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Application Note

Quantitative Analysis of Sugars in Fruit Samples Using Refractive Index Detection (RID-20A)

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Food Beverages

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1. Introduction

In recent years, the consumption of sugars, particularly in the forms of sucrose, glucose, fructose, and lactose, has garnered significant attention due to its implications for health and nutrition. Fructose, found predominantly in fruits and honey, and glucose, present in a range of foods including fruits and vegetables, are fundamental to our metabolism.

When fructose and glucose combine, they form sucrose, commonly known as table sugar, which is prevalent in many processed foods. Conversely, lactose, a disaccharide composed of glucose and galactose, is primarily found in dairy products. These sugars are intricately linked through their roles in nutrition and metabolism, influencing energy levels, blood sugar regulation, and digestive health.

Fruits, despite being naturally sweet, contain a combination of these sugars, primarily fructose and glucose, along with fiber, vitamins, and antioxidants. Unlike added sugars, the natural sugars in fruits are metabolized differently due to their fiber content, which slows absorption and helps regulate blood sugar levels. Understanding the differences between these sugars and their impact on health is essential for making informed dietary choices. Reducing added sugar intake while prioritizing whole foods rich in natural sugars is vital for long-term metabolic health[1].

This research presents a validated approach for detecting four different sugars (refer to Figure 1) in fruits using LC-2060C 3D coupled with RID-20A from Shimadzu Corporation Japan.

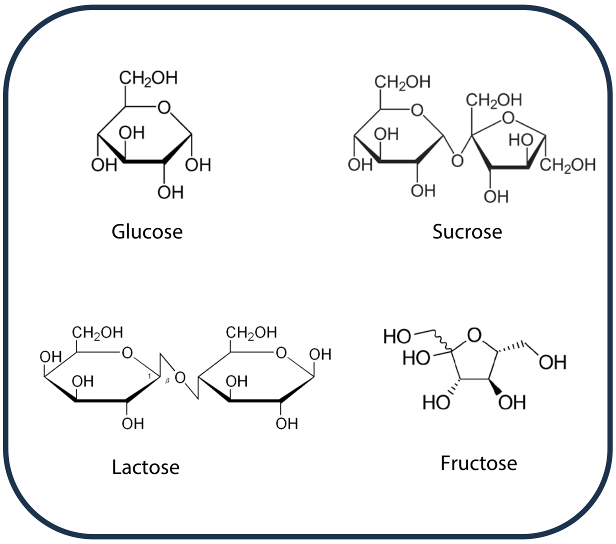

Fig. 1 Representative structures of sugars.

2. Materials and methods

Sugar analysis was conducted using fructose, glucose, lactose, and sucrose standards procured from Himedia to ensure accurate quantification. A total of 11 fruit samples, procured from the local market, were analyzed using the LC-2060C 3D coupled with RID-20A, as shown in Figure 2.

2.1. Analytical Conditions

Table 1: Analytical conditions LC

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

Fig. 2 Shimadzu LC-2060 3D.

2.2. Sample preparation

In this study, standard and sample preparations were conducted to ensure accurate analysis of sugar concentrations. For the standard preparation, approximately 1 g of each sugar (fructose, glucose, lactose, and sucrose) was weighed and transferred into separate 10 mL volumetric flasks. Each flask was filled to the mark with a deionized water solution and sonicated for 10 minutes to guarantee complete dissolution.

A series of calibration standards from 0.2-10% were subsequently prepared by diluting the stock solutions in 50:50 mixture of acetonitrile and deionized water to various concentrations as needed.

For the analysis, fruit samples were first crushed, and 1 gram of the crushed sample was weighed into a 5 mL volumetric flask. A 50:50 mixture of acetonitrile and deionized water was then added up to the mark. The samples were centrifuged at 6000 RPM for 5 minutes to separate any insoluble particles. The resulting clear supernatant was filtered through a 0.45 µm filter to remove any remaining particulate matter before proceeding with chromatographic analysis.

3. Result and Discussion

The quantitative analysis of sugars in eleven fruit samples was conducted using High-Performance Liquid Chromatography (HPLC) equipped with a RID-20A refractive index detector. 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.

This analytical method enabled the precise determination of four sugars—fructose, glucose, sucrose, and lactose. The concentrations of these sugars across the various fruit samples are summarized below.

Fructose, glucose, and sucrose were detected in all eleven fruit samples, while lactose was not detected in any of the samples analyzed, consistent with the general absence of lactose in plant-based foods. The total sugar content exhibited considerable variation among the different fruits, ranging from as low as 3.56% in lemon to as high as 19.48% in grapes.

Among the analyzed fruits, grapes contained the highest total sugar concentration (19.48%), followed by pomegranate (14.21%) and banana (12.87%), all of which showed elevated levels of both fructose and sucrose. Fruits such as apple (11.97%), watermelon (11.77%), and mango (10.88%) also demonstrated relatively high sugar content. On the other hand, guava (4.37%), strawberry (4.56%), and lemon (3.56%) had significantly lower total sugar concentrations.

Across all samples, fructose was the most abundant monosaccharide, typically present in higher concentrations than glucose. Sucrose, a disaccharide, contributed significantly to the overall sugar content, especially in fruits like mango, banana, watermelon, and grapes, where its levels were notably high.

The calibration curves used for quantification are presented in Figure 3 to Figure 6, while a representative chromatogram is shown in Figure 7. A detailed summary of the quantified sugar concentrations for each fruit sample is provided in Table 2.

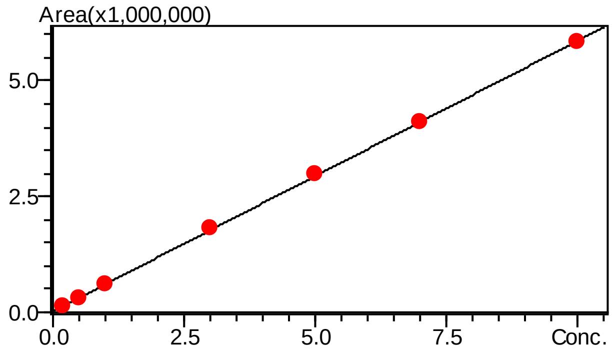

Fig. 3 Calibration curve for fructose.

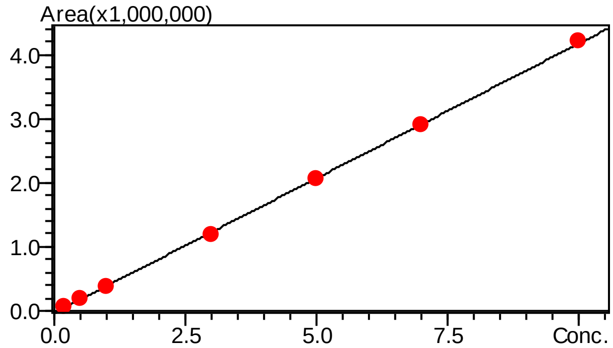

Fig. 4 Calibration curve for glucose.

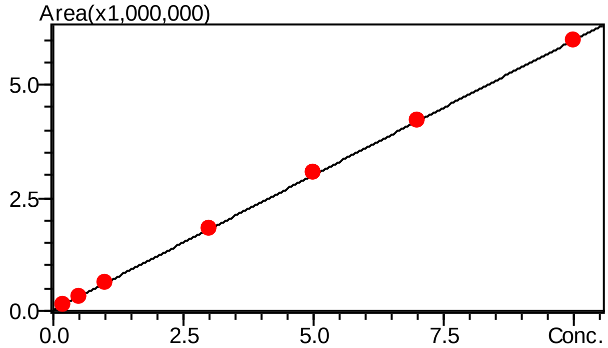

Fig. 5 Calibration curve for Sucrose.

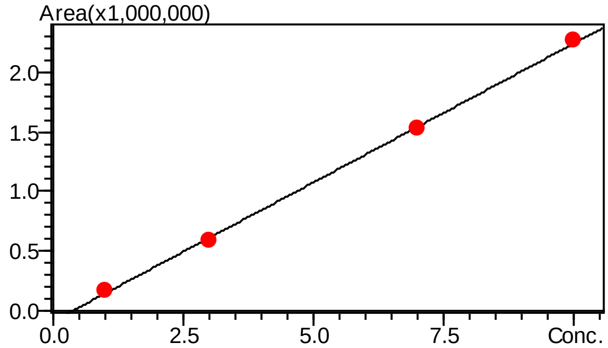

Fig. 6 Calibration curve for Lactose.

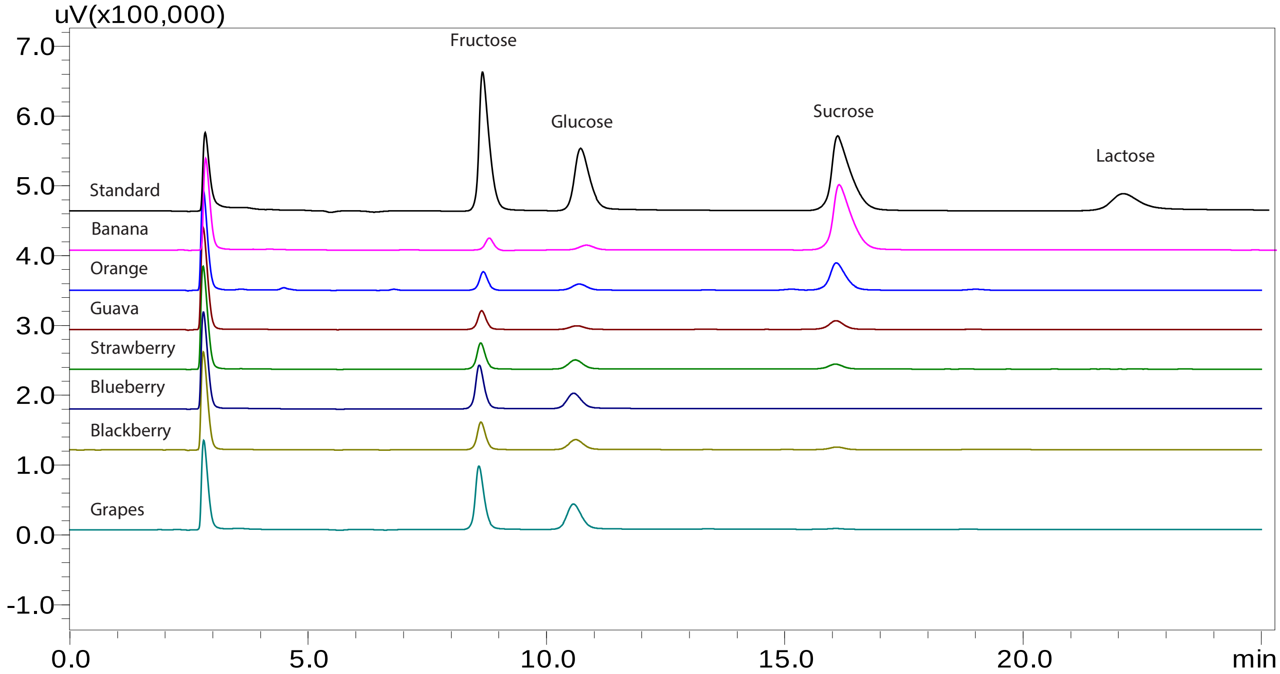

Fig. 7 Comparison chromatograms between standard and samples.

Table. 2 Summary

Sl No	Sample Name	Fructose (%)	Glucose (%)	Sucrose (%)	Lactose (%)	Total (%)
1	Blueberry	6.09	5.50	ND	ND	11.59
2	Grapes	9.96	9.48	0.04	ND	19.48
3	Strawberry	2.89	2.76	0.83	ND	6.48
4	Blackberry	3.77	3.74	0.45	ND	7.96
5	Guava	2.04	1.26	1.60	ND	4.9
6	Lemon	1.27	1.60	0.69	ND	3.56
7	Apple	8.48	2.23	2.37	ND	13.08
8	Watermelon	4.92	3.01	1.23	ND	9.16
9	Mango	2.36	ND	7.94	ND	10.3
10	Banana	1.27	1.55	15.66	ND	18.48
11	Orange	2.07	1.98	5.83	ND	9.88

Note : ND – Not detected

4. Conclusion

This study demonstrates the successful application of High-Performance Liquid Chromatography (HPLC) with a RID-20A detector for the quantification of fructose, glucose, sucrose, and lactose in a variety of fruits. Among the sugars analyzed, fructose, glucose, and sucrose were detected across all fruit samples, while lactose was absent in each, reaffirming its association with dairy sources. The sugar content varied significantly among the fruits, with grapes, bananas, and pomegranates exhibiting the highest total sugar concentrations, while lemon and guava presented the lowest. Fructose was generally the predominant sugar, particularly in fruits with higher sweetness levels.

The method used displayed excellent linearity and precision, confirmed by calibration curves with R^2 values $\geq$ 0.99. These results underscore the importance of individual fruit profiling for nutritional assessment and dietary planning, especially for populations concerned with sugar intake.

Moreover, the study provides a reliable analytical approach that can be extended to processed food products and beverages. The data generated serves as a valuable resource for professionals in food science, nutrition, agriculture, and public health, contributing to better consumer awareness and regulatory compliance in food labeling.

5. Reference

1. Míguez Bernárdez, M., González Pérez, E., & Díaz Villanueva, M. T. (2004). HPLC determination of sugars in varieties of chestnut fruits from Galicia. Journal of Chromatographic Science, 42(2), 101–104.

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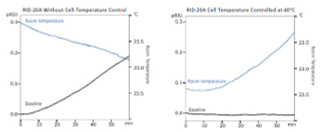

> RID-20A
High-Sensitivity Refractive Index Detector

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