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Application Note

Food Beverages

Quantitative Analysis of Sugars in Commercial Beverages Using High-Performance Liquid Chromatography Coupled with a Refractive Index Detector (RID-20A)

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Food & Beverages

1. Introduction

Sugars are low molecular weight carbohydrates that play a critical role in human metabolism, acting as primary energy substrates[1]. They are commonly present in a wide range of food and beverage matrices, both as naturally occurring components and as added sweeteners.

Sucrose (glucose + fructose) is widely used in beverages and rapidly absorbed, causing glycemic spikes. Glucose is the main cellular energy source, but excessive intake increases diabetes risk. Fructose, mainly metabolized in the liver, may promote fat accumulation and metabolic disorders. Lactose (glucose + galactose) requires lactase for digestion; deficiency leads to lactose intolerance.

Soft drinks and energy beverages often contain high levels of added sucrose and glucose-fructose syrups, contributing to elevated glycemic load. Wine sugars originate from grapes, primarily glucose and fructose, with residual sugars present in sweet varieties. Excess sugar intake is linked to obesity, diabetes, cardiovascular diseases, and fatty liver conditions. Differentiating intrinsic vs. added sugars is essential for accurate nutritional analysis and food regulation.

This research presents a validated approach for detecting four different sugars (refer, Fig. 1) in natural and artificial drinks using LC-2060 3D coupled with RID-20A from Shimadzu Corporation Japan.

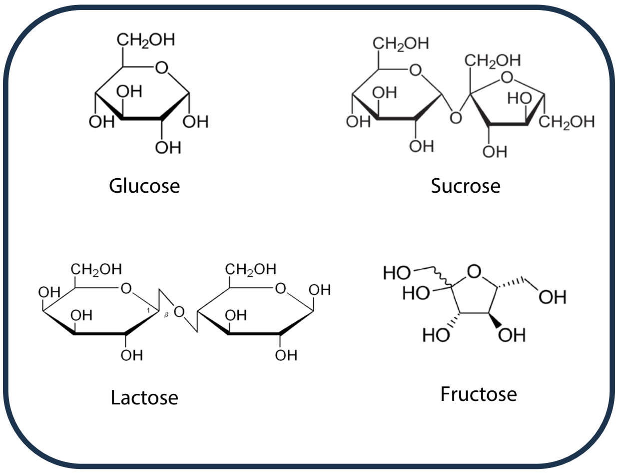

Fig. 1 Representative structures of sugars

2. Materials and methods

Commercially available sugar reference standards were procured from Himedia to ensure accurate quantification of individual sugars during analysis. A total of seventeen commercially available beverages were collected from the local market.

These included a diverse range of products such as fruit juices, aerated soft drinks, and alcoholic beverages. The samples were selected to represent a wide spectrum of commonly consumed beverages, with the objective of analyzing and comparing their sugar profiles.

Calibration standards were analyzed using LC-RID over the concentration range of 0.2% to 10%. Calibration curves were generated using the external standard calibration method. The analytical conditions used for the analysis are summarized in Table 1, which outlines the key parameters and instrument settings employed during the experiment.

Shimadzu LC-2060 3D coupled with RID-20A (Fig. 2) manufactured by Shimadzu Corporation Japan, was used to quantify sugars in different samples.

Fig. 2 Shimadzu LC-2060 3D

The RID-20A refractive index detector is designed to deliver rapid stabilization and a highly stable baseline, thanks to its advanced dual-temperature control system. The optical system is housed within a dual-temperature-controlled block, and the temperature of the incoming mobile phase is regulated in two stages. This configuration significantly reduces the time required for the detector to stabilize after power-on, ensuring quicker readiness for analysis. Additionally, the RID-20A features a four-partition photodetector that enables a wide refractive index detection range from 0.01 to 5000 µRIU. This single, versatile detector supports a broad range of applications—from highly sensitive analytical measurements to preparative-scale separations.

2.1. Analytical Conditions

Table 1 LC Analytical conditions

LC		
Chromatographic mode	:	HILIC
Column	:	Amino (NH2) Column, 80Å, 5 µm, 4.6 mm X 250 mm
Mobile phase	:	Acetonitrile:Water (75:25)
Elution mode	:	Isocratic
Flow rate	:	1.2 mL/min
Column temperature	:	35°C
Injection volume	:	10 µL
Run Time	:	25.0 min

2.2. Sample preparation

In this study, standard and sample preparations were carried out simultaneously and followed the same procedure to ensure consistency and accuracy in the analysis of sugar concentrations.

For the preparation of standards, approximately 1 g each of fructose, glucose, lactose, and sucrose was weighed and transferred into separate 10 mL volumetric flasks. Each flask was filled to the mark with deionized water and sonicated for 10 minutes to ensure complete dissolution.

A series of calibration standards ranging from 0.2% to 10% were then prepared by diluting the stock solutions with a 50:50 mixture of acetonitrile and deionized water.

Similarly, for sample preparation, 1 mL of each beverage sample was taken and diluted at a 1:5 ratio using the same 50:50 acetonitrile-deionized water mixture prior to analysis. Depending on the nature of the sample and the presence of suspended particulate matter, additional preparation steps were performed as needed. For example, freshly crushed fruit juice samples, which typically contain pulp and other solid residues, were filtered through a 0.45 µm membrane filter before injection to prevent clogging of the instrument and to ensure accurate analysis. All prepared standard and sample solutions were subsequently injected into the LC system for analysis.

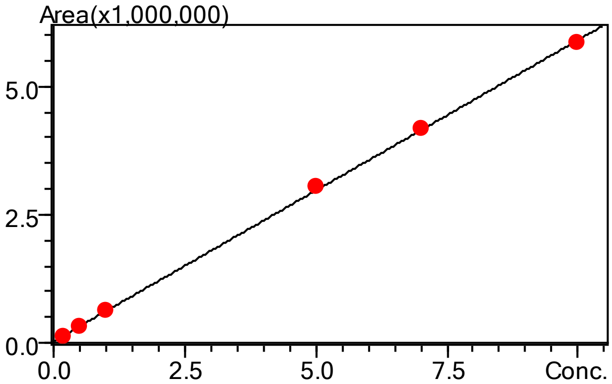

Fig. 3 Calibration curve for fructose

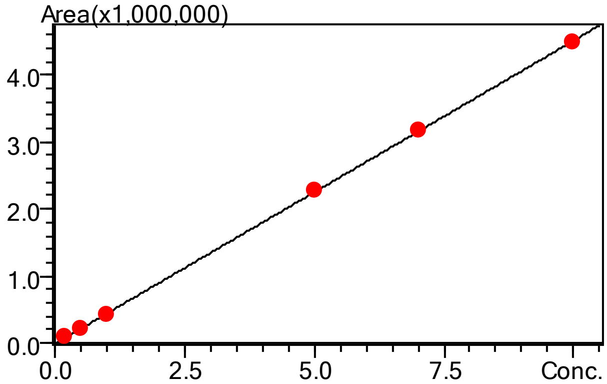

Fig. 4 Calibration curve for glucose

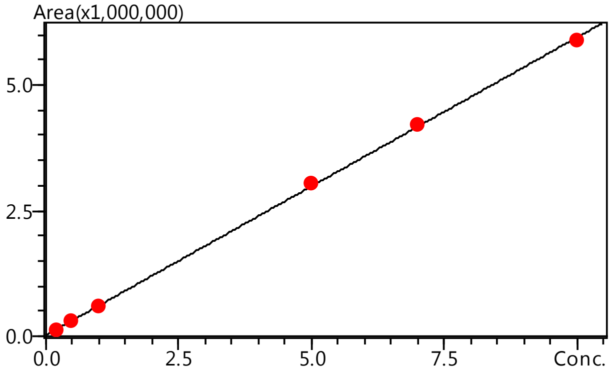

Fig. 5 Calibration curve for sucrose

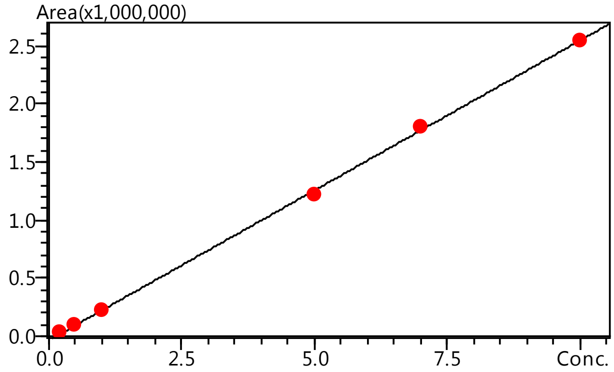

Fig. 6 Calibration curve for lactose

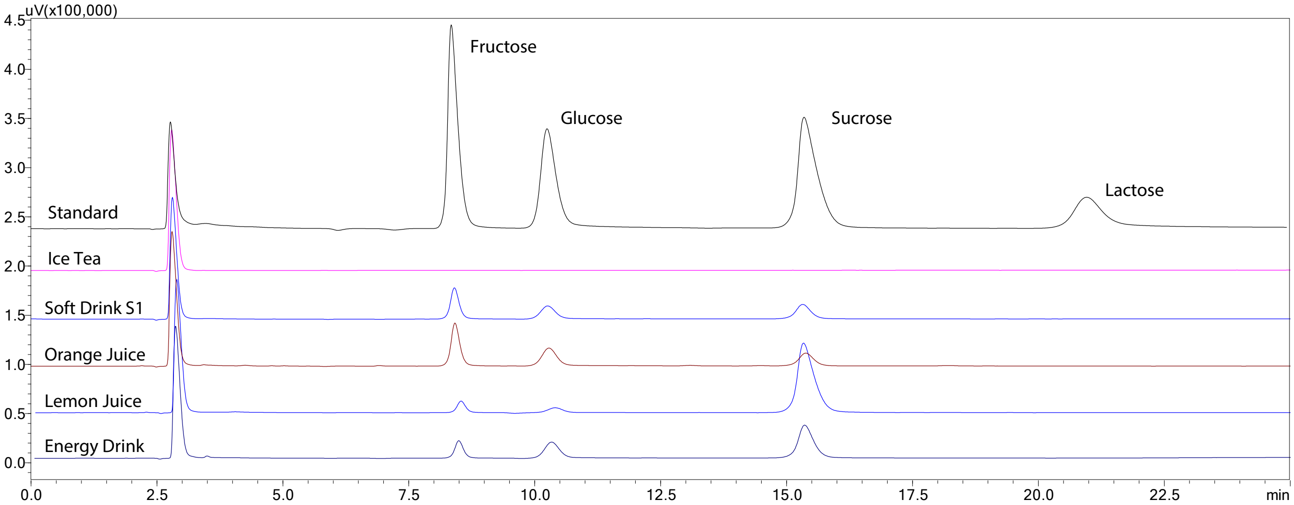

Fig. 7 Comparison chromatograms between standard and samples.

3. Result and Discussion

The calibration curves exhibited excellent linearity, with correlation coefficients (R^2) exceeding 0.99 for all sugars, indicating a high degree of accuracy and reliability in the measurements (Fig. 3 - Fig. 6). A representative chromatogram is shown in Fig. 7.

The study analyzed 17 beverage samples, including fruit juices, energy drinks, red and white wines, various soft drinks (both regular and sugar-free versions. The results indicated significant variations in sugar content across different beverage types (Table. 2). For instance, regular soft drinks (S1, S2, S3, S4) contained higher concentrations of sucrose, glucose, and fructose, contributing to their high caloric content.

In contrast, sugar-free and diet soft drinks showed negligible amounts of these sugars, as expected. Energy drinks also contained notable amounts of sugars, primarily glucose and fructose, which are commonly added for quick energy release. Red and white wines had lower sugar levels, with residual sugars primarily from the fermentation process. Fruit juices, while containing natural sugars from fruits, also showed high sugar content, particularly fructose and glucose, which can lead to blood sugar spikes similar to soft drinks, especially when consumed without fiber. The analysis confirmed that beverages marketed as "sugar-free" or "diet" contained minimal sugar, aligning with their labeling claims.

Table. 2 Summary table

Sl No	Sample Name	Fructose (%)	Glucose (%)	Sucrose (%)	Lactose (%)	Total (%)
1	Lemon Juice	0.97	1.52	14.10	ND	16.59
2	Grape Juice	8.99	8.14	ND	ND	17.13
3	Orange Juice	4.40	4.34	2.14	ND	10.88
4	Ice Tea	ND	ND	ND	ND	ND
5	Energy Drink	1.57	4.03	5.97	ND	11.57
6	Sugar Free Energy Drink	ND	ND	ND	ND	ND
7	Red Wine	2.63	2.94	ND	ND	5.57
8	White Wine	ND	ND	ND	ND	ND
9	Soft Drink S1	6.10	6.48	4.81	ND	17.39
10	Soft Drink S2	3.58	4.14	5.63	ND	13.35
11	Soft Drink S3	0.11	0.97	12.58	ND	13.66
12	Soft Drink S4	4.04	4.40	5.60	ND	14.04
13	Soft Drink Light	ND	ND	ND	ND	ND
14	Soft Drink Zero Sugar S1	ND	ND	ND	ND	ND
15	Soft Drink Zero Sugar S2	ND	ND	ND	ND	ND
16	Soft Drink Zero Sugar S3	ND	ND	ND	ND	ND
17	Soft Drink Diet	ND	ND	ND	ND	ND

Note : ND - Not detected

4. Conclusion

The study successfully quantified the sugar content in a variety of beverages, including soft drinks, energy drinks, wines, and fruit juices, using HPLC with refractive index detection. The calibration curves demonstrated excellent linearity, with R^2 values above 0.99, ensuring the accuracy of the sugar concentration measurements. The results highlighted the significant differences in sugar composition between regular, sugar-free, and diet beverages, as well as the high sugar content in fruit juices. Regular soft drinks, energy drinks, and fruit juices were found to contain high levels of sugars, which could contribute to health issues such as obesity and diabetes if consumed excessively. In contrast, sugar-free and diet beverages offered a low-sugar alternative, though their long-term health impacts remain a topic of debate. The findings underscore the importance of understanding sugar content in beverages, including fruit juices, to make informed dietary choices. Reducing the intake of high-sugar beverages and opting for low-sugar or sugar-free alternatives can help mitigate the health risks associated with excessive sugar consumption.

5. Reference

1. Zielinski, A. A. F., Braga, C. M., Demiate, I. M., Nogueira, A., & Wosiacki, G. (2014). Development and optimization of an HPLC-RI method for the determination of major sugars in apple juice and evaluation of the effect of the ripening stage.

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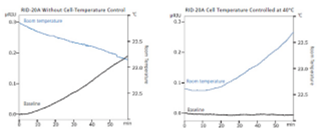

RID-20A
High-Sensitivity Refractive Index Detector

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