APPLICATION NOTE

Liquid Chromatography

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Analysis of Sugars in Honey Using the PerkinElmer Altus HPLC System with RI Detection

Introduction

Honey consumption has grown significantly during the last few decades due to its high nutritional value and unique flavor. The price of natural bee honey is much higher than other sweeteners making it susceptible to adulteration with cheaper sweeteners, primarily sucrose. Besides lower levels of non-sugar ingredients, natural honey primarily consists of glucose and fructose and may contain low levels of sucrose and/or maltose.[1,2] However, according to the international regulations, any commercially available "pure"-labeled honey products that are found to have in excess of 5% by weight of sucrose or maltose are considered to be adulterated.[3]

With the focus on possible honey adulteration, this application highlights the LC separation of various sugars found in honey and the analysis of these components in four store-bought honey samples. Method conditions and performance data, including linearity and repeatability, are presented.

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Experimental

Hardware/Software

For all chromatographic separations, a PerkinElmer Altus™ HPLC system was used, including the Altus A-10 Solvent and Sample Module, Column Module, integrated vacuum degasser/column oven and an Altus A-10 RI Detector. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 CDS platform.

Method Parameters

The HPLC method parameters are shown in Table 1

Table 1. HPLC Method Parameters.

HPLC Conditions	
Column:	PerkinElmer Brownlee™ Analytical Amino 3 µm, 4.6 x 150 mm (Part# N9303505)
Mobile Phase:	Solvent A: 65:35 acetonitrile/water Solvent program:

Time (min)	Flow Rate (mL/min)	%A	%B	%C	%D	Curve
Initial	1.000	100.0	0.0	0.0	0.0	Initial

Analysis Time	6 min.
Flow Rate:	1.0 mL/min. (2300 psi)
Oven Temp.:	25 °C
Detection:	Altus A-10 RI; cell temp.: 35 °C
Injection Volume:	5 µL
Sampling (Data) Rate:	10 pts./sec

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade and filtered via 0.45-µm filters.

The sugar standards were obtained from Supelco® (Irvine, CA) and consisted of fructose, glucose, maltose and sucrose. Stock sugar standards were made using 65:35 acetonitrile/water as diluent. For the 1333 µg/mL (ppm) stock solution, the standards were first dissolved in 17.5 mL of water before adding 32.5 mL of acetonitrile. The lower level standards were then prepared from this stock solution.

All commercially available honey products were purchased at local stores. They were labeled Honey W, Honey X, Honey Y and Honey Z. Each honey was prepared by dissolving 2.5 g into 50 mL of 65:35 acetonitrile/water, followed by another 1:1 dilution using the same solvent.

Prior to injection, all calibrants and samples were filtered through 0.45-µm filters to remove small particles.

Results and Discussion

Figure 1 shows the chromatographic separation of the 1333-µg/mL (ppm) sugar standard containing the four target sugars using the optimized conditions described above. The analysis time was under six minutes.

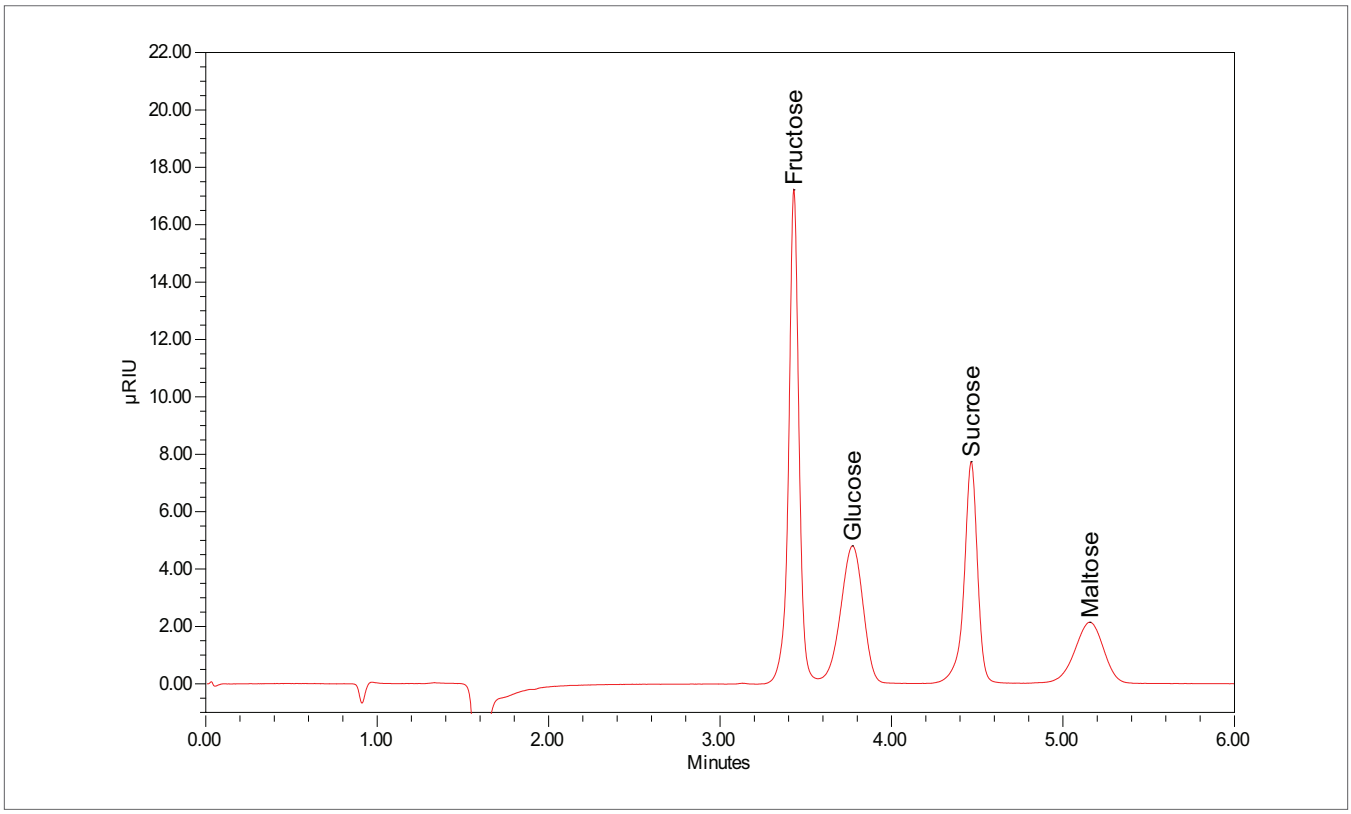

Figure 1. Chromatogram of the 1333 µg/mL sugar standard.

Figure 2 shows the overlay of 12 replicate 667-µg/mL sugar standard injections, demonstrating exceptional reproducibility. Retention time % RSDs were also quite exceptional, exemplified by 0.026% RSD for fructose.

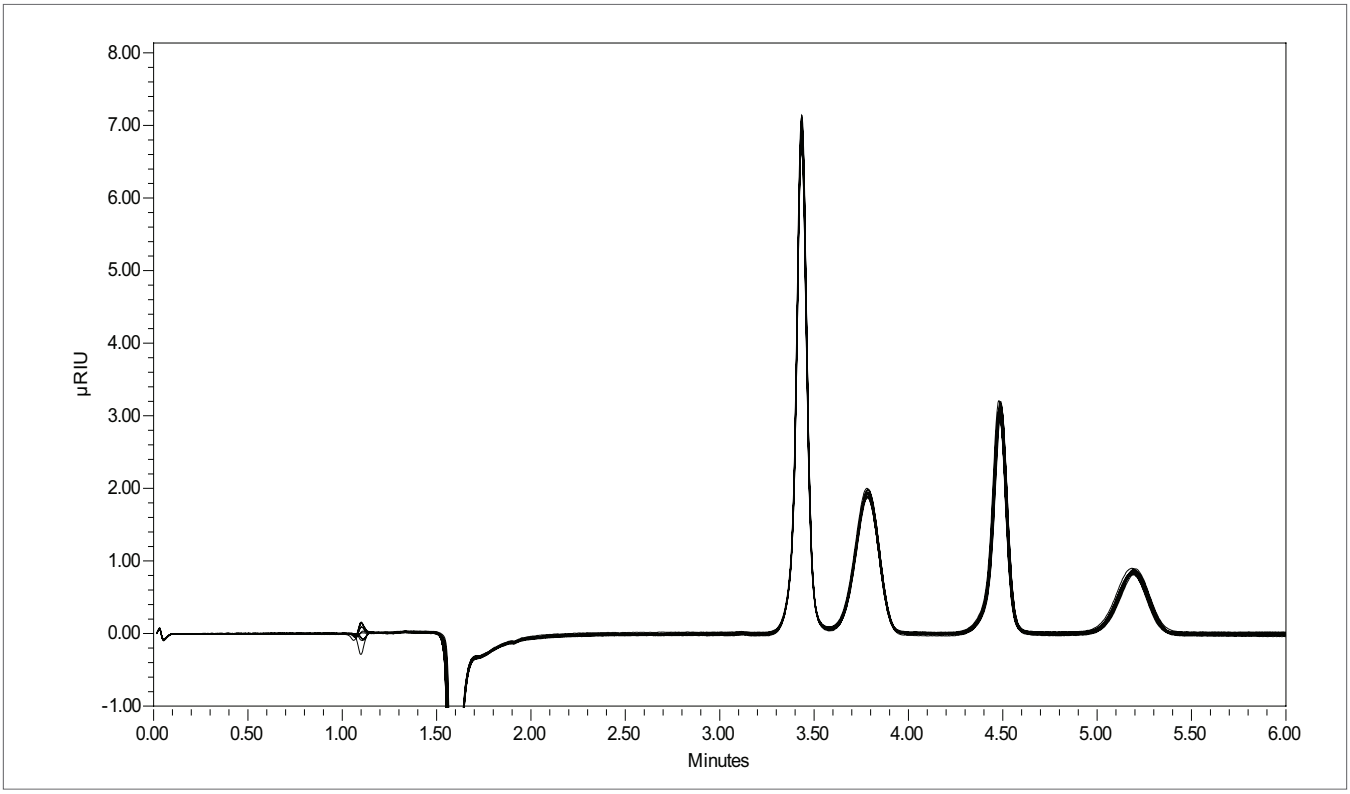

Figure 2. Overlay of 12 replicates of the 667 µg/mL sugar standard.

Figure 3 shows the calibration results for all four sugars over a concentration range of 133 to 1333 µg/mL. All four sugars followed a quadratic (2nd order) fit and had R^2 coefficients > 0.999 (n = 3 at each level).

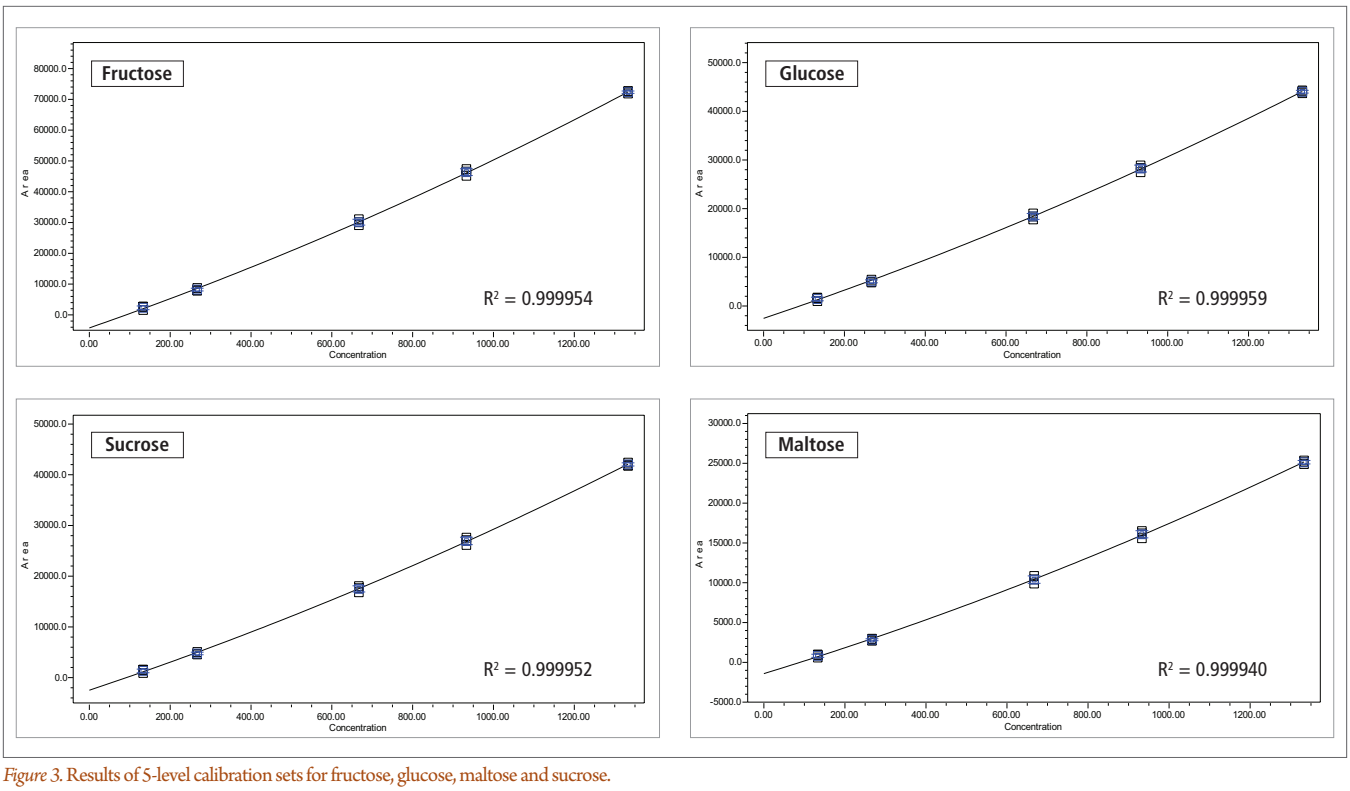

Figure 3. Results of 5-level calibration sets for fructose, glucose, maltose and sucrose.

Using the same chromatographic conditions, four honey samples were analyzed. The chromatographic results for Honey X, Honey Y and Honey Z are shown in Figure 4. Comparing the chromatograms of these honey samples with the sugar standards, it can be observed that all three honey samples contain the same three sugars: fructose, glucose and small amounts of sucrose.

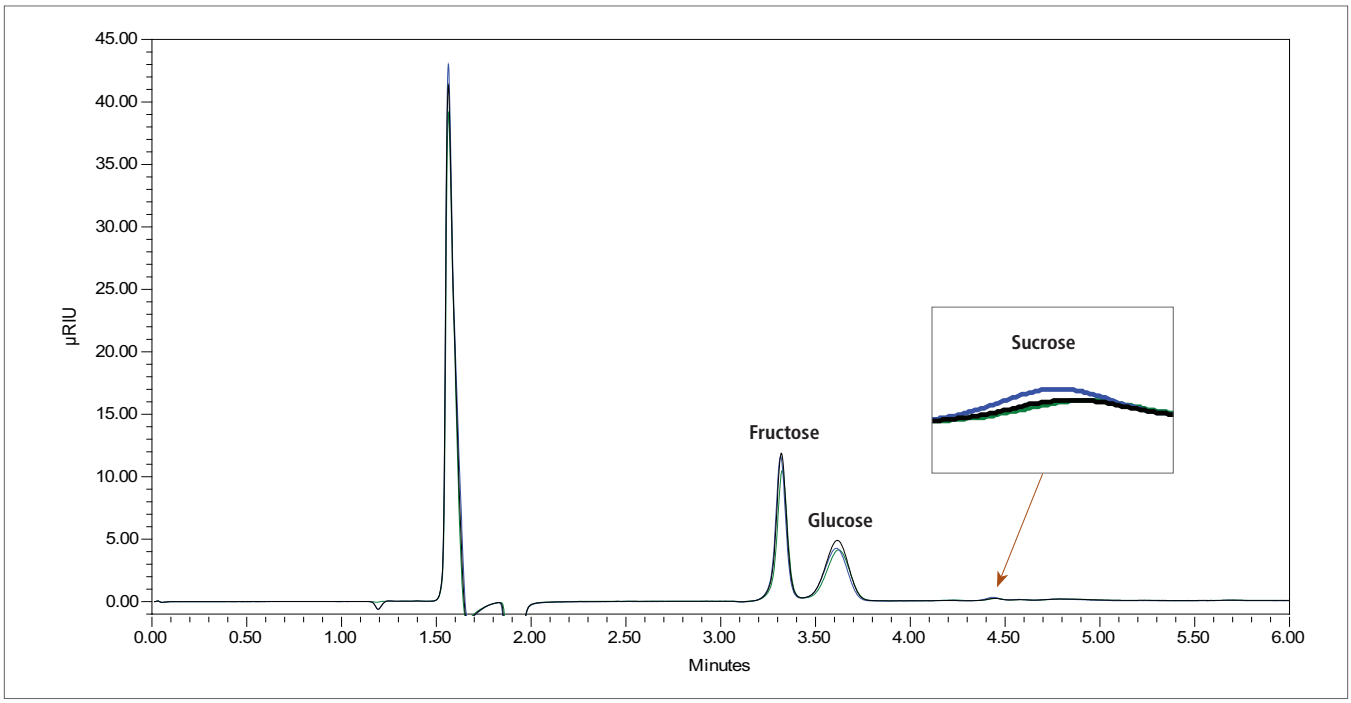

Figure 4. Overlaid chromatograms of Honey X (green), Honey Y (black) and Honey Z (blue).

Based on standard calibration, the quantitative results for each honey sample are shown in Table 2. Combining the fructose and glucose percentages for each honey sample, the overall fructose and glucose content for Honey X, Y, and Z was determined to be 50.90%, 57.13%, and 53.60%, respectively. These results are consistent with the accepted overall content of fructose and glucose in honey, expected to be somewhere around 60%.[1] The sucrose content for each honey sample was determined to be 3.20%, 3.26% and 3.90%, respectively. These values are all below the 5% mass ratio limit for sucrose that is allowed in unadulterated honey. Based on the data presented, the three store-bought honey samples do not appear to be adultered with cheaper sweeteners.

Upon closer examination of the chromatogram of Honey W, a smaller but significant peak was observed at about 5.10 minutes (Figure 5). This matched the elution time for maltose in the standard mix. The amount of maltose was calculated to be 43.85 mg, and the percent sugar was calculated to be 1.75% (w/w). Considering the 5% (by weight) limit that is allowed in commericially available "pure"-labeled honeys, the resulting maltose level found in Honey W suggests it was not adultered.

Table 2. Quantitative Results.

Honey X:		
Component	**Amount (mg)**	**Percent Sugar (w/w)**
Fructose	556.05	22.24
Glucose	716.48	28.66
Sucrose	79.875	3.20

Honey Y:		
Component	**Amount (mg)**	**Percent Sugar (w/w)**
Fructose	610.23	24.41
Glucose	817.95	32.72
Sucrose	81.525	3.26

Honey Z:		
Component	**Amount (mg)**	**Percent Sugar (w/w)**
Fructose	602.30	24.09
Glucose	737.78	29.51
Sucrose	97.525	3.90

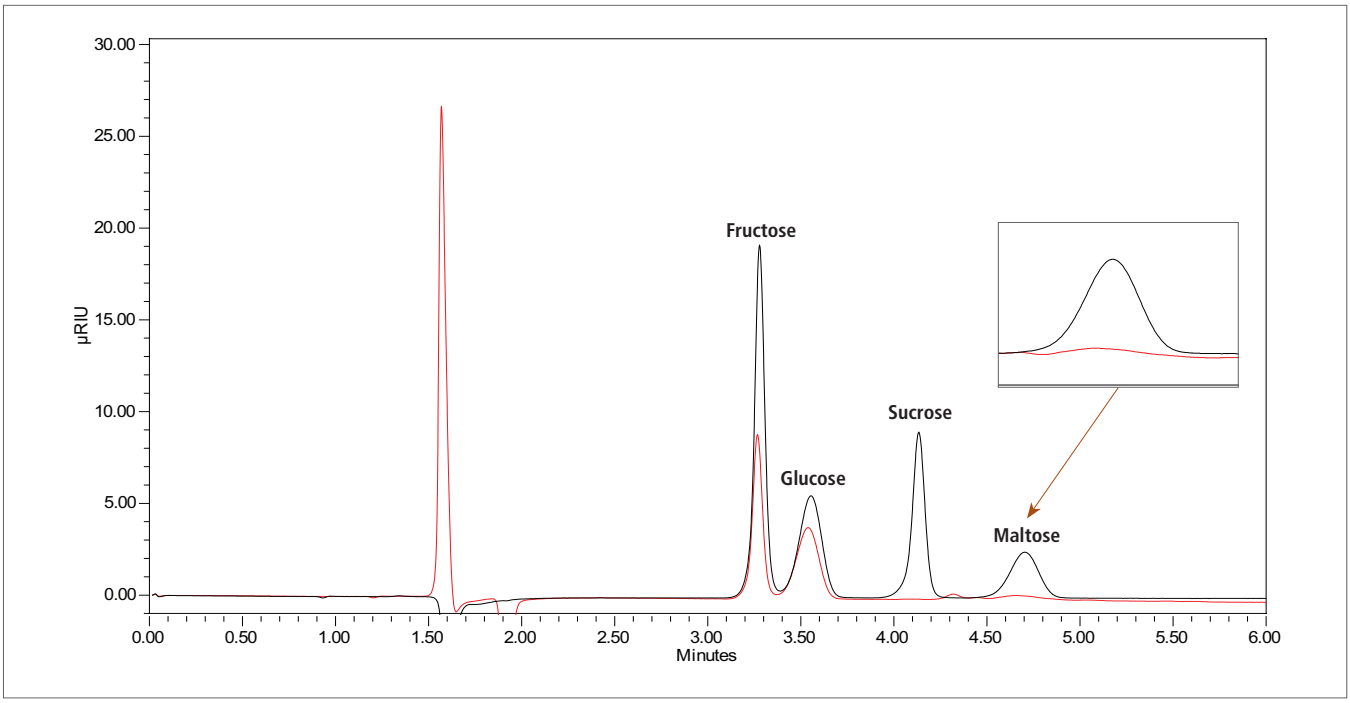

Figure 5. Overlay chromatograms of Honey W (red) and the 133 ppm sugar standard (black), zooming in on last eluting peak.

Conclusion

This work has demonstrated the effective chromatographic separation of four sugars using a PerkinElmer Altus HPLC System with RI detection. The results exhibited very good retention time repeatability as well as excellent linearity over the tested concentration ranges.

From a food quality perspective, there is an ever growing emphasis on food monitoring. This is especially the case pertaining to the adulteration of honey. With this in mind, this work focused on the sugar analysis of four store bought honeys, identifying the particular analytes contained in each of the honey samples, as well as comparing the sugar profiles, both chromatographically and quantitatively.

References

1. W. Guo, Y. Liu, X. Zhu and S. Wang, "*Dielectric properties of honey adulterated with surcrose syrup*", Journal of Food Engineering, pp. 1-7, 2011.
2. A. Moussa, D. Noureddine, A. Saad and S. Douichene, "*The Relationship between Fructose, Glucose and Maltose Content with Diastase Number and Anti-Pseudomonal Activity of Natural Honey Combined with Potato Starch*", Organic Chemistry Current Research, vol. 1, no. 5, pp. 1-5, 2012.
3. Codex Alimentarius Commission, 2001; GB18796-2005, 2005

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