Quercetin Content HPLC Assay Kit

Note: Choose 2-3 sample with large expected differences for prediction before formal determination.

Detection Equipment: High performance liquid chromatography(HPLC)

Catalog Number : BC4994

Size: 50T/48S

Components:

Extract I: Liquid 80 mL ×1, Storage at 4℃.

Extract II: Liquid 90 mL ×1, Storage at 4℃

Standard: Powder ×1 , Storage at 4℃. Before use, 1 mL methanol was added to prepare a standard solution of 2 mg/mL quercetin, which was sealed and stored at 4℃to avoid direct sunlight.

Description:

Quercetin, also known as quercus, quercetin. It can be used as a drug with good expectorant, antitussive effects, and a certain antiasthmatic effect. Quercetin and its derivatives are a kind of flavonoids that widely exist in various plants. As a natural antioxidant, quercetin has a wide range of pharmacological effects and physiological activities, such as anti-tumor, anti-oxidation, anti-inflammation, anti-allergy, and cardiovascular protection.

Quercetin has an absorption peak at 360 nm, and its content can be determined by HPLC.

Reagents and Equipment Required but Not Provided:

High performance liquid chromatograph (C18 column (4.6×250 mm), UV detector (VWD)), benchtop centrifuge, adjustable pipet gun, mortar/homogenizer, brown EP tube, needle filter (50, organic, 0.45 μm), syringe, suction filter, filter membrane (organic system, water system),brown sample bottle (50 pieces, 1.5 mL)， acetonitrile (chromatographic pure, 500 mL), ultrapure water, phosphoric acid (analytical pure), methanol (analytical pure), 5 mL brown plastic reagent bottle, 2 mL EP tube.

Preparation before The Experiment:

1. Add 1.18 mL phosphoric acid to 500 mL ultrapure water and mix thoroughly to obtain mobile phase A.
2. 500 mL chromatographic pure acetonitrile (mobile phase B) and 500 mL prepared mobile phase A were filtered by filter membrane to remove impurities in the solvent to prevent blocking the chromatographic column. (The acetonitrile was filtered by 0.45 μm organic filter membrane, and the mobile phase A was filtered by 0.22 μm aqueous filter membrane).
3. The prepared mobile phases A and B were sonicated for 20 min, and then used.
4. Preparation of standard substance: 2 mg/mL quercetin standard solution was diluted with methanol into 0.5 mg/mL, 0.1 mg/mL, 0.05 mg/mL, 0.01 mg/mL, 0.005 mg/mL quercetin standard solution. It was stored at 4℃ (sealed) and filtered into a brown injection bottle using an organic needle type filter before testing.

Protocol:

I. Sample Extraction

1. Free quercetin extraction:

The tested plant sample were dried in a blast oven at 60℃, ground into powder, and passed through a 20 to 40 mesh screen. According to the mass (g) : extract one volume (mL) 1:10~20 ratio (it is recommended to weigh 0.15 g of dried sample and add 1.5 mL of extract one), add it into 2 mL EP tube, seal it, mix it evenly, and then put it into ultrasonic cleaner at room temperature (25~35℃, $P < 0.05$). The temperature should not be too high when ultrasonic.) under the condition of ultrasonic extraction for 60 min. The sample were centrifuged at 10000 rpm for 10 min at ℃, and the supernatant was removed (if there was still a solid sample in the supernatant)

It can be centrifuged again), stored at 4℃ (sealed), filtered 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 to be tested).

2. Total quercetin extraction:

The tested plant sample were dried in a blast oven at 60℃, ground into powder, and passed through a 20 to 40 mesh screen. According to the mass (g) : extract 2 volume (mL) 1:10~20 ratio (it is recommended to weigh 0.15 g dried sample and add 1.5 mL extract 2) into reagent bottle, weigh, mix evenly, then put into 80℃ constant temperature water bath, extract 2 h (it is recommended to use spiral mouth 5 mL plastic reagent bottle, tighten, Prevent bottle opening during heating). After cooling, the mixture was weighed again and the difference was made up by adding extract two. Sealed, put into the ultrasonic cleaner, ultrasonic extraction under the condition of room temperature (25~35℃, pay attention to the temperature can not be too high) for 60 min. Then the extracted turbidity was transferred to an EP tube, centrifuged at 10000 rpm for 10 min at 4℃, the supernatant was taken (if there were still solid sample in the supernatant, the supernatant could be centrifuged again), stored at 4℃ (sealed), filtered into a brown injection bottle using an organic needle type filter before testing. It can be diluted and filtered again to be measured).

II. Measurement Steps

1. Turn on the computer, turn on the switch button of each module of the liquid chromatograph, install the chromatographic column, and open the software. In the method group, the injection volume is set as 10 μL, the column temperature is 30℃, the flow rate is 0.8 mL/min, and the wavelength is 360 nm. The out-of-sample time of a single sample was 30 min, and the out-of-sample time of a complete sample was 70 min. The preservation method group was set up.
2. Clean the column with the corresponding mobile phase, balance the column with the ratio of acetonitrile: mobile phase A = 20:80, and start adding sample after the baseline is stable.
3. To detect the standard solution to be tested, the injection volume is 10 μL, quercetin can be separated within 30 min, and the retention time of quercetin is about 14.6 min (the retention time is different due to different systems, columns, mobile phase pH, temperature, etc., which is only used as a reference).
4. Detect the sample solution to be tested with an injection volume of 10 μL and detect the peak area of quercetin at the corresponding retention time. (If you need continuous detection, you can press the following table for experiment)

5. Complete sequence loading table: (contains column cleaning and rebalancing process after single sample determination is completed)

Time	Mobile phase	
	Mobile phase B	Mobile phase A
0 min	20%	80%
30 min	60%	40%
30.1 min	70%	30%
50 min	70%	30%
50.1 min	20%	80%
70 min	20%	80%

III. Calculations

The standard curve of quercetin was drawn with the standard concentration (mg/mL) as the abscissa and the peak area as the ordinate. The peak area of the sample was substituted into the standard curve to calculate the concentration x (mg/mL) of quercetin in the extract.

The amount of quercetin (mg/g) = $x \times V_{extraction} \div W = 1.5x \div W$

$V_{extraction}$: volume of extraction solution, 1.5 mL; W: sample quality, g.

Note: sample tested after dilution need to be multiplied by the corresponding dilution before calculation.

Note:

1. After the test, turn off the column temperature chamber heating system, and stop the mobile phase until the column temperature drops to room temperature or below.
2. After the test, it is necessary to wash the column with a high concentration of ultrapure water phase (about 20-30 column volume) to prevent blocking the column, and then wash the column with a high concentration of organic phase, and finally wash the column according to the type of column specification to prevent damage to the column.
3. The dilution of the standard should be determined according to the concentration of quercetin in the sample. The peak area of quercetin in the sample must be within the peak area of the standard solution of different concentrations. If the concentration of quercetin in the sample is too high, it is recommended to dilute it before testing.
4. If the sample size is too large, it is recommended to test the standard solution once a day (one standard solution is enough) to determine the corresponding retention time. All the solutions to be tested should be placed at room temperature before testing.