APPLICATION NOTE

Liquid Chromatography

Author:

Wilhad M. Reuter

PerkinElmer, Inc.
Shelton, CT

The Qualitative and Quantitative Analysis of Sugars and Erythritol by LC-ELSD in Energy/Vitamin Drinks

Introduction

With the abundance of energy/vitamin drinks to choose from, combined with an emphasis on lower calories, more and more individuals are focusing on their daily sugar intake before consuming these drinks. With this consideration, this application highlights both the LC separation of various sugars and erythritol (sugar alcohol) as well as the analysis of these components in a selection of three energy/vitamin drinks. As both sugars and sugar alcohols are inherently poor UV absorbers, refractive index (RI) has historically been the detector of choice for these compounds. However, when using an RI detector, scientists are limited by both sensitivity and the fact that it cannot be used with any solvent gradients. By comparison, when using an Evaporative Light Scattering Detector (ELSD) for detection, researchers are afforded both greater sensitivity and the ability to run solvent gradients. This application takes particular advantage of the ELSD's solvent gradient flexibility. As the seven sugars and sugar alcohol to be analyzed have widely differing column retention, a solvent gradient was targeted to provide the optimal separation in the least amount of time.

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Experimental

Hardware/Software

A PerkinElmer Flexar Binary HPLC System (binary pump, autosampler, vacuum degasser, and column oven with an Imtakt® 3 µm 250 x 3 mm Unison UK-Amino column) was used for all separations. Detection was accomplished with PerkinElmer's ELSD TSII. All instrument control, analysis, and data processing was done via Chromera™ software.

Method Parameters

Table 1. HPLC Conditions

HPLC Conditions	
Column	Imtakt 3 µm 250 x 3 mm Unison UK-Amino
Mobile Phase Gradient	Equilibrate for 6 min. at 3:97 water/acetonitrile Step 1: Hold for 7 min. at 3:97 water/acetonitrile Step 2: Linear gradient to 25:75 water/acetonitrile in 10 min. Step 3: Hold for 3 min. at 25:75 water/acetonitrile
Flow Rate	0.8 mL/min.
Oven Temp.	60 °C
ELSD Conditions	Spray Chamber Temp.: 35 °C Drift Tube Temp.: 60 °C
Injection Volume	5 µL

Solvents, Standards, and Samples

All solvents and diluents used were HPLC grade.

All sugar and erythritol standards were obtained from Sigma-Aldrich®. Sugars included arabinose, xylose, fructose, mannose, glucose, sucrose, and maltose. All standard dilutions were made using 10:90 water/methanol. Methanol was used instead of acetonitrile due to the solubility of the sugars. Even with methanol, for the starting high concentration stock solution, the standards were first dissolved in 5 mL of water before adding 45 mL of methanol.

Samples included three different energy/vitamin drinks purchased at a local grocery store. All samples were first diluted 1/40 with 10:90 water/methanol and then filtered via 0.2 µm filters to remove small particles.

Results and Discussion

Figure 1 shows a 400 ppm standard (Std) chromatogram of seven common sugars plus erythritol, a sugar alcohol, run under the optimized conditions described above.

Using glucose and sucrose as examples, Figure 2 shows the calculated linearity within a 25 – 400 ppm and 50 – 800 ppm concentration range, respectively. Both sugars were found to be quite linear within their ranges. It should be noted that the upper concentrations were chosen to accommodate the expected high-end sugar concentrations within the analyzed samples and that the actual upper limit of linearity may be higher.

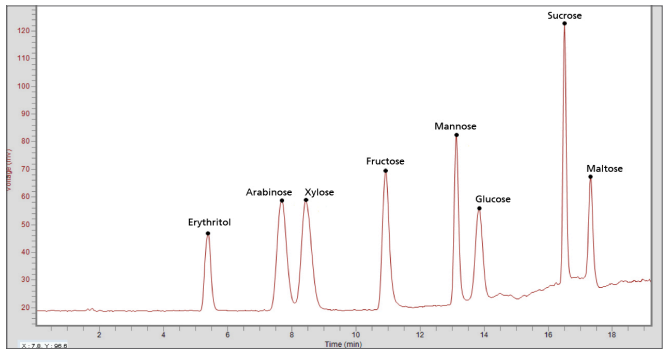

Figure 1. Chromatogram of standard solution containing erythritol and seven common sugars

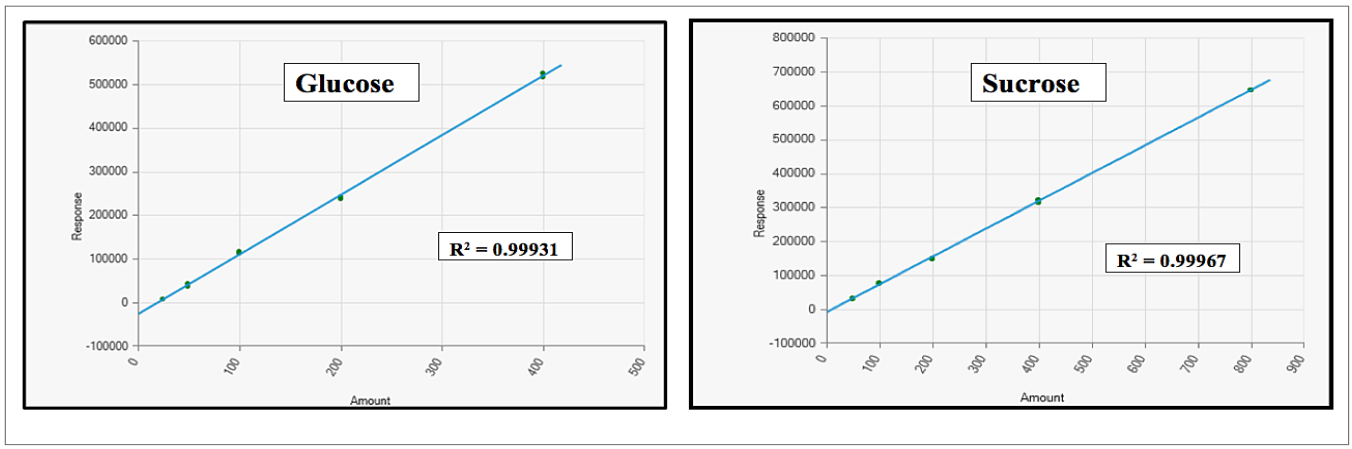

Figure 2. Linearity plots of glucose (25 - 400 ppm concentration range) and sucrose (50 - 800 ppm concentration range)

Subsequently, using the same chromatographic conditions, three energy/vitamin drinks were analyzed: Drink X, Drink Y, and Drink Z. The results for Drinks X and Y are shown in Figure 3, each overlaid upon the 400 ppm standard chromatogram. Comparing the chromatograms of these drinks, (highlighted in Figures 3a and 3b), it can be observed that Drinks X and Y contain basically the same three sugars, namely fructose, glucose (also known as dextrose), and sucrose, with some minor differences in proportion.

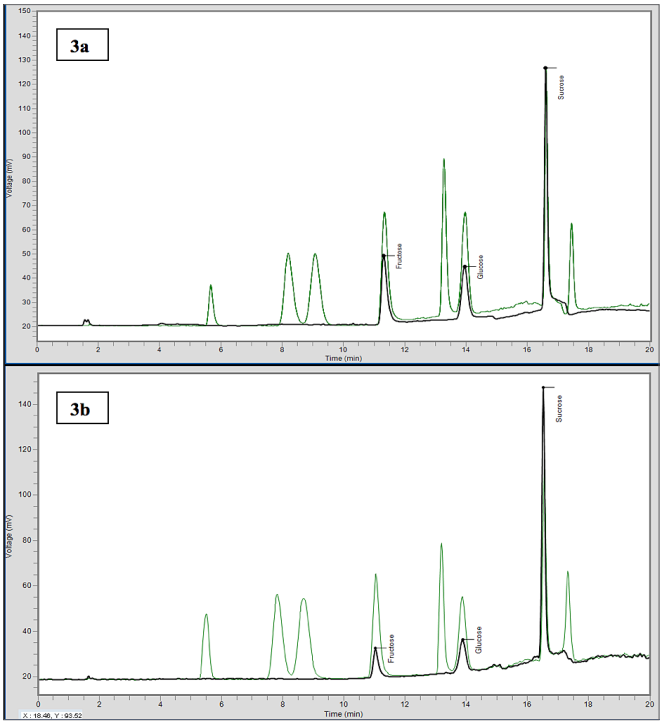

Figure 3. a) Overlaid chromatogram of Drink X (in black) with 400 ppm standard chromatogram b) Overlaid chromatogram of Drink Y(in black) with 400 ppm standard chromatogram

Based on standard calibrations, the quantitative results per injected sample are shown in Table 2.

Table 2. Quantitative Results

Drink X:

Component	Area	Amount (ppm)	Corrected Amounts for 1/40 dilution (ppm)
Fructose	376704.35	231.13	9245.2
Glucose	265239.31	213.74	8549.6
Sucrose	706187.45	873.23	34929.2

Drink Y:

Component	Area	Amount (ppm)	Corrected Amounts for 1/40 dilution (ppm)
Fructose	167061.81	111.04	4441.6
Glucose	228723.71	186.96	7478.4
Sucrose	835912.45	1031.61	41264.4

Adding up the individual corrected sugar amounts for each drink and converting the total from ppm to percent, the overall sugar content for Drinks X and Y was determined to be 5.27 % and 5.32 %, respectively. Both of these results were consistent with the label claim of overall grams of sugar per bottle. Per label claim, Drink X was calculated to contain 5.35 % sugar and Drink Y was calculated to contain 5.41 % sugar.

The results for Drink Z are shown in Figure 4. The upper chromatogram shows the drink injected by itself, with only one prominent peak being observed at about five minutes. The lower chromatogram shows the chromatographic results of 1/40 diluted Drink Z spiked 1:1 with the 400 ppm sugar standard. The solitary peak that appears in the 1/40 diluted Drink Z is seen to perfectly overlay the erythritol peak in the standard. It should be noted that although ribose elutes very close to erythritol, it was observed that an erythritol standard spiked with an equal concentration of ribose resulted in a prominent shoulder on the upslope of the erythritol peak (data not shown). As the spiked Drink Z did not display such a shoulder, this supported the identification of the component in Drink Z as erythritol. This was also supported by Drink Z's label claim, claiming erythritol as a significant component. Interestingly, regarding Drink Z's zero-calorie label claim, as opposed to true sugars, a sugar alcohol, such as erythritol, contributes no significant calories and qualifies as both a sugar-free and calorie-free component in foods and beverages. Also, it should be pointed out that per label claim, Drink Z is reported to contain Reb A, a stevia plant extract, now being increasingly introduced into products as an effective zero-calorie sugar substitute. Reb A was not analyzed in this study.

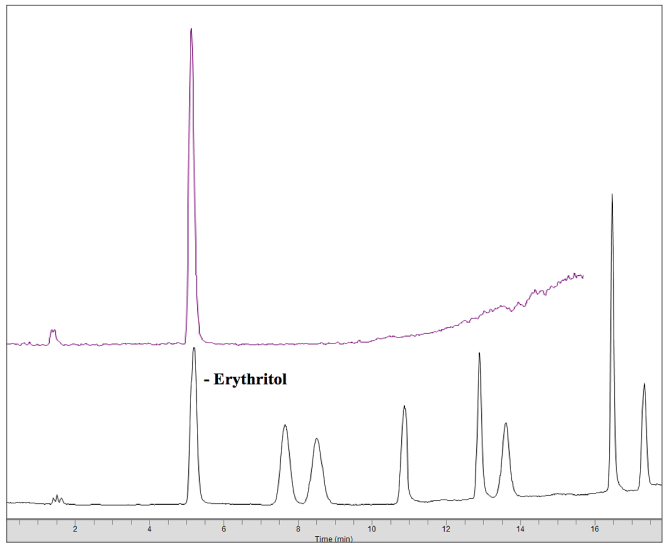

Figure 4. Upper chromatogram: Drink Z, diluted 1/40 with 10% water in methanol; Lower chromatogram: 1/40 diluted Drink Z spiked 1:1 with 400 ppm sugar standard

Conclusion

This work has demonstrated the effective chromatographic separation of seven sugars and erythritol using a PerkinElmer Flexar/Chromera LC-ELSD system. The results exhibited very good linearity over the tested concentration ranges. By choosing an ELSD as the detector, advantage was taken of the ELSD's inherent compatibility with running solvent gradients, allowing for the optimal separation of erythritol and all seven sugars in under 18 minutes.

From a food quality perspective, there is an ever growing emphasis on food monitoring. With this in mind, this work also focused on the sugar/erythritol analysis of three energy/vitamin drinks, identifying the sugars/sugar alcohol contained in each drink, as well as comparing the three drinks' similarities and differences, both chromatographically and quantitatively.

References

1. Unison UK-Amino application brochure (Imtakt, Anoka, MN, USA)

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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