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Application Note

Evaluation of XBridge™ BEH Amide Columns for the Analysis of Five Sugars in Food and Beverages Using an Arc HPLC, Coupled to a 2414 RI Detector

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Abstract

Sugars such as monosaccharides (fructose and glucose), and disaccharides (sucrose, maltose, and lactose) are routinely tested for in foods and beverages. This testing supports product development and reformulation, as well as confirming label claims, and ensuring product consistency, safety, and authenticity.

High Performance Liquid Chromatography (HPLC) is a common technique used for the analysis of sugars in food and beverages. The use of HPLC allows for the separation of monosaccharides and disaccharides, providing information on the sugars present in a sample and their quantities.

The purpose of this application note is to compare the performance of two XBridge BEH Amide Columns with different particle sizes and column lengths for the analysis of five sugars, using a simple isocratic method. The Waters™ Column Calculator tool was used to transfer a previously published method from a 4.6 mm x 250 mm, 3.5 µm column to a 4.6 mm x 150 mm, 2.5 µm column. The result of this was a significant decrease in run-time and a reduction in mobile phase consumption. Method performance criteria such as linearity, USP peak resolution, and the precision of retention time and peak areas are compared for each column. An Arc™ HPLC coupled with a 2414

RI Detector was used to evaluate the performance of the isocratic method on both XBridge BEH Amide Columns.

Benefits

- Use of the XBridge BEH Amide Column allows for a simple isocratic method for the retention and separation of fructose, glucose, sucrose, maltose, and lactose
- The combination of the XBridge BEH Amide Column, Arc HPLC, and 2414 Detector enabled the profiling and quantitation of sugars present in various food and beverage samples
- An XBridge BEH Amide XP Column was tested to demonstrate the possibility of reducing run-time and solvent consumption when using the Arc HPLC

Introduction

Sugars are naturally present or added to food and beverages to provide an array of functions. Sugars not only provide sweetness but also provide texture, bulking, act as a preservative as well as providing a substrate for yeast in fermentation.[1]

Sugars such as monosaccharides (fructose and glucose), and disaccharides (sucrose, maltose, and lactose) are routinely tested for in foods and beverages to support product development and reformulation as well as confirming label claims, and ensuring product consistency, safety, and authenticity.

High Performance Liquid Chromatography (HPLC) is a common technique used for the analysis of sugars in food and beverages. The use of HPLC allows for the separation of monosaccharides and disaccharides, providing information on the sugars present in a sample and their quantities. Very little sample preparation is typically required for several food and beverage types prior to HPLC analysis, allowing for an accurate, reliable, and efficient approach to ensure products are within specification and meet label claims.

The analysis of monosaccharides and disaccharides does have some challenges as they do not retain on commonly used reversed-phase C_{18} columns and lack strong chromophores within their compound structures. However, these challenges can be overcome through use of alternative detectors to ultraviolet-visible light (UV-Vis) such as refractive index (RI) detection, and an alternative chromatographic separation technique such as hydrophilic interaction liquid chromatography (HILIC).

This application note will demonstrate and compare a simple isocratic method for the retention and separation of five sugars using two different XBridge BEH Amide Columns with different particle sizes and column lengths. The analysis was performed using an Arc HPLC coupled with a 2414 Refractive Index (RI) Detector.

Experimental

Materials and Reagents

Fructose, glucose, sucrose, maltose, and lactose were obtained from Sigma Aldrich.

Acetonitrile and methanol (HPLC grade) were obtained from Honeywell Research Chemicals.
Triethylamine was obtained from Sigma Aldrich.
Water from PureLab flex ELGA system (LabWater United States of America).
Products containing the sugars were purchased from local retailers (Massachusetts).

Sample Preparation

Preparation of calibration standards

Sugar standards were prepared at a concentration of 100 mg/mL in water and stored at 4 °C. A mixed standard of sugars was prepared at 5 mg/mL via serial dilution in 50:50 acetonitrile: water. The calibration curves ranged from 0.08 to 5 mg/mL.

Four replicates of children's organic mix juice, orange juice, sports drinks, and non-fat milk were diluted 1:4 with 50:50 acetonitrile and water. The samples were then vortexed for 1 minute, centrifuged for 5 minutes at 4000 rpm, and filtered using a 0.2 µm PVDF syringe filter.

For the bread sample, 3 g was weighed out and dissolved in 25 mL 50:50 acetonitrile and water. The sample was then vortexed for 1 minute, centrifuged for 5 minutes at 4000 rpm, and filtered using a 0.2 µm PVDF filter.

For column 1, 15 µL of the samples were injected and for column 2, 9 µL of the samples were injected.

Further dilution was required to bring the beverage samples into the concentration range of the calibration curve. The type and amount of sugars vary between different food and beverage products, so optimization of the sample dilution step will be required to ensure samples are within the concentration range of the solvent standard

calibration curve.

LC Conditions

LC system:	Arc HPLC System
Detection:	RI Detection (Sampling rate: 10 points/sec)
Detector temp:	35 °C
Vials:	LCGC Certified Clear Glass 12 x 32 mm Screw Neck Max Recovery Vial, with Cap and PTFE/Silicone Septum, 1.5 mL volume (p/n: 186007201C)
Filter:	Syringe Filter 0.2 µm PVDF (p/n: WAT200806)
Column(s):	XBridge BEH Amide Column, 130 Å, 3.5 µm, 4.6 mm x 250 mm p/n: 186004870 (column 1) XBridge BEH Amide Column, 130 Å, 2.5 µm, 4.6 mm x 150 mm p/n: 186006726 (column 2)
Column temp:	35 °C
Sample temp:	25 °C
Injection volume:	15 µL and 9 µL
Flow rate:	1.0 mL/min and 1.4 mL/min
Mobile phase:	75:25 Acetonitrile: Water 0.2% triethylamine
Sample diluent:	50:50 Acetonitrile: Water

Seal wash:	5:95 Acetonitrile: Water
Needle wash:	5:95 Acetonitrile: Water
Purge solvent:	50:50 Acetonitrile: Water

Data Management

Software:	Empower CDS

Results and Discussion

The objective of this short study was to compare two XBridge BEH Amide Columns with different particle sizes and lengths for the analysis of five common sugars. The same HPLC system, mobile phase, and samples were used to compare the performance of both columns. The Waters Column Calculator (Figure 1) was used to transfer previously published method conditions from the 4.6 mm x 250 mm, 3.5 µm column (column 1) to the 4.6 x 150 mm, 2.5 µm column (column 2).[2–3]

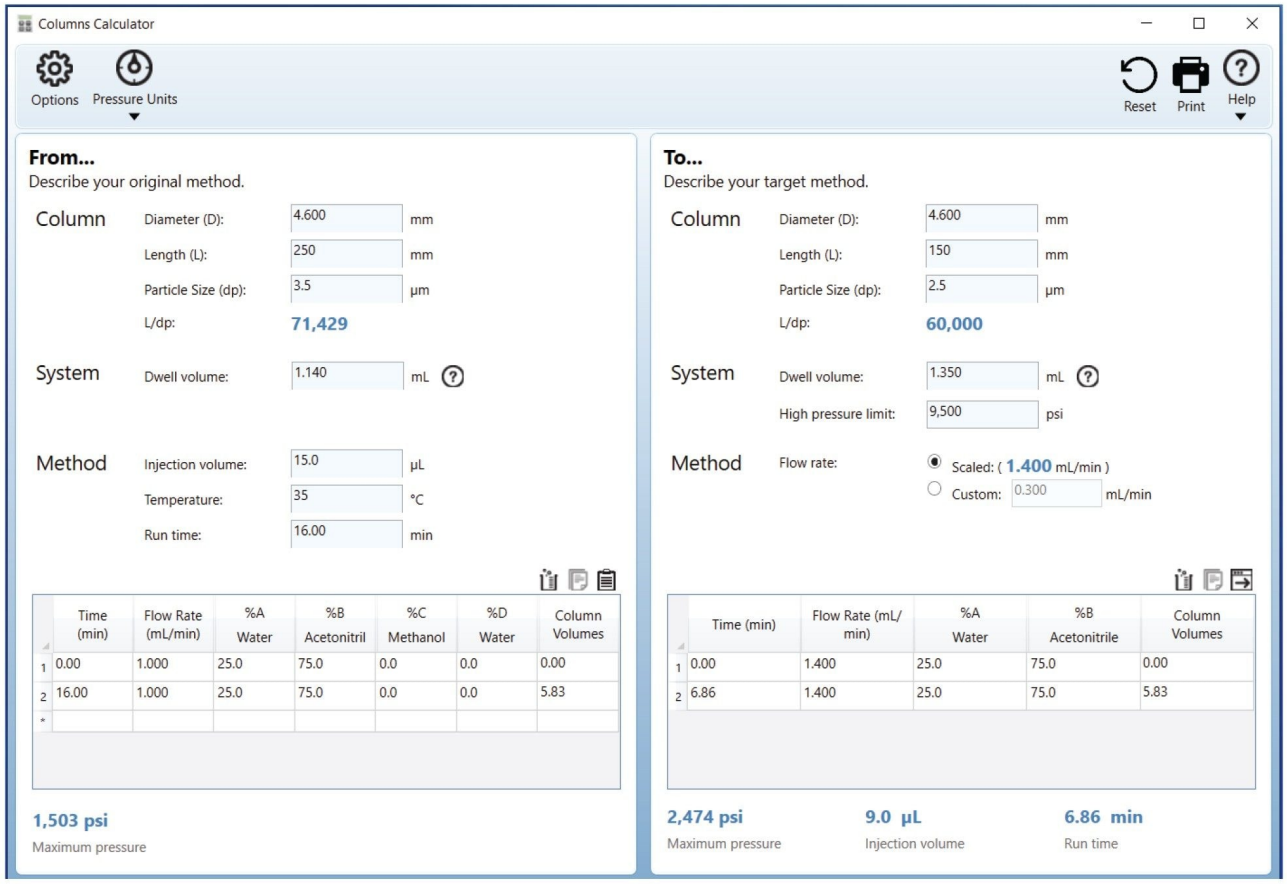


Figure 1. Scaled method transfer example from Waters Column Calculator.

Modern LC columns using stationary phases with smaller particle sizes provide greater chromatographic efficiency. To take advantage of smaller particle sizes the column dimensions should be appropriate to the dispersion volume of the LC system used. Smaller particles also generate higher back pressure so the system must be capable of running the smaller particle at the correct flow rate for optimum performance. The method that was scaled for the smaller particle size results in a higher flow rate of 1.4 mL/min; however, the expected backpressure is well within the system pressure limits of the Arc HPLC (9,500 psi). When running an isocratic method, the calculation of the column length divided by the particle size (L/dp) gives an estimation of the maximum resolving power of a column.[4] Column 1 has an L/dp of 71,400, and column 2 has an L/dp of 60,000, which results in less separation between the sugars. The estimated run time for column 2 is 6.86 minutes, which is a reduction of around 57%. The reduced run time, even with the increase in flow rate for column 2, would also result in a reduction of mobile phase consumption of ≤6 mL per injection.

Figure 2 shows a comparison of the same solvent standard injected onto each of the columns. As indicated by the column calculator tool, the run time can be greatly reduced using column 2 with the last peak (lactose) eluting at a retention time of 6 minutes, before the first peak on column 1 (fructose), which elutes at 6.9 minutes.

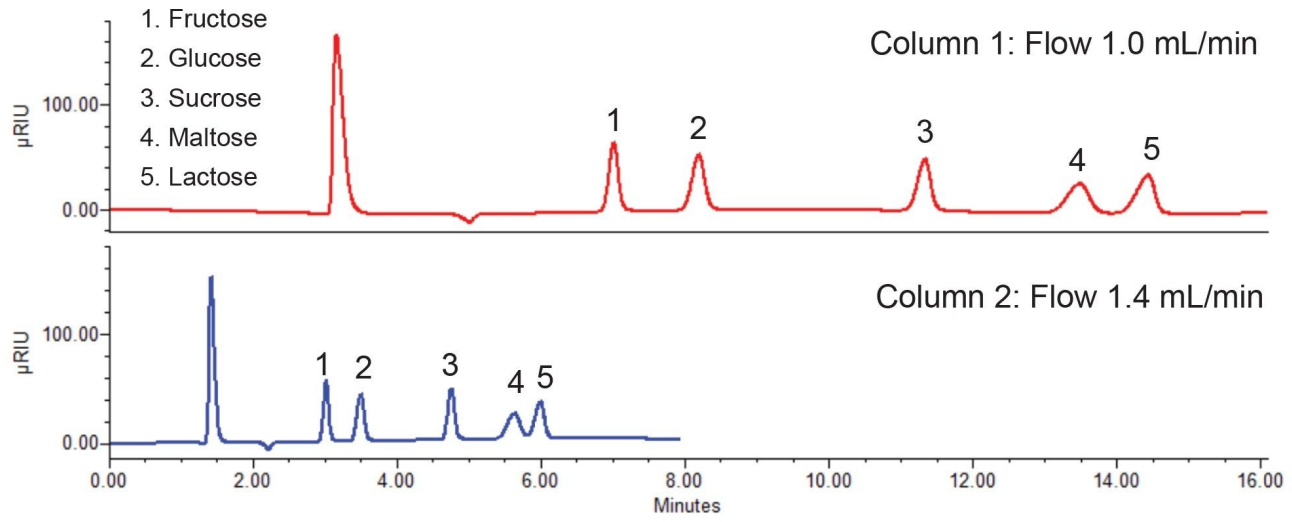


Figure 2. Comparison of solvent standard (5 mg/mL) chromatograms from the two XBridge BEH Amide Columns.

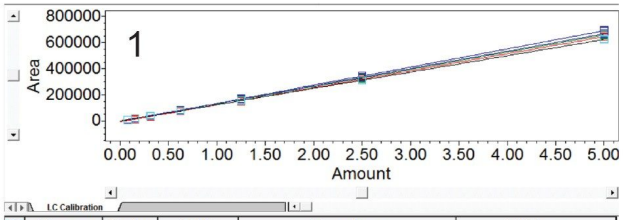
Table 1 is a comparison of peak retention times and USP resolution, which was calculated using the Empower™ CDS Software. When evaluating a separation between closely eluting peaks a typical minimum value used for the resolution between peaks is 2.0 (Rs >2.0), based on FDA guidance.[5] Column 1 was able to achieve this for all the peak separations, whereas column 2 was only able to achieve a resolution of 1.3 between maltose and lactose (peaks 4 and 5), for samples which do not contain both lactose and maltose, column 2 should be a practical option to implement. A reduction in the USP resolution was seen in column 2 for all separations, which was expected as a result in the reduced resolving power (L/dp).

Peak number	Retention time Column 1	USP resolution Column 1	Retention time Column 2	USP resolution Column 2
1. Fructose	6.9		3.0	
2. Glucose	8.2	4.2	3.5	2.9
3. Sucrose	11.3	9.6	4.8	6.9
4. Maltose	13.4	5.3	5.6	3.7
5. Lactose	14.3	2.0	6.0	1.3

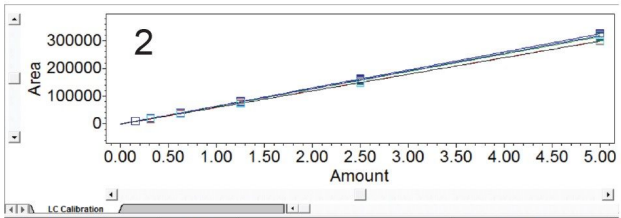
Table 1. Comparison of retention time and peak resolution between the two XBridge BEH Amide Columns.

Calibration Curve: Linearity

A multi-point calibration curve for the tested sugars was prepared in duplicate via serial dilution in 50:50 water and acetonitrile. The calibrated range was from 0.08 mg/mL to 5.0 mg/mL. Both columns showed excellent linearity (R^2 >0.999). Figure 3 shows the calibration curves for both the columns tested.



	Name	Order	R^2	Equation	Fit Type
1	Fructose	1	0.999965	Y = 1.25e+05 X - 7.05e+02	Linear (1st Order)
2	Glucose	1	0.999951	Y = 1.33e+05 X + 4.59e+01	Linear (1st Order)
3	Sucrose	1	0.999957	Y = 1.32e+05 X - 1.33e+03	Linear (1st Order)
4	Maltose	1	0.999694	Y = 1.30e+05 X - 6.60e+03	Linear (1st Order)
5	Lactose	1	0.999829	Y = 1.39e+05 X - 4.60e+03	Linear (1st Order)



	Name	Order	R^2	Equation	Fit Type
1	Fructose	1	0.999977	Y = 5.98e+04 X - 4.98e+02	Linear (1st Order)
2	Glucose	1	0.999978	Y = 6.38e+04 X - 2.03e+02	Linear (1st Order)
3	Sucrose	1	0.999973	Y = 6.31e+04 X - 2.04e+02	Linear (1st Order)
4	Maltose	1	0.999908	Y = 6.01e+04 X - 1.04e+03	Linear (1st Order)
5	Lactose	1	0.999936	Y = 6.53e+04 X - 8.28e+02	Linear (1st Order)

Figure 3. Calibration curves for the tested sugars on the 4.6 mm x 250 mm, 3.5 µm column (1) and the 4.6 mm x 150 mm, 2.5 µm column (2).

Reproducibility of Area, and Retention Time

Six replicate injections of the 5 mg/mL standard were injected on each column to evaluate the retention time

and area reproducibility for each of the 5 sugars tested. Table 2 shows the results of this evaluation.

Peak number	Retention time % RSD Column 1	Area % RSD Column 1	Retention time % RSD Column 2	Area % RSD Column 2
1. Fructose	0.1	0.2	0.2	0.4
2. Glucose	0.1	0.1	0.2	0.4
3. Sucrose	0.1	0.4	0.3	0.3
4. Maltose	0.1	1.1	0.3	0.4
5. Lactose	0.1	0.9	0.3	0.5

Table 2. Comparison of % RSD for retention time (n=6) and peak area (n=6) between the two XBridge BEH Amide Columns.

Analysis of Sugars in Products

Samples were prepared in four replicates, the measured amount of the sugars in each sample are shown in table 3.

Column 1	Total claimed gram per serving	Total detected gram per serving	Fructose detected (% RSD)	Glucose detected (% RSD)	Sucrose detected (% RSD)	Maltose detected (% RSD)	Lactose detected (% RSD)
Organic mix juice	9.0	8.9	5.3 (2)	3.6 (1)	N/A	N/A	N/A
Orange juice	24.0	21.6	5.7 (2)	4.8 (1)	11 (4)	N/A	N/A
Sport drink	21.0	22.6	7.9 (2)	8.9 (2)	5.7 (1)	N/A	N/A
Non-fat milk	12.0	11.2	N/A	N/A	N/A	N/A	11.2 (1)
Bread	1.0	1.5	0.7 (2)	0.4 (2)	0.0	0.4 (3)	N/A

Column 2	Total claimed gram per serving	Total detected gram per serving	Fructose detected (% RSD)	Glucose detected (% RSD)	Sucrose detected (% RSD)	Maltose detected (% RSD)	Lactose detected (% RSD)
Organic mix juice	9.0	8.8	5.1 (0.9)	3.7 (0.7)	N/A	N/A	N/A
Orange juice	24.0	20.7	5.3 (4.2)	4.8 (3.9)	10.6 (4.0)	N/A	N/A
Sport drink	21.0	21.7	7.3 (1.8)	8.8 (1.6)	5.6 (1.6)	N/A	N/A
Non-fat milk	12.0	12.5	N/A	N/A	N/A	N/A	12.5 (1.8)
Bread	1.0	1.5	0.7(2.6)	0.4 (2.3)	0.0	0.4 (4.4)	N/A

Table 3. Amount of sugar detected in the tested samples for column 1 and column 2.

The calculated amount of each sugar was combined to a total amount and compared against the amount of sugar on the product labels (g per serving). All the samples, except the bread sample required additional dilution to fit into the concentration range of the calibration curve. For this short evaluation, lower injection volumes for the different samples were used to achieve this. Optimization of the sample dilution off-line would allow for the injected on-column amounts of the samples to fit the concentration range of the solvent standard calibration curve, using the same injection volume for the standards and samples. The dilution factor can be added into the Empower CDS Software sample list, allowing the automatic calculation of the un-diluted sample concentration during data processing. Example chromatograms of the tested samples for each column are shown in figure 4.

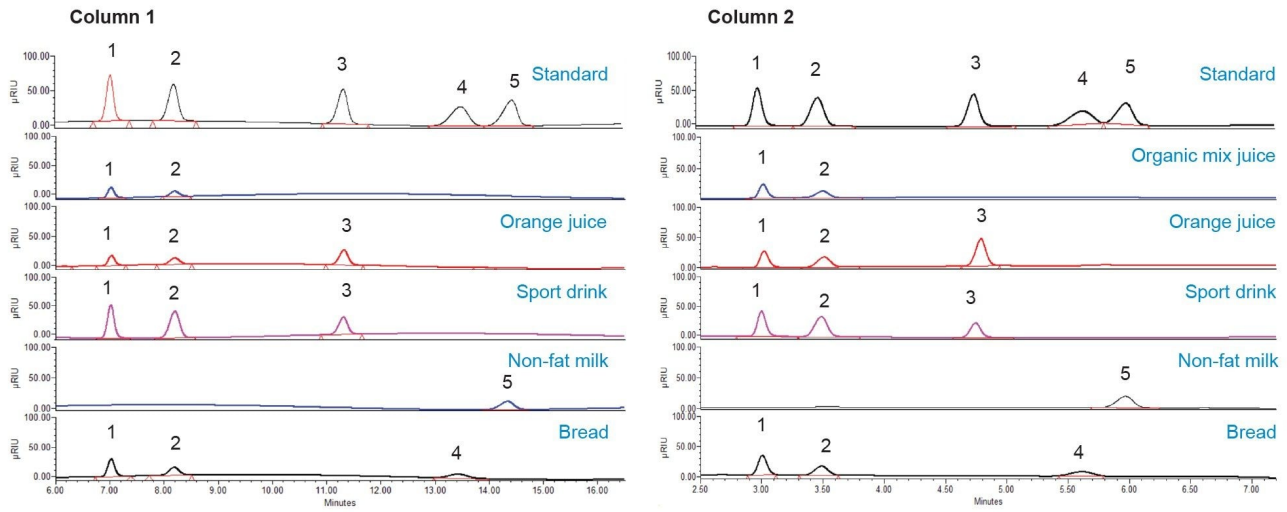


Figure 4. Example chromatograms for tested samples on column 1 and column 2. The peak elution order is 1. fructose, 2. glucose, 3. sucrose, 4. maltose, 5. lactose.

Conclusion

The purpose of this application note was to compare the performance of two XBridge BEH Amide Columns with different particle sizes and column lengths for the analysis of five sugars. The Waters Column Calculator tool was used to successfully transfer the method from a 4.6 mm x 250 mm, 3.5 µm column to a 4.6 mm x 150 mm, 2.5 µm column. A simple isocratic method was used for both columns, using an Arc HPLC coupled with a 2414 refractive index detector. The scaled transfer of the method to the 4.6 mm x 150 mm, 2.5 µm column resulted in a significant decrease in run-time and a reduction in mobile phase consumption. The method showed excellent linearity on both columns as well as good precision for retention times and peak areas for the five sugars. The combination of the XBridge BEH Amide Column, Arc HPLC and 2414 Detector was demonstrated for the profiling and quantitation of sugars present in various food and beverage samples.

References

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