

W: Sample weight(g).

Note:

1. The dilution factor of the standard is determined according to the concentration of chlorogenic acid in the sample, and the peak area of the chlorogenic acid in the sample must be within the peak area of the chlorogenic acid standard of different concentrations, and the dilution factor of this standard is only a reference.
2. After use, the column needs to be rinsed with a high concentration of organic phase and then rinsed according to the type of column specification to prevent damage to the column.

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