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Determination of Sugars in Sports Drinks

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Abstract

Mono and disaccharides are routinely quantified with a differential refractometer. However, we developed a simpler analytical method involving pre-derivatization and high- performance liquid chromatography to achieve a high recovery rate. The purpose of our study was to understand the amount of sugar in sports drinks in order to determine marked consumption leads to tooth decay. Therefore, we quantified sugars in sports drinks.

Keywords: Glucose; Pre-derivatization; High-Performance Liquid Chromatography

Introduction

Sugars are one of the five essential nutrients, being indispensable for human activities. Sugars are a direct energy source for activities such as exercise. Of the different sugars, monosaccharides (glucose and fructose) are essential for brain activities. Monosaccharides are often analyzed with a differential refractometer [1] (RI) using an amide column. In addition, a post-derivatization (post-column) method [2] can be used. However, this method requires expensive and complicated instruments. Here, we quantified mono- and disaccharides employing an inexpensive and simpler pre-derivatization (pre-column) method. Our purpose was to understand the amount of sugar in sports drinks to determine how marked consumption leads to tooth decay [3]. To achieve this, we quantified sugars in sports drinks. The study purpose, to reveal the Concentration of sugars in sports drinks.

Materials and Methods

Reagents

1. Aminobenzoate ethyl ester
2. Phosphoric acid
3. Acetic acid
4. Phenylhydrazine (Wako Pure Chemical Industries, Ltd.).
5. Sodium cyanoborohydride (Nacalai Tesque)

All reagents used were high grade.

Instruments

1. HPLC: LC2 0A-PDA and RF (Shimadzu)

HPLC conditions

a. **Chromatography conditions for glucose and maltose**

Column: COSMOSIL 3 x 100 Mobile phase: Acetonitrile and methanol (1: 1): 0.5% Acetic acid =3: 7

Flow rate: 0.2ml/min

Column temperature: 45°C

UV 307 nm

b. **Chromatography conditions for fructose and sucrose**

Column Intersil Ph-3 4.6 x 150

Mobile phase: Acetonitrile and methanol (1: 1): Water =35: 65

Flow rate: 1.0ml/min

Column temperature: 45°C

Fluorescence detector: 330 nm

Emission: 470 nm

Derivatization of glucose and maltose (UV)

To 5ml of 100-µg/ml glucose, We added 400 µl of 1.4-M sodium cyanoborohydride, 400 µl of acetic acid, and 2ml of 0.6-M aminobenzoate ethyl ester (methanol), followed by heating at 80°C for 10 minutes. The solution was subsequently cooled to room temperature. Then, 2ml of distilled water was added to the solution. The aqueous phase was washed with 4ml of chloroform to remove the aminobenzoate ethyl ester. Then, the aqueous phase was injected into a high performance liquid chromatography (HPLC) column.

Derivatization of fructose and sucrose (Fluorescence)

To 1ml of 100-µg/ml fructose, was added 1ml of hydrazine solution (phosphoric acid: acetic acid: phenylhydrazine =110: 90: 3), followed by reaction at 150°C for 10 minutes. Then, the solution was cooled to room temperature and injected into an HPLC column. This pre-derivatization and HPLC condition were developed independently adjusted. The derivatized with each method divided into two sports drinks, and the aqueous phase was determined with HPLC. Figure 1 show the reaction of making (UV) to the derivatization. The fluorescence derivatization followed the well-known "Fischer synthesis" method.

Results and Discussion

Mono- and disaccharides were measured using the absolute calibration method. The calibration curve of five points (UV and

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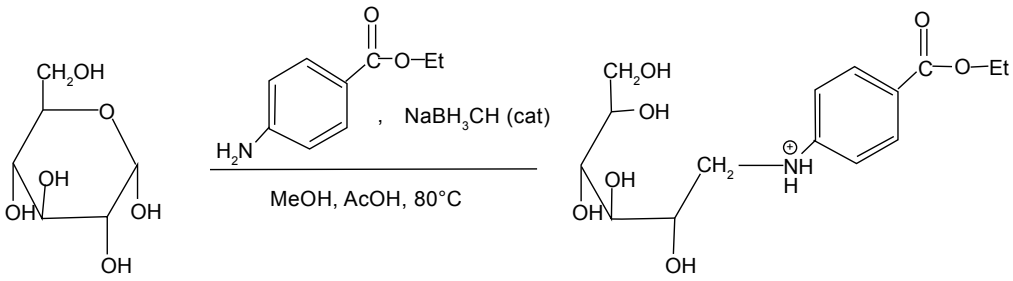

Figure 1: Labelling reaction of glucose with p-aminobenzoic ethyl ester.

fluorescence) was the first regression line. As for r, 0.9999 was obtained (1, 10, 100, 500, and 1000 mg/L).

The results of addition-recovery (sugar-free drink) experiments (1, 10 and 100 mg in 1 L) of glucose, maltose, fructose, and sucrose are shown in Table 1. The recovery rate was as high as 90%. The precision of quantification was marked.

This time, the method to determine the developed sugar was the fixed limit of the quantification value of 0.1 mg/dl. The amount of sugar in the sports drinks is shown in g/dl. The method to determine the sugar developed this time is barely an influenced by impurities. This time, the determination method of the developed sugar is a method that can be used enough in honey (mono- and disaccharides are contained in large quantities). The validation is relevant.

A fructose-glucose or glucose-fructose solution is used in commercial sports drinks. The excessive intake of sports drinks causes dental problems [3]. Thus, quantification of the exact sugar concentrations is important. Here, the four sugars were quantified using our method. The results are given in Table 2. Chromatogram of HPLC to separate sugars in sample was complete. The coefficient of variation was 0.1% or less.

Substance	Trials	Added	Added
Glucose	5	100 mg	98.5
		10 mg	98.2
		1 mg	99.1
Fructose	5	100 mg	98.3
		10 mg	97.4
		1 mg	99.2
Maltose	5	100 mg	97.9
		10 mg	99.6
		1 mg	98.9
Sucrose	5	100 mg	99.1
		10 mg	98.4
		1 mg	97.3

Table 1: Recoveries of glucose fructose, maltose, and sucrose.

	Glucose	Fructose	Maltose	Sucrose (g/dl)
Drink a	1.154±0.003	1.195±0.003	0.001±0.000	4.415±0.004
Drink b	0.412±0.001	0.895±0.001	0.064±0.000	4.381±0.004
Drink c	0.007±0.000	1.245±0.002	0.075±0.000	0.716±0.001

Table 2: Sugar in low-calorie sports drinks.

The correlation coefficient with the already-known (RI method and post-column) method [1,2] and this new method were 0.96.

It fully agreed with the fixed quantity value specified in the law [1,2]. 100ml of a low-calorie sports drink (drink c) contained approximately 1g (less than 4 kcal) of the four sugars combined, as indicated on the label (Carbohydrate). The two other drinks contained the same amount of sugar as indicated for carbohydrates on the label.

The advantages of the newly developed determination method are ease of operation and the use of an inexpensive instrument [1,2], Up to 40% can be reduced due to the simplicity of the operation, while up to50% can be reduced as a result of the economy of the machine [4,5]. References of statistical analysis were used.

In conclusion, a large amount of glucose and fructose is contained in sports drinks (exact amounts of glucose and fructose are not displayed).

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