Waters™

Application Note

Analysis of Food Sugars in Various Matrices Using UPLC with Refractive Index (RI) Detection

Mark E. Benvenuti

Waters Corporation

Abstract

The use of UPLC Technology enables highly efficient, rapid sugar separations in less than 3.5 minutes. The BEH Amide column chemistry allows for the use of basic modifiers and higher column temperatures to improve peak shape and quantification. The ACQUITY Refractive Index Detector's low internal volume delivers low dispersion that is compatible with narrow UPLC peak volumes, while still delivering stable baseline performance for reliable quantitative results, evident in the sugar analysis.

Benefits

Using UPLC with RI detection enables the detection of the food sugars fructose, glucose, sucrose, maltose, and lactose in a variety of matrices resulting in the following benefits:

- Run time of less than 3.5 minutes increases throughput.
- Isocratic elution eliminates the need for column re-equilibration between injections.
- Method allows the use of robust UPLC BEH Amide column chemistry for sugar separation.

Introduction

Sugars are found in a variety of food matrices as either naturally occurring or artificially added. Fructose, glucose, and sucrose are important constituents of various fruit juices. Maltose is found in products derived from corn and grain products. Lactose, also known as milk sugar, exists in dairy products. This set of sugars is known as the five food sugars.

Analysis of these sugars is important for quality control purposes, or to determine authenticity or adulteration of food products. Due to the lack of chromophores on these molecules, typical LC methods use evaporative light scattering (ELS), electrochemical, or refractive index (RI) detection. In many laboratories, RI is the detection method of choice because it is simple to use and needs no equilibration between injections. RI provides excellent repeatability and shows a linear response for quantification. There is no need for a clean nitrogen supply, which provides an economic benefit and is ideal for a manufacturing facility. In laboratories where RI detection is the preferred detection method for these samples, a UPLC-compatible version was required to take advantage of the benefits of sub-2-µm particle technology.

This application note shows the efficacy of the Waters ACQUITY UPLC H-Class System with the ACQUITY RI Detector to analyze sugars in several different food matrices with a run time of less than 3.5 minutes.

Figure 1. ACQUITY UPLC H-Class System with RI Detector.

Experimental

LC conditions

System:	ACQUITY UPLC H-Class

Run time:	3.5 min
Column:	ACQUITY BEH Amide 2.1 x 50 mm, 1.7µm
Temp.:	85 °C
Mobile phase:	77:23 acetone/water with 0.05% triethylamine (TEA)
Flow rate:	0.15 mL/min
Injection volume:	1.0 µL
Detection:	ACQUITY Refractive Index Detector
Data acquisition:	20 points/s
Time constant:	Normal
Flow cell:	40 °C

Standard Preparation

A stock solution of fructose, glucose, sucrose, maltose, and lactose was prepared by dissolving ~1 gram of each of these sugars in water and diluting to 100 mL with same. From this stock, six dilutions were made in 50:50 acetonitrile/water to create working standards with concentrations listed in Table 1. The fructose high standard was ~1400 ppm greater than that in the other sugars due to the amount weighed for the stock, extending the curve for this analyte compared to the other sugars.

Sample Preparation

Individual samples of orange, white grape, and pineapple juices were centrifuged at 4000 rpm for 30 min to remove solids. The supernatants were diluted 1:5 with 50:50 acetonitrile/water, and injected. A corn syrup sample was prepared by dissolving 1.024 g syrup in water, and further diluting to 50 mL. 500 µL of this was diluted with the same amount of 50:50 acetonitrile/water, then injected. A sample of low fat milk was diluted 1:10 with 50:50 acetonitrile/water, and centrifuged at 4000 rpm for 30 min to remove the precipitated lipids. A

portion of the supernatant was collected for injection.

Std	Fructose	Glucose	Sucrose	Maltose	Lactose
1.0	6505.0	5125.0	5110.0	5065.0	5165.0
2.0	2602.0	2050.0	2044.0	2026.0	2066.0
3.0	1301.0	1025.0	1022.0	1013.0	1033.0
4.0	650.5	512.5	511.0	506.5	516.5
5.0	520.4	410.0	408.8	405.2	413.2
6.0	260.2	205.0	204.4	202.6	206.6

Table 1. Concentrations of the mixed sugar standards in ppm (mg/kg).

Results and Discussion

The resulting chromatograms from injections of each of the six standards are shown in Figure 2. Note that all target analytes elute in less than 3.5 minutes. Figure 3 shows the linear calibration curves for each of the five food sugars where R^2 values are greater than 0.999 for all compounds.

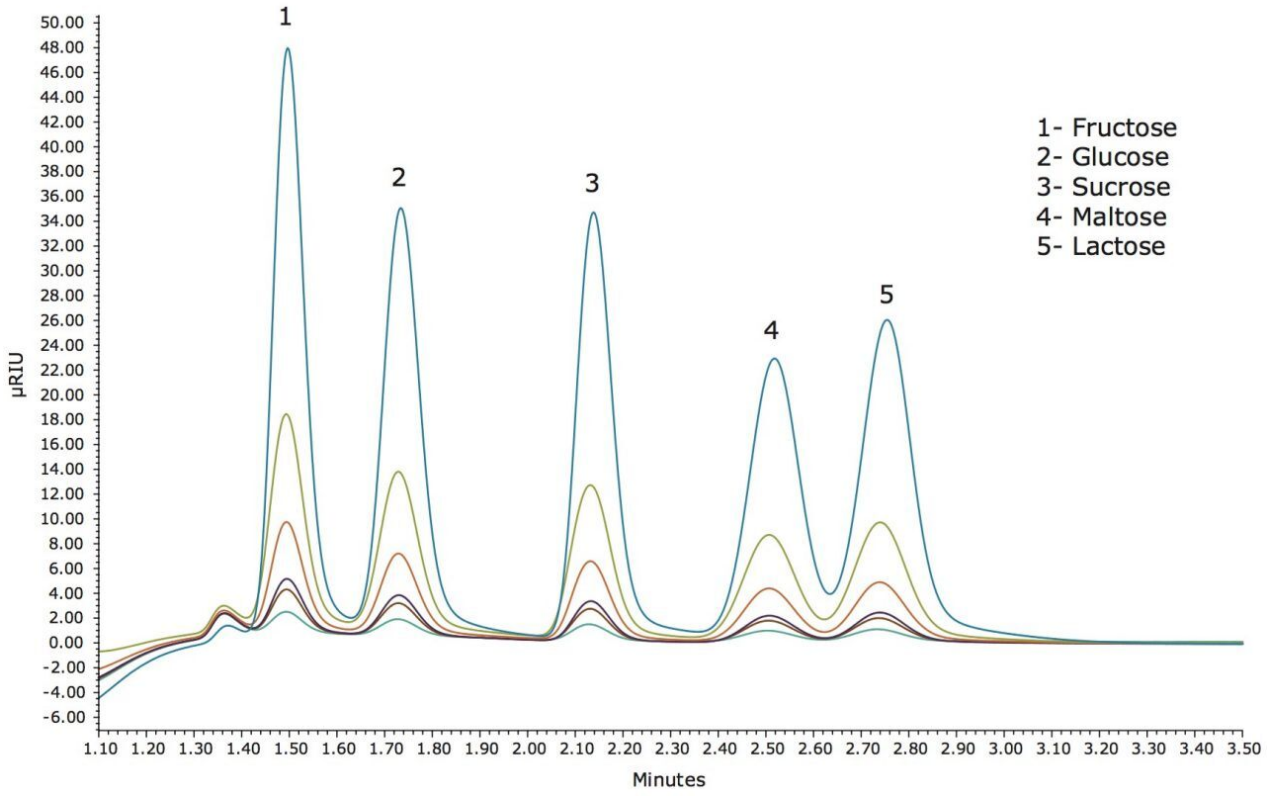

Figure 2. Overlay of the chromatograms from the six standards of differing concentrations.

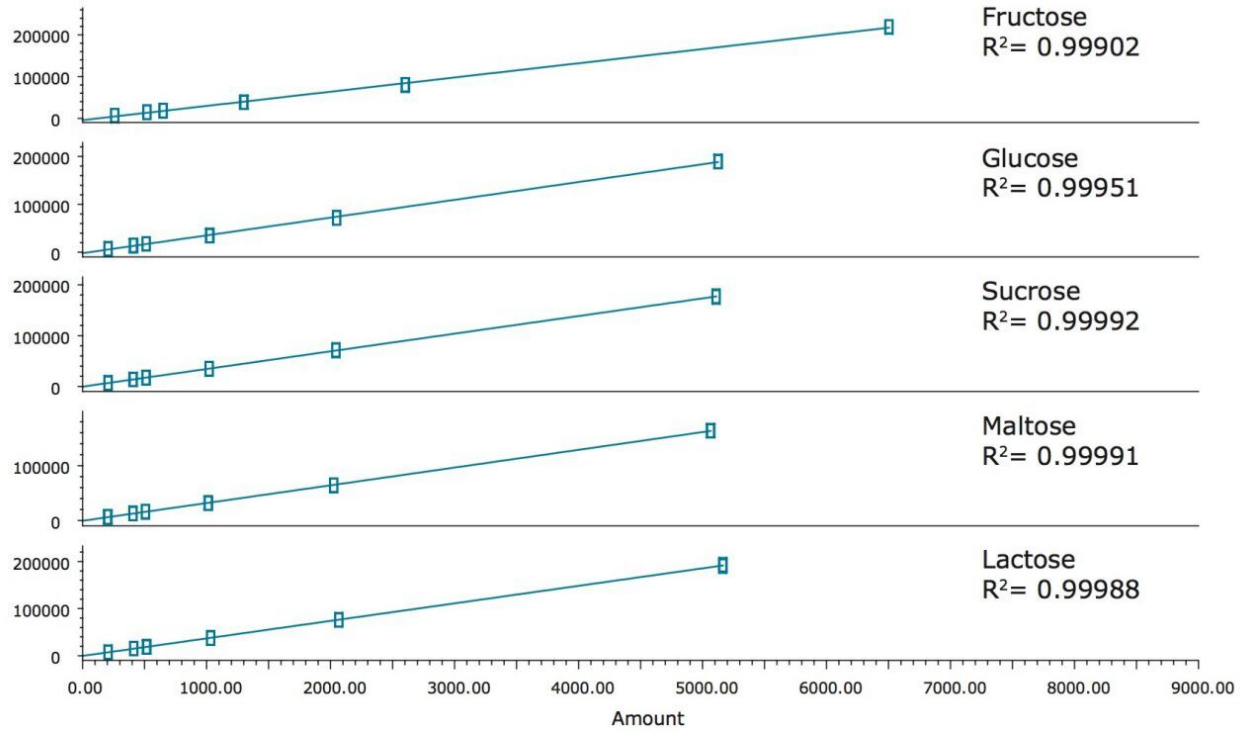

Figure 3. Calibration curves for the five food sugars.

To assess the utility of the method for the quantification of sugars in fruit juices, three fruit juice samples were analyzed, and the resulting chromatograms are shown in Figure 4. Differences in sugar content and ratios for each juice were quite apparent. The ratio of fructose, glucose, and sucrose was ~1:1:2, while the white grape juice contains fructose and glucose, but negligible sucrose. This is consistent with the literature.[1] To assess the retention time and amount repeatability, each juice was injected three times. The resulting data are shown in Table 2. The %RSD for retention time and amount for all analytes was less than 0.11 and 1.25, respectively.

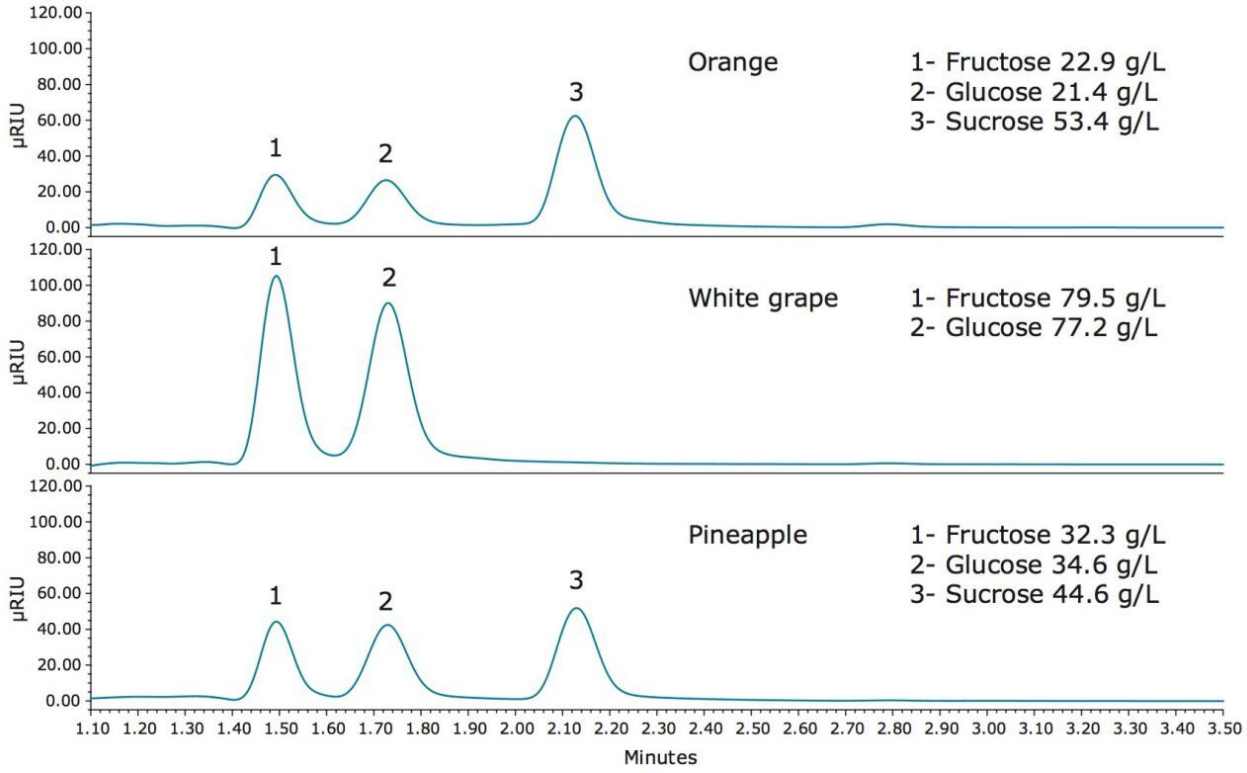

Figure 4. Chromatograms of orange, white grape, and pineapple juices.

Sugar	Orange		White grape		Pineapple	
	RT	Amount	RT	Amount	RT	Amount
Fructose	0.104	1.218	0.048	0.365	0.066	0.371
Glucose	0.038	0.827	0.035	0.659	0.049	0.321
Sucrose	0.035	0.588	N/A	N/A	0.043	0.429

Table 2. Reproducibility data (%RSD) for retention time and amount for three injections of each fruit juice.

The chromatograms for corn syrup and low fat milk are shown in Figures 5 and 6. These samples show very different profiles from those expected for fruit juices. The supermarket corn syrup sample used here consists primarily of glucose and maltose, as shown in Figure 5. Unlike high fructose corn syrups, where cornstarch has been enzymatically converted to glucose and then to fructose, this sample still has high levels of maltose. Lactose is a food sugar found in dairy products and is present in the chromatogram of the milk sample in Figure 6.

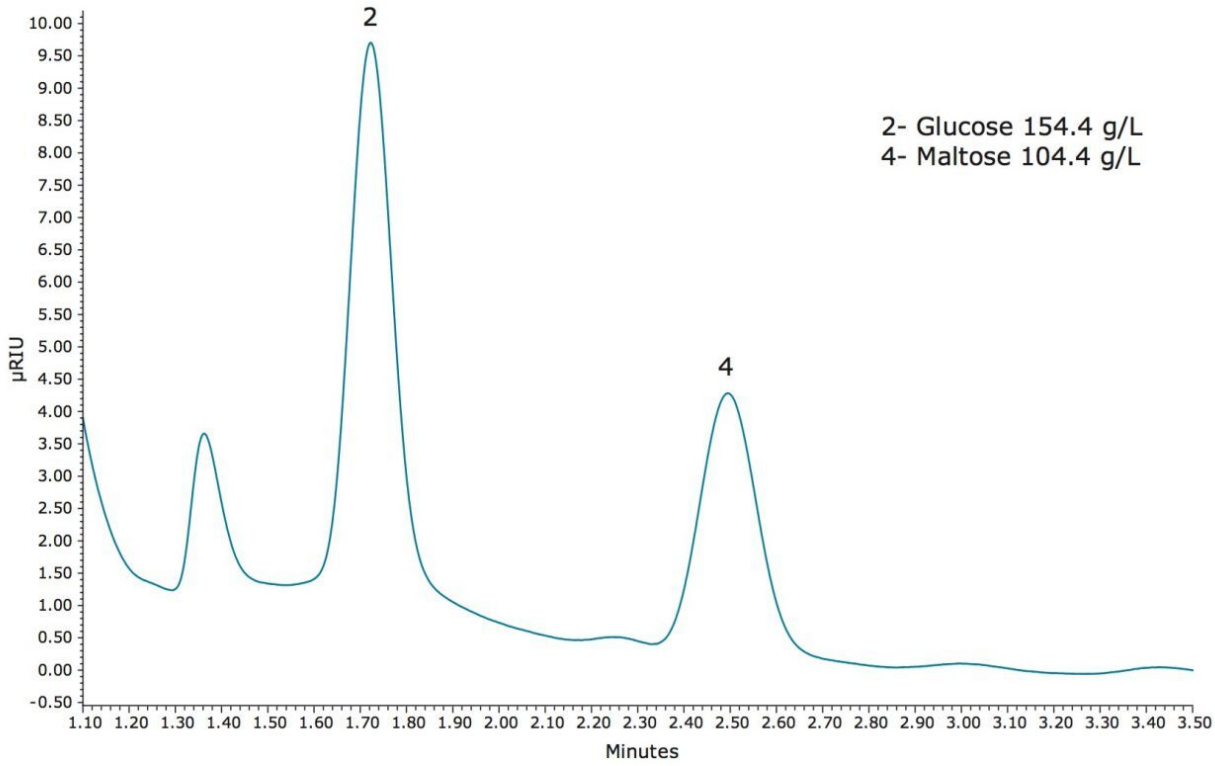

Figure 5. Chromatogram of corn syrup showing calculated concentration of the two sugars; glucose and maltose.

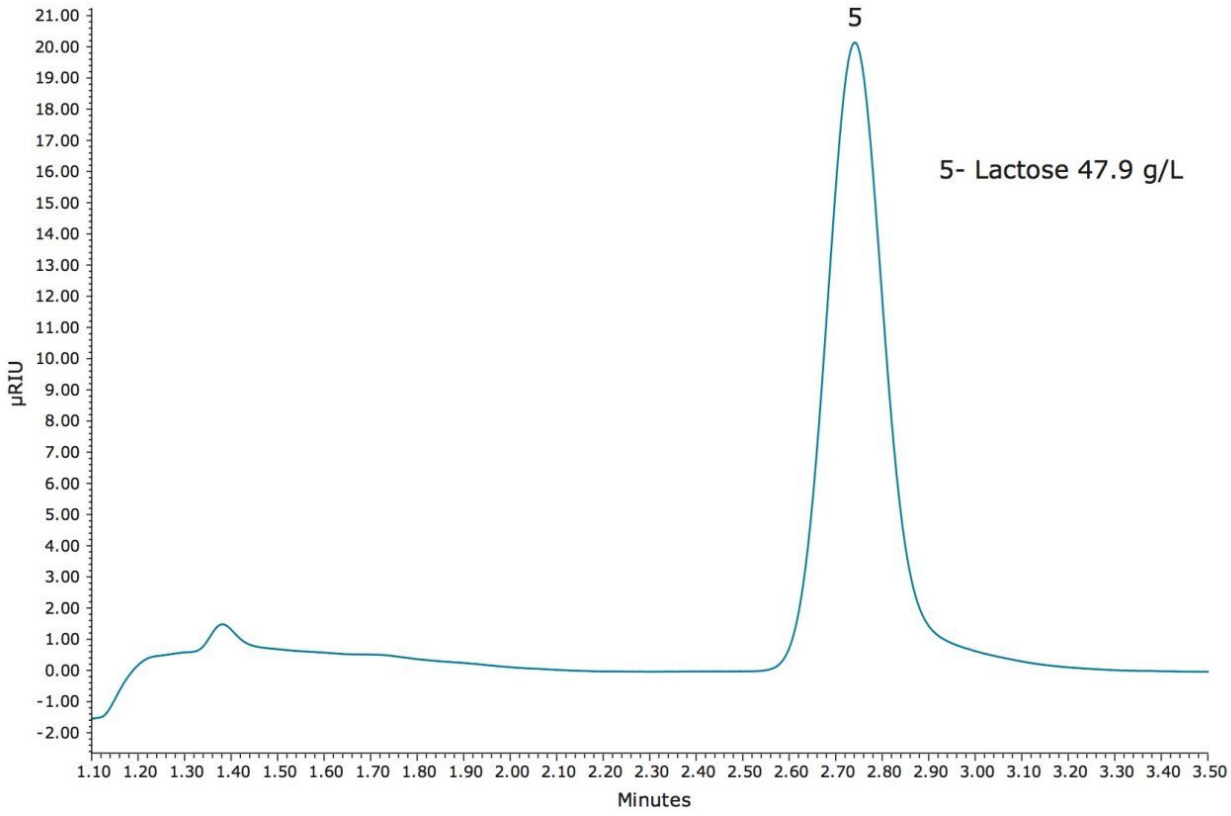

Figure 6. Chromatogram of low fat milk showing the presence of lactose.

The use of UPLC Technology enables highly efficient, rapid sugar separations in less than 3.5 minutes. The BEH Amide column chemistry allows for the use of basic modifiers and higher column temperatures to improve peak shape and quantification. The ACQUITY Refractive Index Detector's low internal volume delivers low dispersion that is compatible with narrow UPLC peak volumes, while still delivering stable baseline performance for reliable quantitative results, evident in the sugar analysis.

Conclusion

This application note demonstrates a rapid isocratic method for the analysis of food sugars.

Waters ACQUITY UPLC H-Class System with RI detection provides the following benefits:

- Rapid analysis time with no column equilibration necessary

- A reduction in turnaround time resulting in increased throughput
- Low eluent flow rate reduces solvent cost and waste disposal

References

1. Sanz *et al*. Inositols and Carbohydrates in Different Fresh Fruit Juices. *Food Chemistry*. 2004; 87: 326.

Featured Products

ACQUITY UPLC H-Class System <https://www.waters.com/10138533>

ACQUITY Refractive Index Detector <https://www.waters.com/134726507>

Available for purchase online

ACQUITY UPLC BEH Amide Column, 130Å, 1.7 µm, 2.1 mm X 50 mm <
https://www.waters.com/waters/partDetail.htm?partNumber=186004800>

720004677, May 2013