Sinapic Acid Content Test Kit

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

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4504

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 I	Liquid 80 mL×1	2-8℃
Extract solution II	Liquid 90 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. Add 1.18 mL of phosphoric acid to 500 mL of ultrapure water, mix thoroughly to obtain mobile phase A.

2. Filter 500 mL of chromatographic-grade acetonitrile (Mobile Phase B) and 500 mL of the prepared Mobile Phase A through filters to remove impurities from the solvents and prevent blockage of the chromatographic column. (Use a 0.45 μm organic filter for acetonitrile and a 0.22 μm aqueous filter for the prepared Mobile Phase A.)

3. Subject the prepared Mobile Phases A and B to ultrasonication for 20 minutes to remove gases from the solvents, thereby preventing blockage of the chromatographic column and ensuring accurate experimental results.

4. Preparation of standards: Before use, add 2 mL of methanol to prepare a 1 mg/mL sinapic acid standard solution. Store it at 4℃ in a sealed container, avoiding direct sunlight.1 mg/mL sinapic acid standard solution was diluted into 0.1 mg/mL, 0.05 mg/mL, 0.01 mg/mL, 0.005 mg/mL, and 0.001 mg/mL sinapic acid standard solution with the methanol.(The prepared standard solution concentration is for reference only and can be adjusted according to the actual sample concentration.) Store at 4℃ (sealed), and before testing, filter it into a brown sample vial using an organic needle filter. Keep it for testing (please allow it to reach room temperature before testing to avoid affecting the retention time).

Product Description:

Sinapic acid, also known as 4-hydroxy-3,5-dimethoxycinnamic acid, is a light yellow powder, irritating, usually not harmful to water, and stored separately from acids and edible chemicals. Sinapic acid is widely found in the plant kingdom, and various fruits, vegetables, cereals, oil crops, some spices, medicinal plants, etc. contain sinapic acid. Sinapic acid and its derivatives are potent antioxidants with a wide range of biological activities.

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Sinapic acid has an absorption peak of 360 nm, and its content can be determined by high performance liquid chromatography.

Reagents and Equipment Required but Not Provided:

High Performance Liquid Chromatograph (C18 column (4.6×250 mm), ultraviolet detector (VWD)), benchtop centrifuge, 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, 1.5 mL), acetonitrile (chromatographically grade, 500 mL), phosphoric acid (analytically grade, 500 mL), methanol (analytically pure), 5 mL white plastic reagent bottle, 2 mL EP tube.

Operation procedure

I. Extraction of sinapic acid:

1. Extraction of free sinapic 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) = 1:10~20 ratio (it is recommended to weigh 0.15 g of dried sample, add 1.5 mL of extract solution) to a 2 mL EP tube, seal, mix evenly, and then put it into an ultrasonic cleaning machine, and extract ultrasonically for 60 min at room temperature (25~35 °C, pay attention to the temperature should not be too high during ultrasonic). Centrifuge at 10,000 rpm for 10 min at 4°C, take the supernatant (if there are still solid samples in the supernatant, it can be centrifuged again), store (seal) at 4°C, filter into the brown sample bottle with an organic syringe filter before testing, and wait for testing (place at room temperature before testing, if the supernatant is too dark or too dense, it can be diluted and filtered again for testing).

2. Extraction of total sinapic 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 extract two (mL) is 1:10~20 ratio (it is recommended to weigh 0.15 g of dried sample, add 1.5 mL of extract two) to the reagent bottle, weigh, mix evenly, and then put it into an 80 °C constant temperature water bath for 2 h (it is recommended to use a wire reagent bottle and tighten it to prevent the bottle from collapsing during heating). After cooling, weigh again, and add extract 2 to make up the difference. Seal, put it in an ultrasonic cleaning machine, and extract it at room temperature (25~35°C, pay attention to the temperature should not be too high during ultrasonic) for 60 min. Then transfer the extracted turbidity solution to an EP tube, centrifuge at 10000 rpm at 4°C for 10 min, take the supernatant (if there are still solid samples in the supernatant, it can be centrifuged again), store (seal) at 4°C, filter it into the brown injection bottle with an organic syringe filter before the test, and wait for testing (place it at room temperature before the test, if the supernatant is too dark or too dense, it can be diluted and filtered again for testing)

II. Determination procedure:

1. Turn on the computer, turn on the switch buttons of each module of the liquid chromatograph,

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install the chromatographic column, open the software, set the injection volume to 10 μL, the column temperature: room temperature (about 30°C), the flow rate is 0.8 mL/min, the wavelength is 360 nm, the elution

program is as shown in the following table, the dissampling time is 70 min, and the method set is saved after setting.

2. Wash the column with the appropriate mobile phase, equilibrate the column with acetonitrile: mobile phase A = 20:80 mobile phase, and start loading after the baseline is stable.

3. Detect the standard solution to be tested, the injection volume is 10 μL, the sinapic acid can be separated within 30 min, and the retention time of sinapic acid is about 8.2 min (the retention time is different depending on the system, column, mobile phase pH, temperature, etc., and is only used as a reference).

4. Detect the sample solution to be tested with a 10 μL injection and measure the peak area of the sinapic acid at the corresponding retention time. (If you need continuous testing, you can perform experiments in the table below).

Time	Mobile phase	
	Phase B	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. Calculation:

Plot a standard curve for sinapic acid with the concentration of the standard solution (mg/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 (mg/mL) of sinapic acid in the extraction solution.

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

V: The volume of extract solution, 1.5 mL;

W: Sample weight(g).

For samples tested after dilution, multiply by the corresponding dilution factor before calculation.

Note:

1. After the test, turn off the heater heating system and wait for the column temperature to drop to room temperature or below before stopping the mobile phase.

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2. After the test, you need to rinse the column with a high concentration of ultrapure aqueous phase (about 20-30 column volumes) to prevent blocking of the column, then rinse the column with a high concentration of organic phase, and finally rinse according to the type of column specification to prevent damage to the column.

3. The dilution factor of the standard should be determined according to the concentration of sinapic acid in the sample, and the peak area of the sinapic acid in the sample must be within the peak area of the standard solution of different concentrations, and the dilution factor of the standard is only a reference. If the concentration of sinapic acid in the sample is too high, it is recommended to dilute it and test again.

4. If the sample volume 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, and all the solutions to be tested must be left at room temperature before testing.

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