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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, the researchers developed a simpler analytical method involving pre-derivatization and high-performance liquid chromatography to achieve a high recovery rate. The purpose of the study is to understand the amount of sugar in sports drinks in order to determine how large doses lead to tooth decay. To dose so the researchers quantified sugars in sports drinks.

Key words: Glucose, pre-derivatization, high-performance liquid chromatography, sports drinks, dose, Japan

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, mono-saccharides (glucose and fructose) are essential for brain activities. Mono-saccharides are often analyzed with a differential Refractometer (Gomis *et al*., 2001) (RI) using an amide column. In addition, a post-derivatization (post-column) method can be used. However, this method demands expensive and complicated instruments. Here, we quantified mono and disaccharides employing an inexpensive and simpler pre-derivatization (pre-column) method. The purpose is to understand the amount of sugar in sports drinks to determine how larges lead to tooth decay. To achieve this, we quantify sugars in sports drinks.

MATERIALS AND METHODS

Reagents: Aminobenzoate ethyl ester, phosphoric acid, acetic acid and phenylhydrazine (Wako Pure Chemical Industries, Ltd.). Sodium cyanoborohydride (Nacalai Tesque). All reagents used were high grade.

Instruments:

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

HPLC conditions:

- Chromatography conditions for glucose and maltose
- Column: COSMOSIL 3×100 Mobile phase: Acetonitrile and methanol (1:1): 0.5% acetic acid = 3:7
- Flow rate: 0.2 mL min^{-1}
- Column temperature: 45°C
- UV 307 nm

Chromatography conditions for fructose and sucrose:

- Column intersil Ph-3 4.6×150
- Mobile phase: Acetonitrile and methanol (1:1): Water = 35:65
- Flow rate: 1.0 mL min^{-1}
- Column temperature: 45°C
- Fluorescence detector: 330 nm
- Emission: 470 nm

Derivatization of glucose and maltose (UV): To 5 mL of 100 μg mL^{-1} glucose were added 400 μL of 1.4 M sodium cyanoborohydride, 400 μL of acetic acid and 2 mL of 0.6 M aminobenzoate ethyl ester (methanol) followed by heating at 80°C for 10 min. The solution was subsequently cooled to room temperature. Then, 2 mL of distilled water was added to the solution. The aqueous phase was washed with 4 mL of chloroform to remove the aminobenzoate ethyl ester. Then, the aqueous phase was injected into an High Performance Liquid Chromatography (HPLC) column.

Derivatization of fructose and sucrose (fluorescence): To 1 mL of 100 μg mL^{-1} fructose was added 1 mL of hydrazine solution (Phosphoric acid: acetic acid: phenylhydrazine = 110:90:3) followed by reaction at 150°C for 10 min. Then, the solution was cooled to room temperature and injected into an HPLC column.

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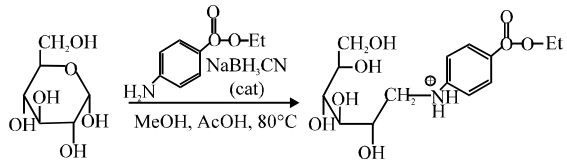

Fig. 1: Labeling reaction of glucose with P-aminobenzoic ethyl ester

dividing the specimen material of the sports drink into two and the aqueous phase was measured with HPLC. Figure 1 shows the reaction of making (UV) to the derivatization. The fluorescence derivatization followed 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 fluorescence) was the first regression line. As for r, 0.9999 was obtained (1, 10, 100, 500 and 1000 mg L^{-1}).

The results of addition-recovery (sugar free drink) experiments (1, 10, 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 determination method of the developed sugar was the fixed limit of quantification value of 0.1 mg dL^{-1}. The amount of sugar in the sports drinks is displayed in g dL^{-1}. The determination method of the sugar developed this time is a method of hardly receiving an influence from impurities. This time, the determination method of the developed sugar is a method that can be used enough in honey that mono and disaccharides are contained in large quantities.

A fructose-glucose or glucose-fructose solution is used in commercial sports drinks. The excessive intake of sports drinks causes dental problems. Thus, quantification of the exact sugar concentrations is important. Here, four sugars were quantified using the method. The results are shown in Table 2. The coefficient of variation was 0.1% or less.

The correlation coefficient with already-known (RI method and post-column) method (Gomis *et al*., 2001) and this new method were 0.96. It completely agreed to the fixed quantity value in the law so far (Gomis *et al*., 2001). About 100 mL of a low-calorie sports drink (drink c) contained approximately 1 g (<4 kcal) of 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.

Table 1: Recoveries of glucose fructose, maltose and sucrose

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

Table 2: Sugar in low-calorie sports drinks

	Sugar (g dL^{-1})			
Drink	Glucose	Fructose	Maltose	Sucrose
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

The advantages of the newly developed determination method are handiness of operation and the use of an inexpensive instrument (Gomis *et al*., 2001). Up to 40% can be reduced through the handiness of the operation while up to 50% can be reduced through the economy of the machine (Blanken *et al*., 1985; Vincken *et al*., 1998).

CONCLUSION

The study shows that a large amount of glucose and fructose are contained in sports drinks (exact amounts of glucose and the fructose are not displayed).

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