Research Paper

“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 (RI) using an amide column. In addition, a post-derivatization (post-column) method can be used. However, this method requires expensive and complicated instruments. Here, we quantified mono- and disaccharides employing an inexpensive and newly 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. 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
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 x 100 Mobile phase: Acetonitrile and methanol (1: 1): 0.5% Acetic acid =3: 7
Flow rate: 0.2 ml/min
Column temperature: 45°C
UV 307 nm

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: 1.0 ml/min
Column temperature: 45°C
Fluorescence detector: 330 nm
Emission: 470 nm

Derivatization of glucose and maltose (UV)
To 5 ml of 100-µg/ml glucose, We 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 minutes. 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 fructose, was added 1 ml 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 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.

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, the four sugars were quantified using our method. The results are given in Table 2. Chromatogram of HPLC to separate sugars in sample were complete. The coefficient of variation was 0.1% or less.

The advantages of the newly developed determination method are ease of operation and the use of an inexpensive instrument. One hundred ml of a low-calorie sports drink (drink c) contained approximately 1 g (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 correlation coefficient with the already-known (RI method and post-column) method and this new method were 0.96. It fully agreed with the fixed quantity value specified in the law.

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The advantages of the newly developed determination method are ease of operation and the use of an inexpensive instrument. Up to 40% can be reduced due to the simplicity of the operation, while up to 50% can be reduced as a result of the economy of the machine. 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
References
2) H.Inoue. Ehimekenkajyukenshi 17(2003)7-17
3)"kenkounaha3” Tsuyama-shika-ishikai 2006

Table 1  Recoveries of glucose fructose, maltose, and sucrose

<table>
<thead>
<tr>
<th>Substance</th>
<th>Trials</th>
<th>Added</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>5</td>
<td>10 mg</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 mg</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 mg</td>
<td>98.3</td>
</tr>
<tr>
<td>Fructose</td>
<td>5</td>
<td>10 mg</td>
<td>97.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 mg</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 mg</td>
<td>97.9</td>
</tr>
<tr>
<td>Maltose</td>
<td>5</td>
<td>10 mg</td>
<td>99.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 mg</td>
<td>98.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 mg</td>
<td>99.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>10 mg</td>
<td>98.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 mg</td>
<td>97.3</td>
</tr>
</tbody>
</table>
Table 2  Sugar in low-calorie sports drinks

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Fructose</th>
<th>Maltose</th>
<th>Sucrose</th>
<th>g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drink a</td>
<td>1.154±0.003</td>
<td>1.195±0.003</td>
<td>0.001±0.000</td>
<td>4.415±0.004</td>
</tr>
<tr>
<td>Drink b</td>
<td>0.412±0.001</td>
<td>0.895±0.001</td>
<td>0.064±0.000</td>
<td>4.381±0.004</td>
</tr>
<tr>
<td>Drink c</td>
<td>0.007±0.000</td>
<td>1.245±0.002</td>
<td>0.075±0.000</td>
<td>0.716±0.001</td>
</tr>
</tbody>
</table>
Fig. 1. Labeling reaction of glucose with p-aminobenzoic ethyl ester.