Curcumin 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: BC4654

Size: 50T/48S

Product Description:

Curcumin is a natural phenolic substance extracted from the rhizomes of plants in the Curcuma genus of the ginger family. Curcumin has a wide range of pharmacological activities, such as anti-inflammatory, antioxidant, anti-tumor, and lipid-lowering effects.

Curcumin has an absorption peak at 425 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 60 mL×1, stored at 2-8 ℃.
2. Reagent I: Liquid 5 mL×1, stored at 2-8 ℃.
3. Standard：Powder×1, stored in the dark at -20 ℃. Before use, add 1 mL of methanol to prepare a 2 mg/mL standard solution of curcumin, seal and store at -20 ℃, and avoid direct sunlight.

Preparation before the experiment:

1. Add 1 mL of phosphoric acid to 500 mL of ultrapure water and mix thoroughly to obtain mobile phase A.
2. Filter 500 mL of chromatographically pure acetonitrile (mobile phase B) and 500 mL of prepared mobile phase A through a filter membrane to remove impurities and prevent column blockage. (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. Use the prepared mobile phase A and B ultrasound for 20 minutes to remove any gas from the solvent and prevent blockage of the chromatographic column, which may affect the experimental results.
4. Preparation of standards: Dilute 2 mg/mL of curcumin standard solution with methanol to 0.1 mg/mL, 0.05 mg/mL, 0.01 mg/mL, 0.005 mg/mL, and 0.001 mg/mL of curcumin standard solution (The

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concentration of the prepared standard sample is for reference only and can be adjusted according to the actual sample concentration). Store at -20 ℃ (sealed), filter with an organic needle filter into a brown injection bottle before testing (please place at room temperature before testing to avoid affecting the retention time).

Operation steps:

I. Extraction of curcumin:

According to the ratio of weight (g): volume of extraction solution (mL) 1:5-10, it is recommended to weigh 0.1 g of the sample. If it is a leaf with the main vein removed, it should be thoroughly crushed, then 1 mL of extraction solution should be added, mixed evenly, weighed, ground, and weighed again. The weight loss should be compensated with the extraction solution, sealed, and then placed in dark conditions or wrapped in tin foil and shaken at room temperature for 4 hours. Centrifuge at 10000 rpm for 10 minutes at room temperature, take the supernatant (if there are still solid samples in the supernatant, centrifuge again), store at -20 ℃ (sealed), and filter it into a brown injection bottle using an organic needle filter before testing (leave it at room temperature before testing, if the color of the supernatant is too dark or the concentration is too high, dilute it and filter it 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 425 nm. The single sample aliasing time is 30 minutes. After setting, save the method group.
2. 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=49:51. Start adding samples after the baseline is stable.
3. Test the standard solution to be tested, with an injection volume of 10 μL. Curcumin can be separated within 15 minutes, and its retention time is about 11.5 minutes (the retention time varies depending on the system, column, mobile phase pH, temperature, etc., and is only for reference).
4. Detect the sample solution to be tested, with an injection volume of 10 μL, and measure the peak area of curcumin at the corresponding retention time.

III. Calculation of Curcumin Content

Plot the standard curve of curcumin using the standard concentration (mg/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 curcumin in the sample x (mg/mL).

The content of curcumin (mg/g) = x × Ve ÷ W = x ÷ W

Ve: volume of Extract solution, 1 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 curcumin in the sample. The peak area of curcumin 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 curcumin 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.