APPLICATION

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In addition to chromatography, Brian also has a passion for ice cream-making, and enjoys experimenting with bold, new flavors.

Fast Analysis of Sucrose, Glucose, and Fructose Composition in Fruit Juices and Processed Beverages using Simplified HPLC Methodology

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Sucrose, glucose, and fructose are naturally found in fruit juices but are also added to various processed beverages. In this study, we present a fast and simple method for the analysis of sugar using Rezex HPLC columns evaluating a variety of commonly consumed beverages including sports/energy drinks, soda, fruit juices, and wine.

Introduction

The quantitation of sucrose and its monosaccharide constituents, glucose and fructose, is of critical importance for beverage manufacturers. High-fructose corn syrup (HFCS) is a sweetener composed primarily of glucose and fructose, which is ubiquitous in processed foods and beverages. There is a concern for HFCS content in processed foods because HFCS has been linked to metabolic disorders, including diabetes[1]. There is also a need for quantitation of sugar in naturally sweetened beverages since sucrose may degrade during the manufacturing process[2].

A number of HPLC methods have been used to quantitate sucrose, glucose, and fructose including HILIC[3] and Ligand Exchange[4]. However, HILIC methods will use high amounts of organic and analysis might require extensive method development. Previous Ligand Exchange methods provide linearity and limits of quantitation that are not practical for real-world applications.

In this study, we demonstrate the use of Rezex™ RCM-Monosaccharide Ca^{+2} to develop a quick and quantitative method under 15 minutes for the analysis of the three main sugars in various beverages and fruit juices: sucrose, glucose, and fructose.

Materials and Methods

Reagents and Chemicals

Sugar standards were purchased from Sigma Chemical (St. Louis, MO). Beverages were obtained from local grocers. A Sartorius® arium® comfort II was used for ASTM Type 1 ultrapure water. Nine standards were prepared in water at concentrations from 0.1 mg/mL to 50 mg/mL. Beverage samples were diluted 1:10 in ultrapure water. Although not performed in this study, the use of a 0.45 µm Phenex™-NY (Nylon) syringe filter prior to injection is encouraged for beverages with high particulate content (e.g. fruit juice with pulp).

Experimental Conditions

HPLC analysis was performed using an Agilent® 1260 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 600 bar, equipped with a binary pump, autosampler, and Refractive Index (RI) detector. Method conditions are below.

Column:	Rezex RCM-Monosaccharide Ca^{+2}
Dimensions:	100 x 7.8 mm
Part No.:	00D-0130-K0
Mobile Phase:	Water
Flow Rate:	0.4 mL/min
Temperature:	80 °C
Detector:	RI (Refractive Index) @ 35 °C
Sample:	Sucrose Glucose Fructose

Results and Discussion

A nine-point standard curve was run for sucrose, glucose, and fructose from 0.1 mg/mL to 50 mg/mL (**Table 1**). Retention times for standards are shown in **Table 2**. Using peak areas, a linear regression line was drawn for each sugar respectively, with correlation coefficients greater than 0.99 for each (**Figures 1-3**).

Column: Rezex™ RCM-Monosaccharide Ca^{+2}
Dimensions: 100 x 7.8 mm
Part No.: 00D-0130-K0
Mobile Phase: Water
Flow Rate: 0.4 mL/min
Temperature: 80 °C
Detector: RI (Refractive Index) @ 35 °C
Sample: Sucrose
Glucose
Fructose

Table 1. Standard concentrations

Standards	Conc. (mg/mL)
1	0.1
2	0.25
3	0.5
4	1
5	2.5
6	5
7	10
8	25
9	50

Figure 1. Standard curve for sucrose, 0.1-50 mg/mL

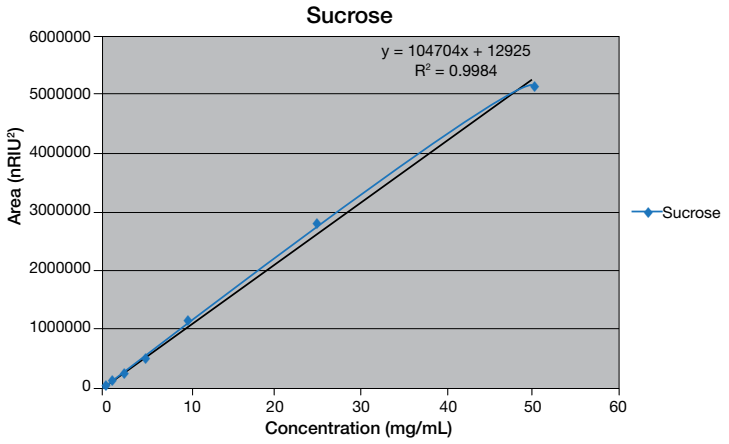


Figure 2. Standard curve for glucose, 0.1-50 mg/mL

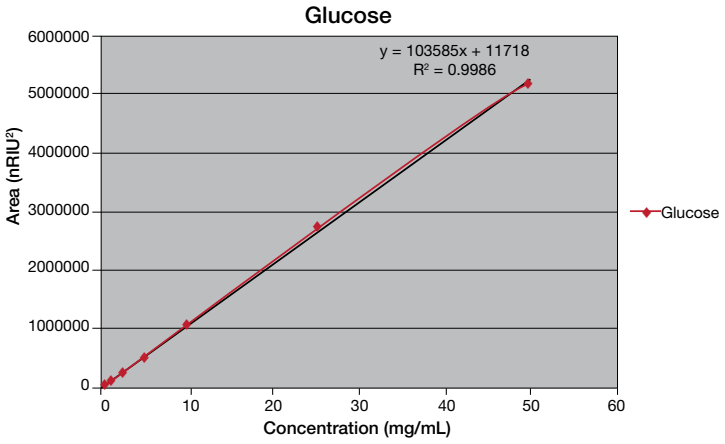
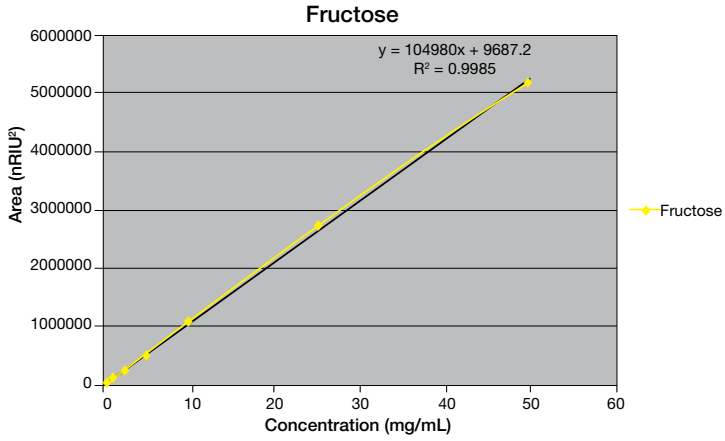


Figure 3. Standard curve for fructose, 0.1-50 mg/mL



Typically in ion exclusion and ligand exchange, a minimum of a one minute retention time difference is required for baseline separation. Baseline separation is clearly observed in **Figures 4 & 5**, at 0.1 mg/mL and 50 mg/mL, respectively.

Concentrations for each beverage were calculated using the calibration curve then multiplied by 10 to adjust for the dilution factor (**Table 3**).

Figure 4. 0.1 mg/mL Sugar Standard, prepared in Water

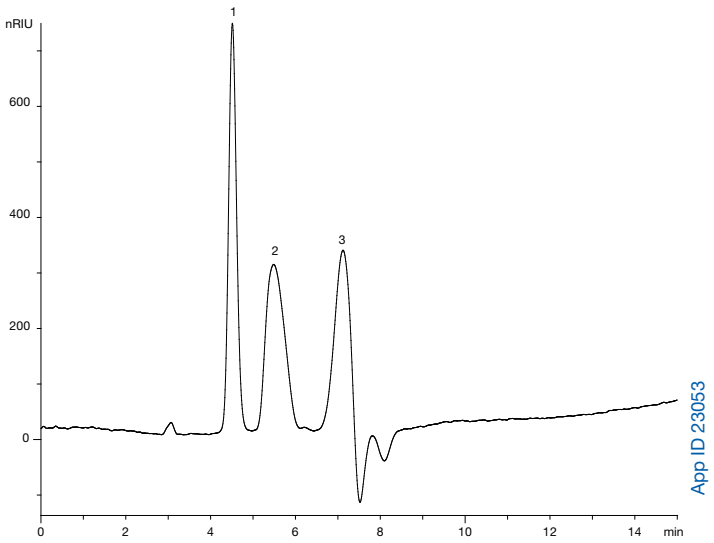


Figure 5. 50 mg/mL Sugar Standard, prepared in Water

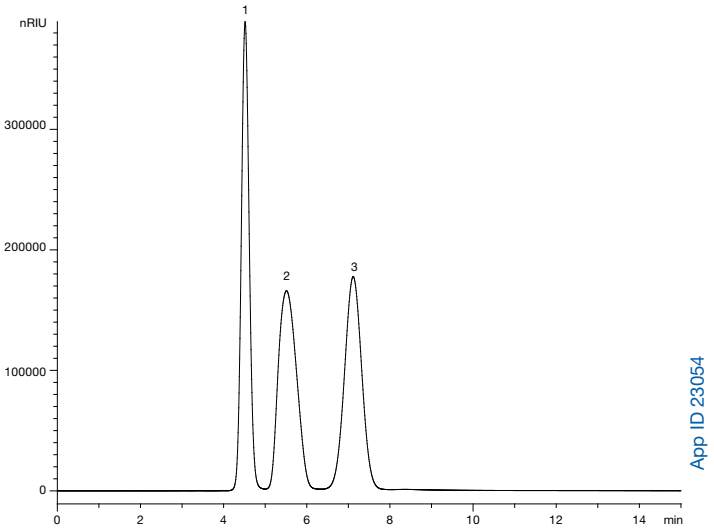


Table 2. Standard Retention Times

Sugar	Retention Time (min)
1. Sucrose	4.5
2. Glucose	5.5
3. Fructose	7.1

Column: Rezex RCM-Monosaccharide Ca^{+2}
Dimensions: 100 x 7.8 mm
Part No.: 00D-0130-K0
Mobile Phase: Water
Flow Rate: 0.4 mL/min
Temperature: 80 °C
Detector: RI (Refractive Index) @ 35 °C
Sample: Sucrose
Glucose
Fructose

Percent deviations were determined by comparing the concentration of the three sugars combined to expected concentration of sugars according to the nutrition facts on the beverage label. **Figures 6 &7** show sugar content in red and white wine, respectively. Red wine in particular can have fructose content which gives an undesirable sweetness[5]. It has been reported that between 1-2.5 % sugar content[6] can be detected by most palates and the linearity of the assay (i.e. 0.1-5 %) is appropriate for this analysis.

Table 3. Analytes and Sugar Content in Comparison to Reported Nutrition Facts

Beverages	Sucrose (mg/mL)	Glucose (mg/mL)	Fructose (mg/mL)	Total Sugars (mg/mL)	Percent Deviation from Nutrition Facts
Merlot (Red Wine)	0.0	0.0	1.6	2.1	N/A
Moscato (White Wine)	0.0	26.8	51.3	76.9	N/A
Orange Juice 1	52.7	29.2	30.6	112.6	28.63 %
Orange Juice 2	50.9	26.5	29.5	106.8	16.55 %
Fruit Punch	4.3	51.3	69.8	125.4	36.79 %
Pineapple Orange Juice	35.0	34.5	46.9	116.4	3.43 %
Pineapple Mango Juice	35.7	23.5	38.1	97.4	16.90 %
Energy Drink	40.4	54.7	30.6	125.8	10.16 %
Cola	2.5	49.8	66.7	119.0	1.92 %
Sports Drink	38.2	15.8	11.9	65.9	14.52 %

Figure 6. Merlot (Red Wine)

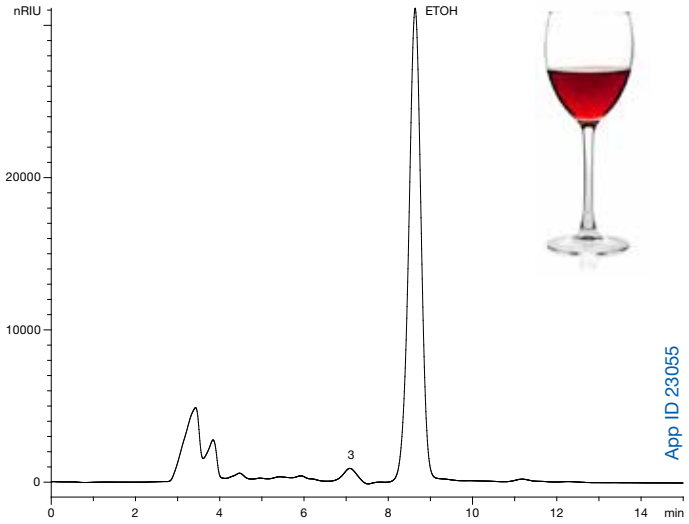
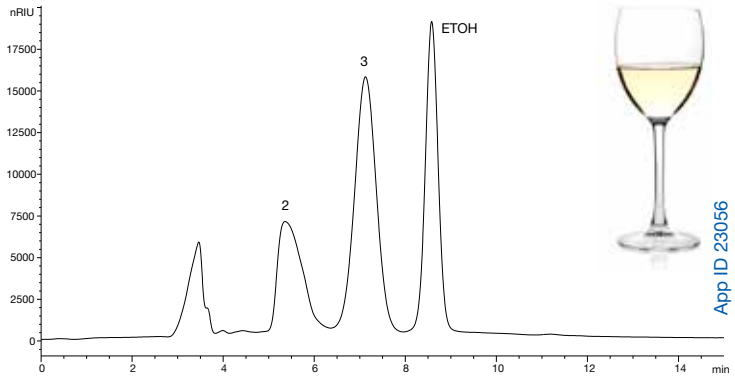


Figure 7. Moscato (White Wine)



Figures 8 & 9 show sugar content for orange juices from two separate sources. Both are similar in sugar content. **Figure 10** shows an artificially flavored "fruit punch." Only 4.3 mg/mL sucrose is detected, indicating sweetening primarily with HFCS.

Figure 8. Orange Juice 1

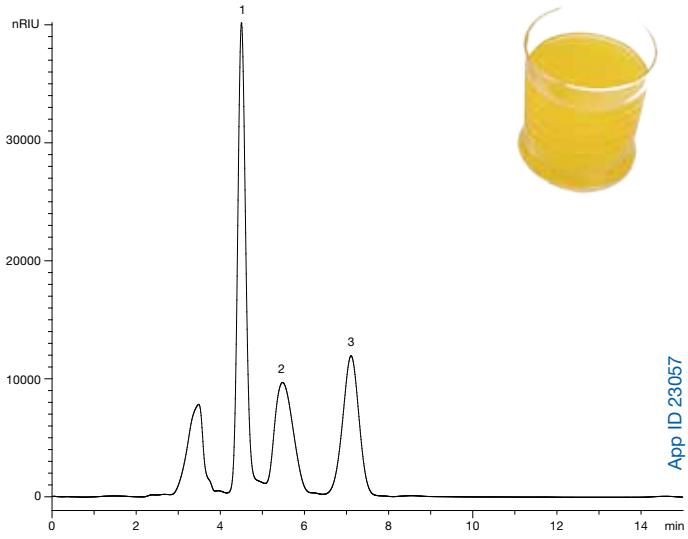


Figure 9. Orange Juice 2

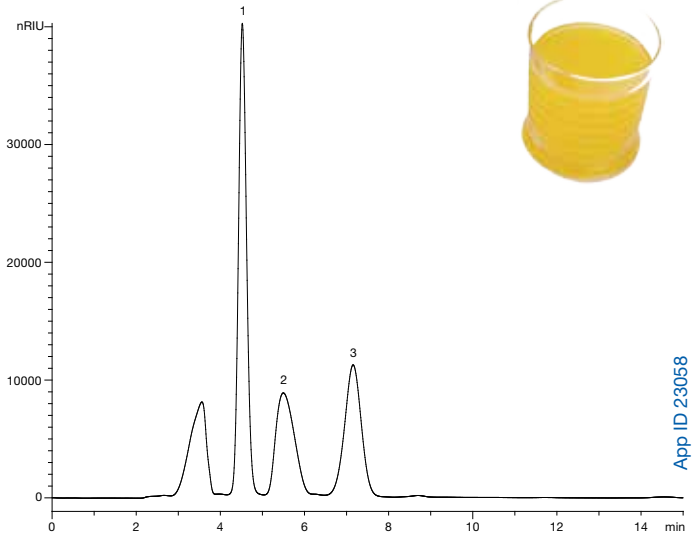
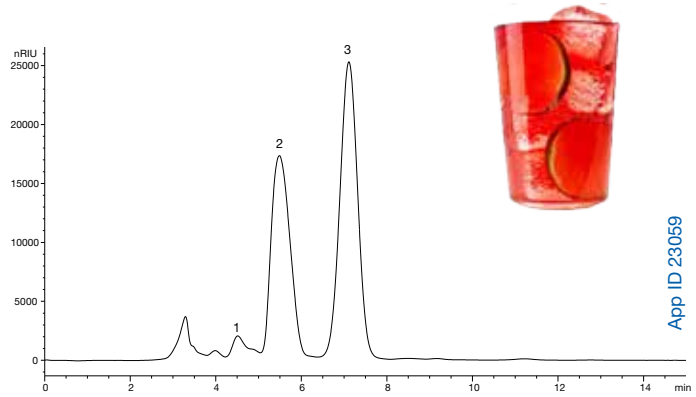


Figure 10. Fruit Punch



Column:	Rezex RCM-Monosaccharide Ca^{+2}
Dimensions:	100 x 7.8 mm
Part No.:	00D-0130-K0
Mobile Phase:	Water
Flow Rate:	0.4 mL/min
Temperature:	80 °C
Detector:	RI (Refractive Index) @ 35 °C
Sample:	Sucrose Glucose Fructose

The two samples “Pineapple Orange Juice” and “Pineapple Mango Juice” are reported to be 100 % juice. These juices can also contain the natural sugar alcohol sorbitol. Note in this method, the sorbitol peak elutes at approximately 12 minutes (**Figures 11 & 12**) Although not the focus of this study, this method could feasibly be used for sorbitol quantitation.

Figure 11. Pineapple Orange Juice

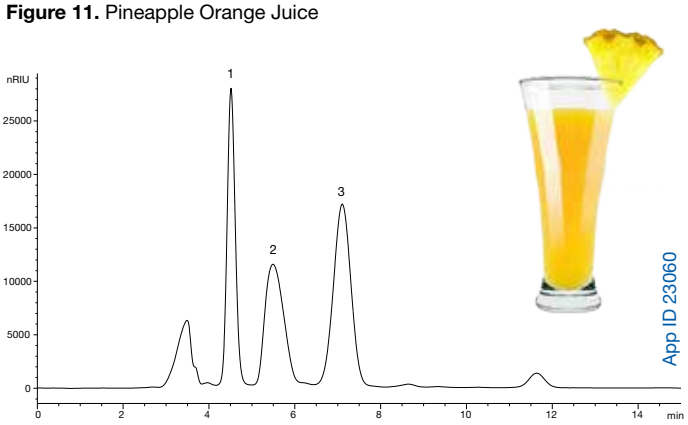
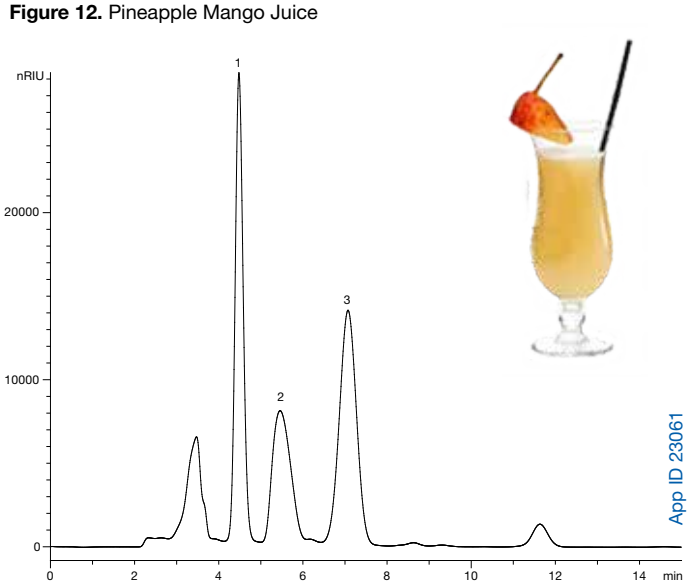


Figure 12. Pineapple Mango Juice



The “Energy Drink” sample contained two unknown peaks eluting at approximately 7-8 minutes (**Figure 13**). Further studies would need to be performed to confirm the identity of these peaks. Cola sample in **Figure 14** shows almost no sucrose, indicating the beverage is sweetened primarily with HFCS. Conversely, “Sports Drink” sample showed it primarily being sweetened with sucrose (**Figure 15**).

Figure 13. Energy Drink

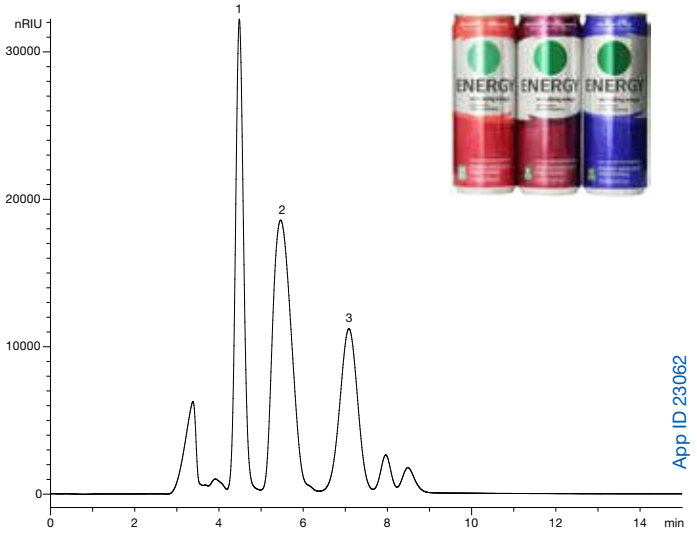


Figure 14. Cola

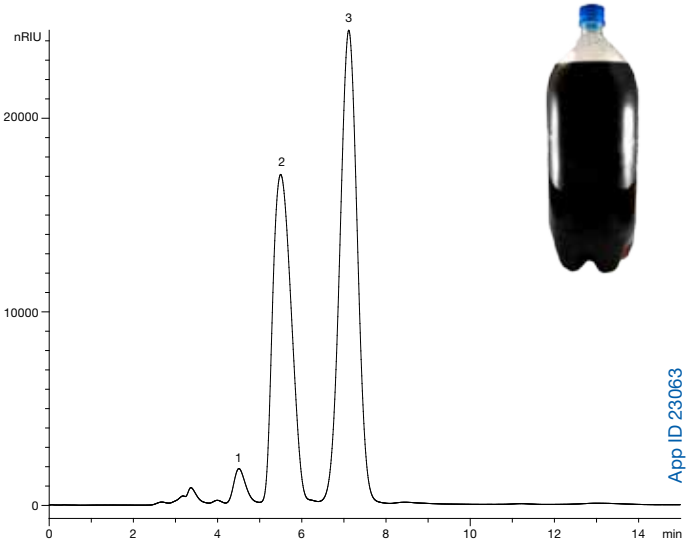
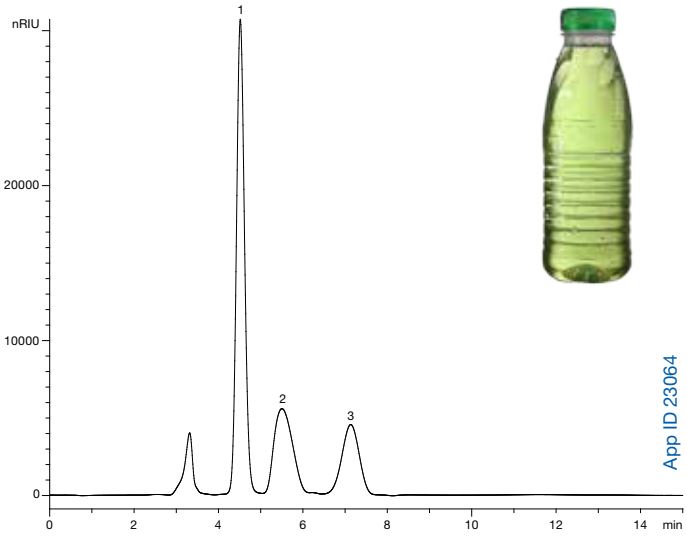


Figure 15. Sports Drink



Finally, it is is worth noting that both "Orange Juice 1" and "Fruit Punch" samples were outside the 20 % discrepancy allowed by the FDA[7].

Conclusion

In this study, we demonstrated a method using Rezex™ RCM for the analysis of sucrose, glucose, and fructose content in processed sugary drinks and beverages. The method is quantitative, simple, and robust, being compatible with a variety of sample matrices, including wines, fruit juices, and sports drinks. Further studies could include the inclusion of sorbitol, as well as other sugar alcohols.

References

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7. Bender, Mary M, Jeanne I. Rader, and Foster D. McClure. "Guidance for Industry: FDA Nutrition Labeling Manual—A Guide for Developing and Using Data Bases." US Dept of Health and Human Services, Food and Drug Administration Center for Food Safety and Applied Nutrition. Web. 17 May 2015

Rezex Ordering Information

Columns					Guards		SecurityGuard™ Cartridges (mm)
Description	Part No.	Cross Linkage	Ionic Form	Size (mm)	Part No.	Size (mm)	4 x 3.0*
RCM-Monosaccharide	00D-0130-K0	8 %	Calcium	100 x 7.8	03B-0130-K0	50 x 7.8	AJ0-4493
RCM-Monosaccharide	00F-0130-K0	8 %	Calcium	150 x 7.8	03B-0130-K0	50 x 7.8	AJ0-4493
RCM-Monosaccharide	00H-0130-K0	8 %	Calcium	300 x 7.8	03B-0130-K0	50 x 7.8	AJ0-4493
RHM-Monosaccharide	00H-0132-K0	8 %	Hydrogen	300 x 7.8	03B-0132-K0	50 x 7.8	AJ0-4490
RAM-Carbohydrate	00H-0131-K0	8 %	Silver	300 x 7.8	—	—	AJ0-4491
RSO-Oligosaccharide	00P-0133-N0	4 %	Silver	200 x 10.0	03R-0133-N0	60 x 10.0	—
RNO-Oligosaccharide	00P-0137-N0	4 %	Sodium	200 x 10.0	03R-0137-N0	60 x 10.0	—
RPM-Monosaccharide (for USP procedure)	00H-0135-K0	8 %	Lead	300 x 7.8	03B-0135-K0	50 x 7.8	AJ0-4492
	00D-0135-K0	8 %	Lead	100 x 7.8	03B-0135-K0	50 x 7.8	AJ0-4492
RNM-Carbohydrate	00H-0136-K0	8 %	Sodium	300 x 7.8	03B-0136-K0	50 x 7.8	—
ROA-Organic Acid	00F-0138-E0	8 %	Hydrogen	150 x 4.6	—	—	AJ0-4490
ROA-Organic Acid	00G-0138-E0	8 %	Hydrogen	250 x 4.6	—	—	AJ0-4490
ROA-Organic Acid	00F-0138-K0	8 %	Hydrogen	150 x 7.8	03B-0138-K0	50 x 7.8	AJ0-4490
ROA-Organic Acid	00H-0138-K0	8 %	Hydrogen	300 x 7.8	03B-0138-K0	50 x 7.8	AJ0-4490
RKP-Potassium	00H-3252-K0	8 %	Potassium	300 x 7.8	—	—	—
RFQ-Fast Acid	00D-0223-K0	8 %	Hydrogen	100 x 7.8	03B-0223-K0	50 x 7.8	AJ0-4490
RCU-USP Sugar Alcohols	00G-0130-D0	8 %	Calcium	250 x 4.0	—	—	AJ0-4493
							for ID: 3.2-8.0 mm

*SecurityGuard Analytical Cartridges require universal holder Part No.: KJ0-4282

APPLICATION

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