Fructose 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 : BC2454

Size: 50T/48S

Components:

Reagent I: Liquid 2.5 mL ×1, Storage at 2-8℃.

Reagent II: Powder ×1 , Storage at 2-8 ℃. Dissolve thoroughly by adding 2.5 mL distilled water before application.

Standard: Powder ×1 , Storage at 2-8℃. A standard solution of 20 mg/mL fructose was prepared by adding 5 mL distilled water before use, and the solution was sealed and storage at 2-8℃.

Description:

Fructose is a monosaccharide composed of six carbon atoms and is the isomer of glucose. It is abundant in the free state in fruit juices and honey. D-fructose is the sweetest monosaccharide and can also combine with glucose to form sucrose.

Fructose content was determined using a differential refractive detector.

Instruments and reagents required in the test:

Reagents and Equipment Required but Not Provided:

High performance liquid chromatograph (Agilent ZORBAX Carbohydrate column (4.6×250 mm, sugar analysis column)), differential refractive detector (RID), desktop centrifuge, adjustable pipet gun, mortar/homogenizer, EP tube, needle filter (organic system, water system, 0.45 μm), syringe, suction filter, filter membrane (organic system, water system), brown injection bottle (1.5 mL), acetonitrile (chromatography pure), ultra-pure water, ultrasonic cleaner.

Preparation before The Experiment:

1. The chromatographic pure acetonitrile was filtered by organic filter membrane and ultra-pure water was filtered by aqueous filter membrane, and 75% acetonitrile-aqueous solution was configured by the extracted acetonitrile and ultra-pure water as mobile phase. (Acetonitrile was pumped with 0.45μm organic filter membrane, and ultrapure water was pumped with 0.22μm water filter membrane).
2. The prepared mobile phase was sonicated for 20 min to remove the gas in the solvent.
3. Preparation of standard substance: 20 mg/mL fructose standard solution was diluted into 10mg/mL, 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL fructose standard solution with distilled water. (The prepared standard concentration is for reference only and can be adjusted according to the actual sample concentration). Seal and store at 2-8 ℃ in the dark. Filter into brown injection bottle by water needle type filter before testing (please put it in room temperature before testing).

Protocol:

I. Sample Extraction

1. Low Protein Content Sample (plant tissue, etc.)

1) **Solid sample:** The tested sample were fully ground, added into 2 mL EP tubes according to the ratio of mass (g): distilled water volume (mL)1:10~20 (it is recommended to weigh 0.1 g

sample and add 1 mL distilled water), sealed, mixed evenly, and then put into an ultrasonic cleaner, ultrasonic extraction at room temperature for 30 minutes. After centrifugation at 15000 rpm for 15 min, the supernatant was taken (if necessary, the filter residue was extracted again, and the supernatant was combined), filtered into a brown injection bottle through a water needle type filter, and tested.

2) **Liquid sample:** According to the ratio of sample volume (mL): distilled water volume (mL)1:10~100 (it is recommended to weigh 50 μL of sample and add 0.95 mL of distilled water), add it to 1 mL EP tube, seal it, mix it well, and centrifuge at 15000 rpm for 10 min. The supernatant was filtered into a brown injection bottle using a water needle filter and tested (if there was still turbidity in the supernatant, the supernatant could be diluted and centrifuged again).

2. Protein-rich sample (animal tissues, etc.) : If the sample contains a lot of protein, after extraction or dilution according to the above methods, take 900 μL supernatant, add 50 μL reagent 1, 50 μL reagent 2, let it stand for 10 min, centrifuge at 15000 rpm for 15 min, take the supernatant, filter it into a brown injection bottle through a water needle filter, and be tested.

3. Cell sample: According to the ratio of cell number (10^4): distilled water volume (mL)=1000:1 (it is recommended to weigh 10 million cells and add 1 mL distilled water), add them into 1 mL EP tubes, seal, mix evenly, and extract them by ultrasound at room temperature for 30 minutes. After centrifugation at 15000 rpm for 10 min, the supernatant was filtered into a brown injection bottle using a water needle filter and tested (if there was still turbidity in the supernatant, the supernatant could be diluted and centrifuged again).

II. Measurement Steps

1. Turn on the computer, turn on the switch buttons of each module of the liquid chromatograph, install the chromatographic column, and open the software. In the method group, set the sample size to 10μL, column temperature to 35℃, flow rate to 1 mL/min, detection pool temperature to 35℃, sampling time to 12 min, and the preservation method group was set.
2. Use the corresponding mobile phase to clean the column, use the prepared mobile phase balance column, and start adding sample after the baseline is stable.
3. Test the standard solution to be measured, the sample size is 10μL, the fructose can be separated within 12 min, and the retention time of fructose is about 9 min (The difference of column, mobile phase, temperature, etc., may cause the deviation of the retention time, for reference only).
4. Test the sample solution to be measured, the injection volume is 10 μL, and the peak area of fructose is detected at the corresponding retention time. Repeat Step 2 for the next sample.

III. Calculations

The standard curve of fructose 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 fructose in the extract.

1. Almost no protein sample

Solid sample: Fructose content (mg/g) =x ×$V_{extraction}$ ÷W=x÷W

$V_{extraction}$: total volume of distilled water added, 1 mL; W: sample quality, g.

Liquid sample: Fructose content (mg/mL) = x ×V_{total} ÷V_{sample} =20x

V_{sample}: the total volume of liquid sample added, 0.05 mL; V_{total}: total volume of liquid sample versus added distilled water, 1 mL.

2. Protein-rich sample

Solid sample: Fructose content (mg/g) = x×1.11×V_{total} ÷W =1.11x÷W

Liquid sample: Fructose content (mg/mL) = x×1.11×V_{total} ÷V_{sample} =22.2x

V_{total}: the total volume of the first sample treatment, 1 mL; 1.11: sample dilution, (0.9+0.05+0.05) ÷0.9=1.11; W: sample quality, g; V_{sample}: sample volume, 0.05mL.

4. **Cell sample**

Fructose content (mg/10^4 cells) =x ×V_{total}l ÷N=x÷N

V_{total}: total volume of distilled water added, 1 mL; N: number of cells added, in units of 10^4.

Note :

1. After the test is completed, close the column temperature box and the detector heating system to cool down, and wash according to the type of column to prevent damage to the column.
2. The dilution ratio of the standard product should be determined according to the concentration of fructose in the sample, and the peak area of fructose in the sample must be within the peak area of the standard solution of different concentrations. The dilution ratio of the standard product is only a reference. If the concentration of fructose in the sample is too high, it is recommended to dilute and then measure.
3. If the sample size is too large, it is recommended to test the standard solution once a day (one standard solution can be used) to determine the corresponding retention time. All the solution to be tested must be placed at room temperature before testing.
4. If necessary, a blank sample can be detected and the influence of reagent factors can be deducted.
5. The sample tested after dilution need to be multiplied by the corresponding dilution multiple before calculation.