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Study on Test Method for the Quantitation of Individual Sugar and Sugar Alcohol in Protein Powder by LC/MS/MS

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Abstract

Sugar and sugar alcohol were added to the health benefit product to give a favorite flavor of the products, but people have conditions that need sugar replacement such as sugar alcohol. Thus, a new LC/MS/MS method was developed to separate and quantify fructose, glucose, saccharose, maltose, lactose, and sorbitol in protein powder and tablet. With using of an MS detector with an ESI source, the mass information of the sugar and sugar alcohol components can be obtained along with the retention times, combination of chromatographic retention time and molecular mass weight improves the selectivity of sugar and sugar alcohol analysis. The linearity range of fructose is 2.13-83.26 μg/mL, sucrose is 2.05-80.12 μg/mL, glucose is 3.07-120.08 μg/mL, maltose is 7.26-283.41 μg/mL, lactose is 7.56-295.41 μg/mL, sorbitol is 1.98-77.42 μg/mL. The recoveries of each sugar component and sorbitol from protein powder product are 91%-110%. The matrix of protein powder, tablets, soft gel, and capsule with a matrix of soy protein isolate was validated, showing that the method is reliable and suitable for routine use.

Keywords

Sugar, sugar alcohol, LC/MS/MS, ESI source

1. Introduction

Sugar and sugar alcohol were added to the health benefit product to enhance the flavor of the products [1-3]. They are one of the important nutrients that people pay attention to [4], but individuals with conditions (such as diabetes) need sugar replacements such as sugar alcohol [5-8]. Sugar alcohols are low in calories and are less sweet than regular table sugar, so they are commonly used as fillers for low-calorie sweeteners or high-sweetness sweeteners, and sugar alcohols can replace sugars that are high in sugar and keep the body's blood sugar levels from rising. But the ratio plus compositional changes will change the taste and flavor, to prevent this happens, an accurate measurement of sugar and sugar alcohol is needed to prevent the adding sugar from having a negative impact on the populations with conditions and protect the exact taste and flavors designed for the products.

Conventional sugar testing methods using HPLC [9, 10], IC [11], UPLC [12, 13], or GC is limited to separating the individual sugars and sugar alcohols [14-16], such as glucose and sorbitol in both of HPLC with RI and GC/MS method. HPLC technology is usually used with RI or ELSD detection [17]. RI detection requires careful control of the mobile phase, there are carbohydrate columns and 75% acetonitrile used, the range of application is relatively narrow and limited to gradient elution, and the results were easily affected by the maltodextrin matrix. ELSD detection is relatively reliable for single composition, the amide column was used, and elution by combined mobile phase of acetonitrile and water with 0.2% triethylamine, but it is often not possible to detect in complex matrices to obtain the sensitivity required for sugar. GCMS has several advantages by using the column of mid-polar 14% cyanopropylphenyl polysiloxane, such as high resolution, high sensitivity and easy coupling to different detectors, including mass spectrometers. However, the GC/MS

method involves the use of various derivatization reactions, there are more byproducts in the reaction process, the derivation procedure takes time and is hard to control for some isomers [18], such as the mixture of sorbitol and mannitol can be obtained by reducing fructose.

In this study, we developed an LC/MS/MS method to separate and quantify fructose, glucose, saccharose, maltose, lactose, and sorbitol. While an ESI detector can obtain the components' mass information, the combination of chromatographic retention time and mass information improves the selectivity of individual sugars and sugar alcohol analysis. Better selectivity and sensitivity can be achieved by mass spectrometry. This method will help us better screen and quantify target individual sugars or sugar alcohols and could reduce false positive results [19]. LC/MS/MS is a technology usually reserved for accurate measurement of low-concentration or sensitive compounds, although it is rarely used for general sugar analysis because of the cost and complexity, its combination of chromatographic retention time and molecular mass weight improves the selectivity and specify of sugars and sugar alcohol analysis. Better selectivity and sensitivity can be achieved by mass spectrometry, in particular accurate quantification of sugars and sugar alcohols as flavoring.

2. Methods

The sample containing 50 mg of Sugar or Sorbitol was extracted by 50/50 Acetonitrile and Water (1:1) with sonication, followed by a dilution with the same solution. An Amide 2.5 μ 3.0 x 150mm column with gradient elution was used for a gradient separation.

MS QQQ Mass Spectrometer

(a) Ion Source: ESI
(b) Gas Temp (℃): 350
(c) Gas Flow (L/min): 12
(d) Nebulizer (psi): 40
(e) Capillary (V): 3000
(f) Scan Segments
(g) Calculate

Spl. Conc. = Sample concentration from the standard curve SS = Serving size

D.F. = Dilution factor for sample ATW = Average tablet weight

Calculate individual sugars (applies to all standards including fructose, sucrose, lactose, and maltose) and sorbitol as follows:

$$\text{(ng/tab or serving)} = \frac{\text{Spl. Conc. (ng/mL)} \times \text{D.F.(mL)} \times \text{ATW or SS (g/tab or serving)}}{\text{Spl. Wt.(g)}}$$

Summation test results for each individual sugar to get the total sugar content in the sample and report the results per serving size.

Table 1. Scan Segments

Component	Prec Ion	Prod Ion	Dwell	Frag (V)	CE(V)	Cell Acc	Polarity
Fructose	179.1	89.1	120	60	5	4	Negative
Glucose	179.1	89.1	120	54	5	4	Negative
Sorbitol	181.1	101.1	120	92	11	4	Negative
Sucrose	341.1	119.0	120	134	15	4	Negative
Maltose	341.1	161.1	120	54	3	4	Negative
Lactose	341.1	161.1	120	116	3	4	Negative

3. Experimental

3.1 Reagents and Equipment

(a) Acetonitrile, HPLC grade or equivalent
(b) Methanol LC-MS grade or equivalent
(c) Guanidine Hydrochloride, AR grade or equivalent
(d) Diethylamine AR grade or equivalent

(e) Isoprapanol HPLC or equivalent
(f) Analytical Balance
(g) Sonicator
(h) Volumetric flasks, volumetric pipettes, graduated cylinders; Class A

3.2 HPLC chromatographic conditions

(a) Mobile Phase: A = Acetonitrile: Isopropanol: Water = 900:50:50
(b) Mobile Phase: B = Acetonitrile: Water = 800:
(c) Gradient conditions:
(d) Column temperature: 80 °C
(e) Sample temperature: ambient
(f) Injection volume: 2 µL

Table 2. Gradient Table

Time min	Flow mL/min	Solv Ratio %A	Solv Ratio %B
0	0.8	100	0
4.5	0.8	100	0
16.0	0.8	80	20
23.0	0.8	80	20
35.0	0.8	0	100
40.0	0.8	100	0

3.3 Standard Preparation

Accurately weigh about 20 mg of Fructose, Sucrose, sorbitol, 30 mg of Glucose, and 75 mg of Maltose and Lactose into a 25 mL volumetric flask, add about 15 mL of extracting solution, and sonicate the solution for 10 minutes. Dilute to volume with extracting solution (conc. 0.8 mg/mL for Fructose, Sucrose, and Sorbitol, 1.2 mg/mL for Glucose, and 3.0 mg/mL for Maltose and lactose). Mix well. Dilute to working standard range with extracting solution (conc. 2.56 µg/mL, 12.8 µg/mL, 64.0 µg/mL for Fructose)

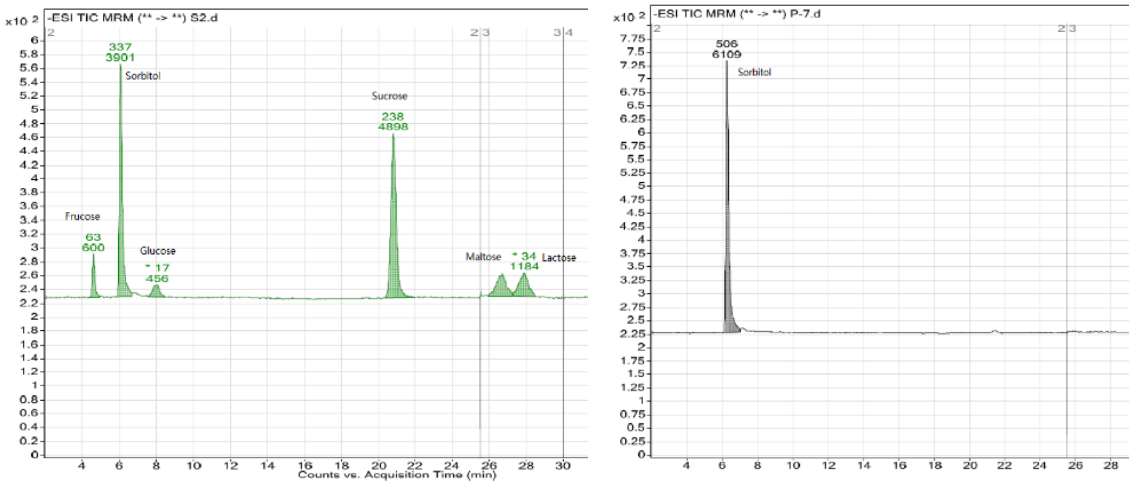
3.4 Sample Preparation

(a) Tablets, Powder or Soft Gel, and Hard capsule: These are sample that contains protein, sugar/ sugar alcohol (about 7%) maltodextrin, vitamins, minerals, caffeine, botanical extracts, and other materials. Grind the tablets to a fine powder using a grinder. Accurately weigh, an amount of sample containing about 50 mg of Sugar or Sorbitol into a 100 mL volumetric flask.
(b) Protein Powder: Those are higher protein-contained samples, the matrix contained soy protein isolate, cellulose, vitamins, minerals, botanical extracts, and other materials, and there is about 36% sugar contained. Accurately weigh, an amount of sample containing about 50 mg of Sugar or sorbitol into a 100 mL volumetric flask.
(c) Extract:
    (1) Add about 50 mL of extracting solution. Vortex the sample for about 30 seconds.
    (2) Sonicate for 15-20 minutes and QS to volume with extracting solution and mix well.
    (3) Pipette 5 mL of the above sample solution into a 100 mL volumetric flask and dilute to volume with extracting solution and mix well.
    (4) Filter a portion of the sample through 0.2 µ syringe filter, discard the first few drops of the filtrate, and then fill an HPLC vial.

4. Results and Discussion

4.1 Specificity

Blank, mixed standard, and sample solutions were prepared and analyzed following the test method. The chromatogram of blank and sample were compared with those of Standard solutions. The detected sugar peaks in the chromatograms of the sample were located by comparing to each standard. No significant peak is present in the chromatogram of the blank at the retention times of each sugar and sorbitol peak from standard solution (Figure 1).



Standard Solution Sample Blank

Figure 1. Chromatogram of Standard and Samples Blank (ACN: Water = 1:1)

4.2 Precision

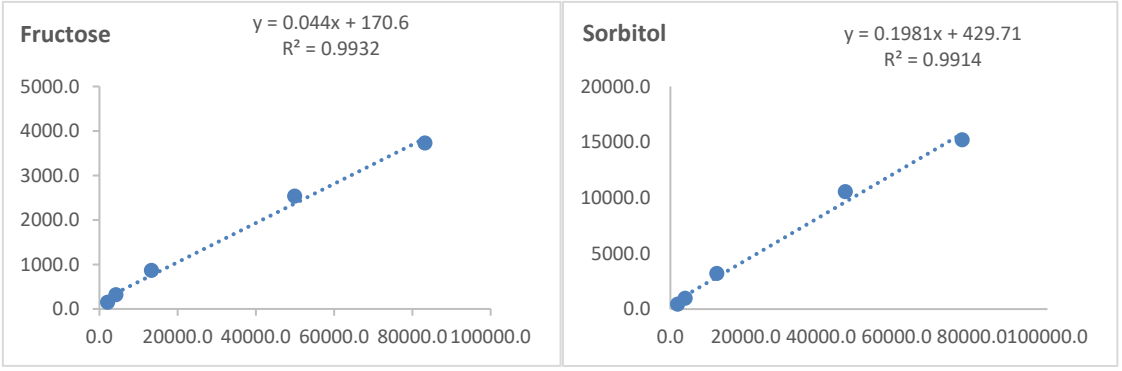
Six replicates of each sample were prepared following the test method. The samples were analyzed versus freshly prepared standard solution. The amounts of total sugars and sorbitol in samples were calculated, the RSDs of six results of total sugars and sorbitol were not more than 4%.

4.3 Accuracy

The spiked samples were prepared by adding known quantities of sugars and sorbitol Standard Solution to samples, Mixed standard solution at concentration (849, 150 ppb of Fructose, 318,944 ppb of Sucrose, 304,727 ppb of sorbitol, 1,180,618 ppb of Glucose, 2,224,379 ppb of Maltose, and 2,814,980 ppb of Lactose) was prepared. Nine (9) replicates of each sample at the normal test concentration were prepared. Known quantities of the mixed stock solution were added to the sample solutions. These spiked samples (three concentrations and three replicates of each concentration) were analyzed according to the test method. The amount of each component in the spiked samples measured versus the spiked amounts of corresponding component in the sample was calculated as % Recovery respectively, the recoveries for all spiked levels of each component were within 91-110%.

4.4 Linearity/Range

Working standard solutions containing 5 levels of each component (Fructose, Sorbitol, Glucose, Sucrose, Maltose and Lactose) were prepared by making dilutions from the mixed stock solution. Three (3) replicate injections were made for each of the five (5) solutions. The average peak areas of each component obtained for each solution were plotted against their corresponding theoretical concentration. Linear regression analyses on the five coordinates were performed. The R^2 of the linear curve were 0.990-1.000 for those components (Figure 2).



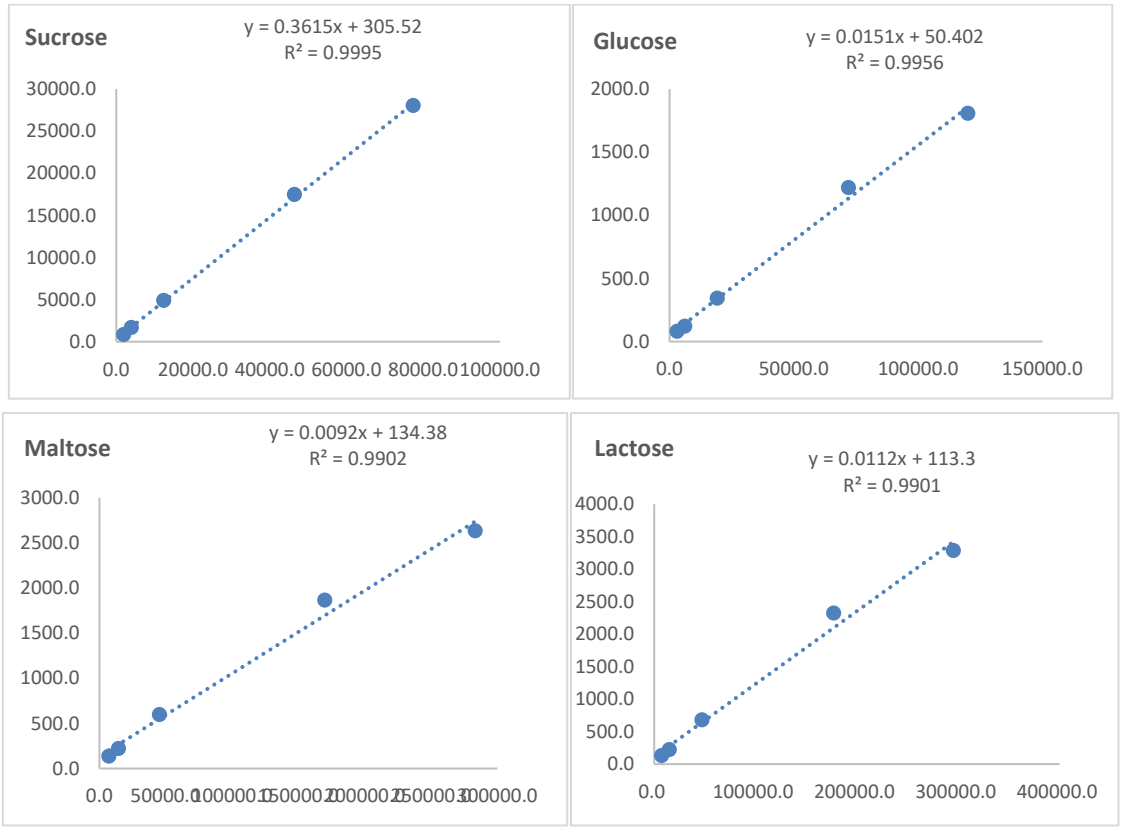


Figure 2. Calibration Curve for each component.

4.5 Ruggedness

The same lots of each sample were analyzed in duplicate by a second analyst on a different day, using a different column. The results were compared to those from the precision test performed by the first analyst. The RSD of total sugar and sorbitol amount from both analysts (6 precision and 2 ruggedness) for each sample were within 2%.

5. Conclusion

A simple and sensitive LC/MS/MS analytical method for the quantitation of sugar and sugar alcohol in a matrix of protein powder, tablets, softgel, and capsule with a matrix of soy protein isolate was validated, showing that this method is reliable and suitable for routine use. This method demonstrated excellent coefficient of determination, precision, accuracy, ruggedness, and specificity. The LC/MS/MS method was validated to quantify fructose, glucose, saccharose, maltose, lactose, and sorbitol in protein matrix samples.

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