Application Note

Food/Beverage Testing, Fermentation Monitoring, Agrochemicals, Biofuels, Alternative Energy

Analysis of sugars using an Agilent InfinityLab Poroshell 120 HILIC-Z column

Authors

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Abstract

An Agilent InfinityLab Poroshell 120 HILIC-Z column was used to separate 11 sugar compounds by both gradient and isocratic elution. The effect of pH and temperature on glucose anomer separation was explored. It was discovered that a combination of high pH and low temperature offered the best solution for both peak shape and column lifetime. The final separation used an ammonium hydroxide mobile phase at 35 °C.

Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. These columns generate high efficiency at lower pressure compared to their totally porous particle column counterparts. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column. The higher efficiency can be used to speed up analyses or improve results by increasing resolution and sensitivity.

To date, superficially porous particles have primarily focused on reversed-phase separations. With the maturation of superficially porous particle technology, applications for further chemistries and chromatographic techniques, such as hydrophilic interaction liquid chromatography (HILIC), are becoming available. HILIC is well suited for the analysis of polar analytes, which are often difficult to retain and separate in reversed-phase mode. This Application Note demonstrates the UHPLC performance of an Agilent InfinityLab Poroshell 120 HILIC-Z, 2.7 µm column, and its ability to resolve 11 sugar compounds by both gradient and isocratic elution.

The InfinityLab Poroshell 120 HILIC-Z phase uses a novel zwitterionic stationary phase bonded to a robust hybrid particle. It is stable up to 80 °C and up to pH 12. This phase is capable of separating analytes over a wide range of polarity. These HILIC-Z phase features make it well suited for challenging high pH separations, offering a robust solution.

Experimental

An Agilent 1260 Infinity binary LC with an Agilent G4218A ELSD was used for this work. All connecting capillaries were short, with a 0.12 mm internal diameter to minimize system dispersion.

Agilent OpenLAB software was used to control the system and process the data. Table 1 shows the chromatographic methods that were used. All compounds were injected as individual standards. Table 2 lists the concentrations and sample solvents.

The 11 sugar compounds analyzed were bought from Sigma-Aldrich. Ammonium formate, formic acid, ammonium acetate, and ammonium hydroxide were also from Sigma-Aldrich. Acetonitrile was bought from Honeywell (Burdick and Jackson). Water was 0.2 µm filtered, 18 molecular weight, from a Milli-Q system (Millipore).

Table 2. Sample preparation and injection volumes.

Sugar	Prepared as a saturated solution in:	Injection volume (µL)
Xylose	CH_3CN/H_2O (9:1)	0.1
Arabinose	CH_3CN/H_2O (9:1)	0.1
Fructose	CH_3CN/H_2O (9:1)	0.1
Mannose	CH_3CN/H_2O (9:1)	0.2
Glucose	CH_3CN/H_2O (9:1)	0.4
Galactose	CH_3CN/H_2O (9:1)	0.4
Sucrose	CH_3CN/H_2O (9:1)	0.5
Maltose	CH_3CN/H_2O (9:1)	1.0
Lactose	CH_3CN/H_2O (9:1)	1.5
Maltotriose	CH_3CN/H_2O (9:1)	3.0
Raffinose	CH_3CN/H_2O (9:1)	7.0

Table 1. Method parameters.

Method	Mobile phase A	Mobile phase B	Mobile phase composition	Flow rate (mL/min)	Column	Column temperature (°C)	ELSD Settings
Figure 1	Water	Acetonitrile	95–80 %B in 12 minutes, 3 minutes re-equilibration	0.4	Competitive HILIC column, 2.1 × 100 mm, 2.7 µm	35, 40, or 80	60 °C, 3.5 bar, 30 Hz
	100 mM Ammonium formate in water pH 4.5						
	100 mM Ammonium acetate in water pH 7.0						
	0.6 % Ammonium hydroxide in water						
Figure 2	0.3 % Ammonium hydroxide in water	Acetonitrile	90 % B Isocratic	0.4	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm (p/n 685775-924)	35	60 °C, 3.5 bar, 30 Hz
	100 mM Ammonium acetate in water pH 7.0					80	
Figure 3	0.3 % Ammonium hydroxide in water	0.3 % Ammonium hydroxide in acetonitrile	85–60 %B in 6 minutes, 3 minutes re-equilibration	0.4		35	60 °C, 3.5 bar, 30 Hz
Figure 4	0.3 % Ammonium hydroxide in water	0.3 % Ammonium hydroxide in acetonitrile	80 %B Isocratic	0.4		35	60 °C, 3.5 bar, 30 Hz

Results and discussion

Sugars can be difficult to analyze by HPLC, as many compounds experience anomer separation. Figure 1 shows that glucose anomer separation can be controlled by either using high pH, high temperature, or some combination thereof. For silica-based LC columns, these conditions are harsh, and can negatively impact column lifetime.

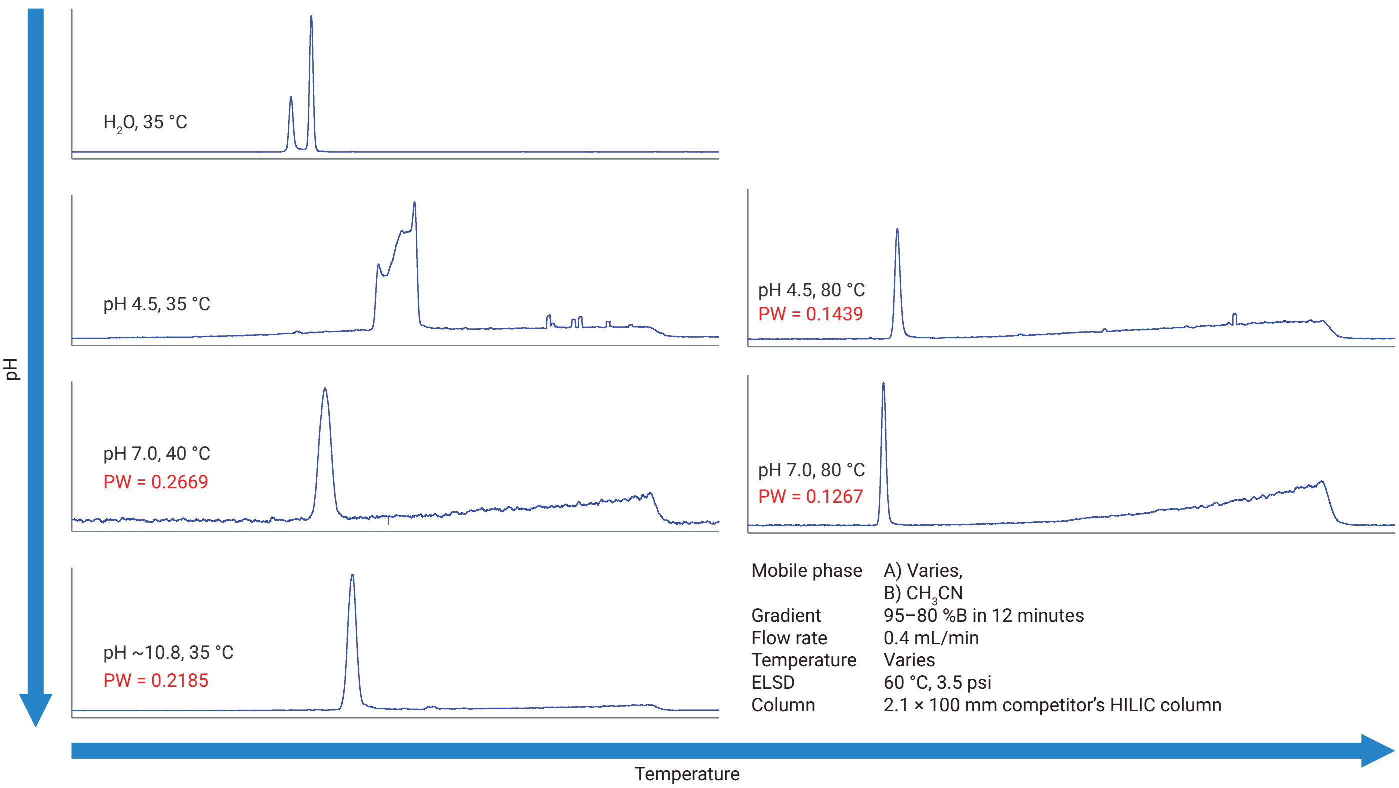
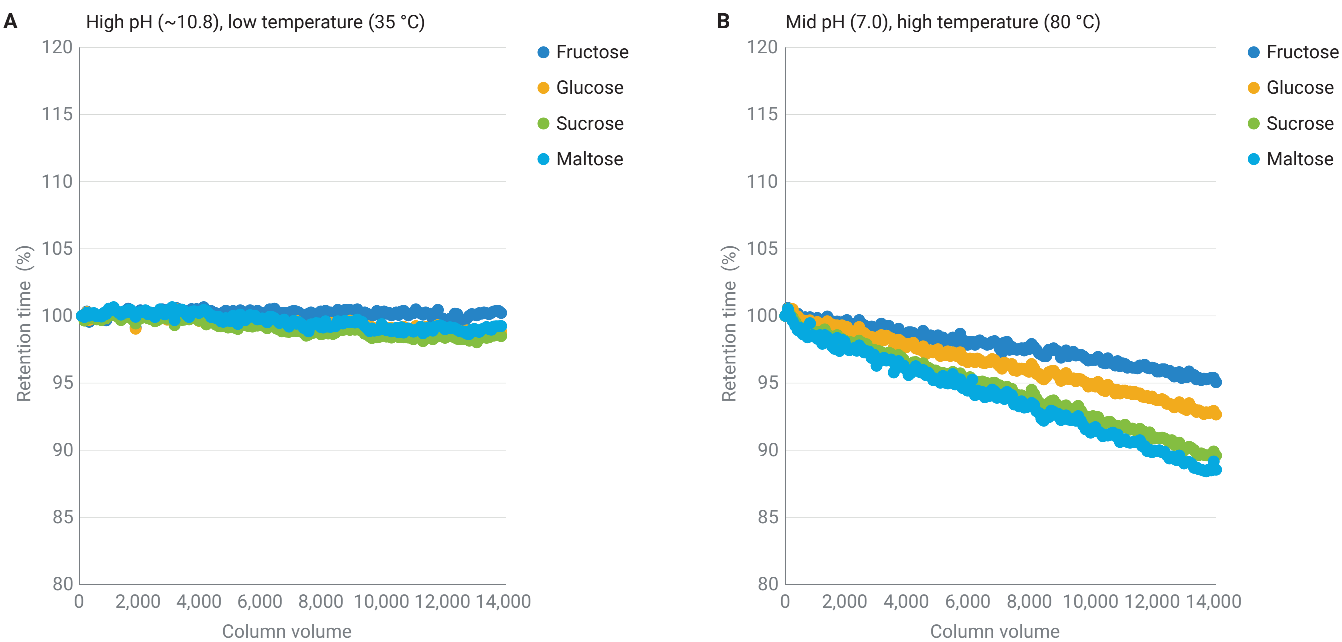


Figure 1. Effect of pH and temperature on anomer separation of glucose.

The Agilent InfinityLab Poroshell 120 HILIC-Z particles are made with a proprietary zwitterionic bonding on hybrid particles, which makes them more stable in high-pH mobile phases. Figure 2 evaluates the Agilent InfinityLab Poroshell 120 HILIC-Z column lifetime under method conditions suitable for sugars. The combination of high pH and low temperature causes no loss of performance over 14,000 column volumes. Elevated temperature combined with mid-pH offers narrower peak widths and lower backpressures, but these conditions also accelerate column degradation. For the InfinityLab Poroshell 120 HILIC-Z column, the high pH and low temperature combination offers a more robust method for sugar analysis.

Figure 3 shows that 11 sugars were resolved on an InfinityLab Poroshell 120 HILIC-Z column, using gradient elution with a high-pH mobile phase. Peak shape was excellent for all compounds, and anomer separation was well controlled using ammonium hydroxide (pH ~10.8) and a 35 °C column temperature. Two critical pairs, xylose/arabinose and glucose/galactose, were not baseline resolved. It is likely that these critical pair separations could be improved with a longer column and a longer analysis time.

Figure 4 shows that an isocratic separation of sugars is also possible. Figure 3 shows that the isocratic separation was accomplished in approximately the same amount of time as the gradient analysis. However, it does not offer as much resolution for the early-eluting compounds, and sensitivity is not as great for the later-eluting compounds. Despite these small issues, the isocratic solution could work well for sugar analyses where gradient elution is not possible. Note that RI detector flow cells are not compatible with high-pH mobile phases. If RI detection is desired, use the combination of mid-pH and high temperature. All other method parameters should remain constant, and similar selectivity is expected.



Mobile Phase	A) 0.3 % Ammonium hydroxide B) CH_3CN
Gradient	90 %B
Flow rate	0.4 mL/min
Temperature	35 °C
ELSD	60 °C, 3.5 psi
Column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm

Mobile Phase	A) 100 mM Ammonium acetate pH 7.0 B) CH_3CN
Isocratic	90 %B
Flow rate	0.4 mL/min
Temperature	80 °C
ELSD	60 °C, 3.5 psi
Column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm

Figure 2. Lifetime comparison of high pH versus high temperature sugar analysis on a 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z LC column.

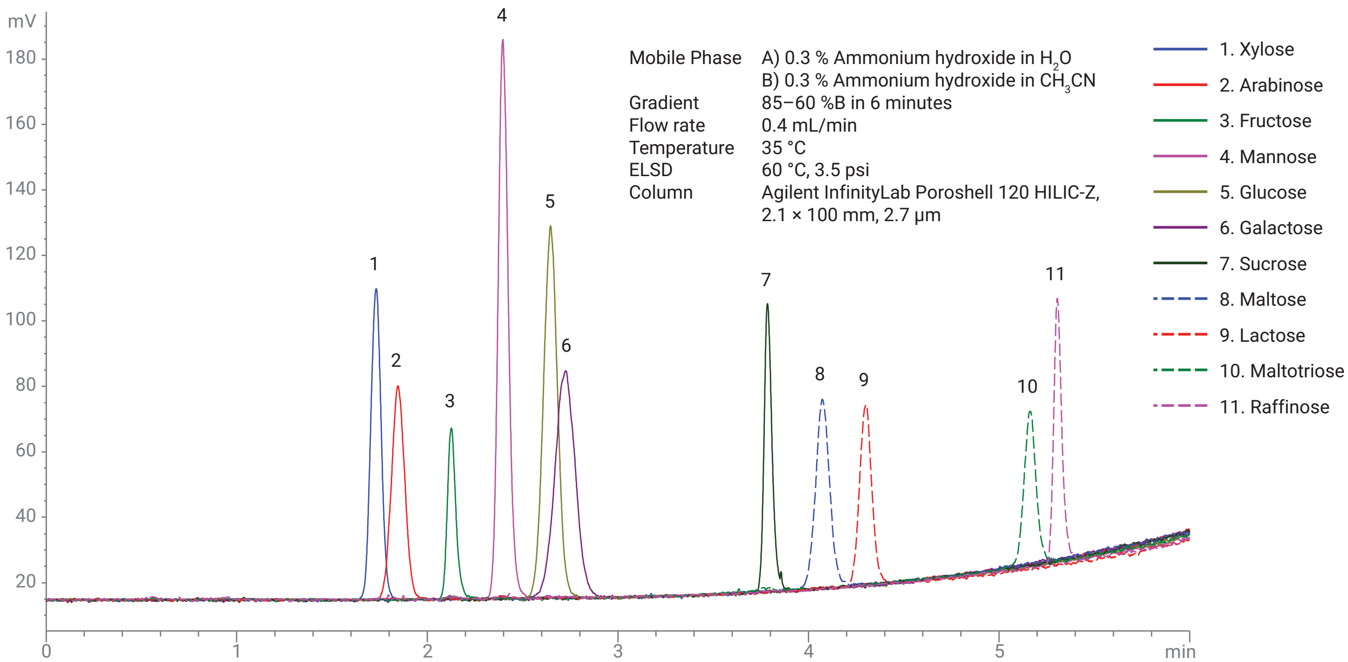


Figure 3. Gradient separation of 11 sugar compounds on an Agilent InfinityLab Poroshell 120 HILIC-Z LC column.

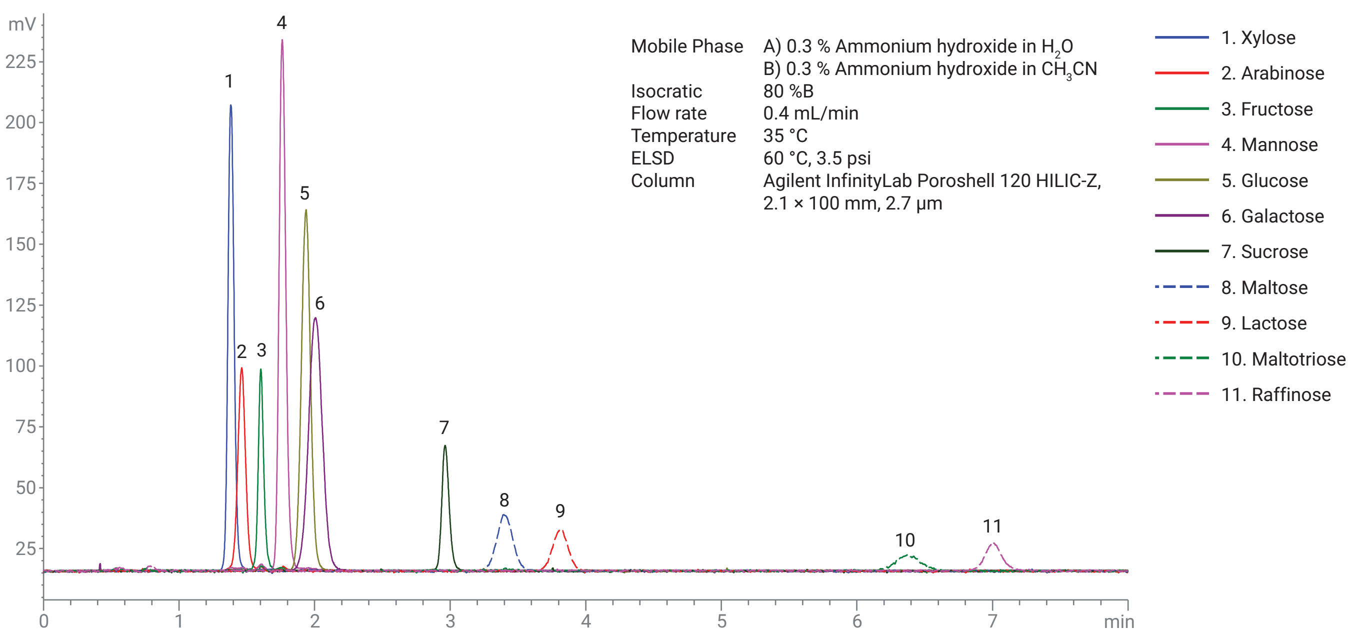


Figure 4. Isocratic separation of 11 sugar compounds on an Agilent InfinityLab Poroshell 120 HILIC-Z LC column.

Conclusions

The Agilent InfinityLab Poroshell 120 HILIC-Z column is well suited for the separation of sugars. This column offers good resolution and peak shape for all compounds, as well as excellent lifetime under high-pH conditions.

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