SCALABILITY OF SOLID-CORE PARTICLES FOR CHROMATOGRAPHIC ANALYSES

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INTRODUCTION

Liquid Chromatography plays a crucial role in many areas of science. It is widely used for quality control of pharmaceuticals and biologics, research and medical purposes (1). Over the last several decades the gold standard of analysis for these areas of science has been High-Performance Liquid Chromatography (HPLC). Typical HPLC systems operate within a 6000 psi pressure limit (2). Consequently, they are commonly paired with columns packed with 3 - 5 µm particles. Although smaller particle-packed columns allow faster separations, their use in HPLC is restricted to shorter lengths due to elevated backpressures (3). Additionally, established methods which are often used in a quality control setting may restrict alterations to particle size or column configuration.

Improved column efficiency on an HPLC system can be achieved using solid-core particles. Solid-core particles have a non-porous "solid" center and a porous outer layer(4). Columns packed with solid-core particles offer distinct advantages over those packed with fully porous particles, including higher efficiency and reduced operating backpressure. Waters offers a line of solid-core particle columns known as CORTECS™ Columns.

By utilizing CORTECS Columns, researchers can increase separation efficiency, achieve faster analysis times, reduced solvent waste and decreased column back pressure. In this poster, we delve into the significance of larger solid-core particles for scalability. We compare columns packed with 5 µm CORTECS Prototype Material, CORTECS UPLC™ (1.6 µm) Material and CORTECS HPLC (2.7 µm) C18 Material with columns packed with fully porous particles. Additionally, we explore how CORTECS Columns differ from similar offerings on the market.

METHODS

STUDY 1: BACKPRESSURE AND EFFICIENCY

LC Systems:	ACQUITY™ UPLC I-Class System with TUV Detector
Columns:	1.6 µm CORTECS UPLC C_{18} Column 2.7 µm CORTECS HPLC C_{18} Column 5 µm CORTECS C_{18} Prototype Column 1.7 µm ACQUITY BEH™ C_{18} Column 3.5 µm XBridge™ BEH C_{18} Column
Flow Rate(s):	0.04 to 1.40 mL/min
Mobile Phase:	Acetonitrile: Water (75:25, v/v)
Injection Volume:	0.4 µL
Sample Mixes (µg/mL)	thiourea (0.01), naphthalene (0.05), acenaphthene (0.20)

STUDY 2: SCALABILITY

LC Systems:	ACQUITY UPLC System with TUV Detector
Columns:	1.6 µm CORTECS UPLC C_{18} Column 2.7 µm CORTECS HPLC C_{18} Column 5 µm CORTECS C_{18} Prototype Column Vendor H Columns—2, 2.7, 5 µm Vendor A Columns— 1.9, 2.7, 4 µm
Flow Rate(s):	0.25 mL/min
Mobile Phase:	Acetonitrile: 15.4 mM Ammonium Formate pH 3.00 (35:65, v/v)
Injection Volume:	3.0 µL
Sample Mixes (µg/mL)	uracil (1), pyrenesulfonic acid (6.5), amitriptyline (8), promethazine (3), butylparaben (3.5), naphthalene (20)

Column configuration was 2.1 x 50 mm for all columns tested. Column temperature was maintained at 30°C for each study. Detection was made using an ACQUITY UPLC TUV Detector at 254 nm.

ACQUITY, UPLC, XBridge, BEH and CORTECS are trademarks of Waters Technologies Corporation.

RESULTS AND DISCUSSION

STUDY 1: BACKPRESSURE AND EFFICIENCY

Limited operating backpressure is one of the greatest disadvantages of an HPLC system. Figure 1 compares the column backpressure at various flow rates of fully porous BEH C_{18} Columns in two particle sizes vs the backpressure of superficially porous CORTECS C_{18} Columns in three particle sizes.

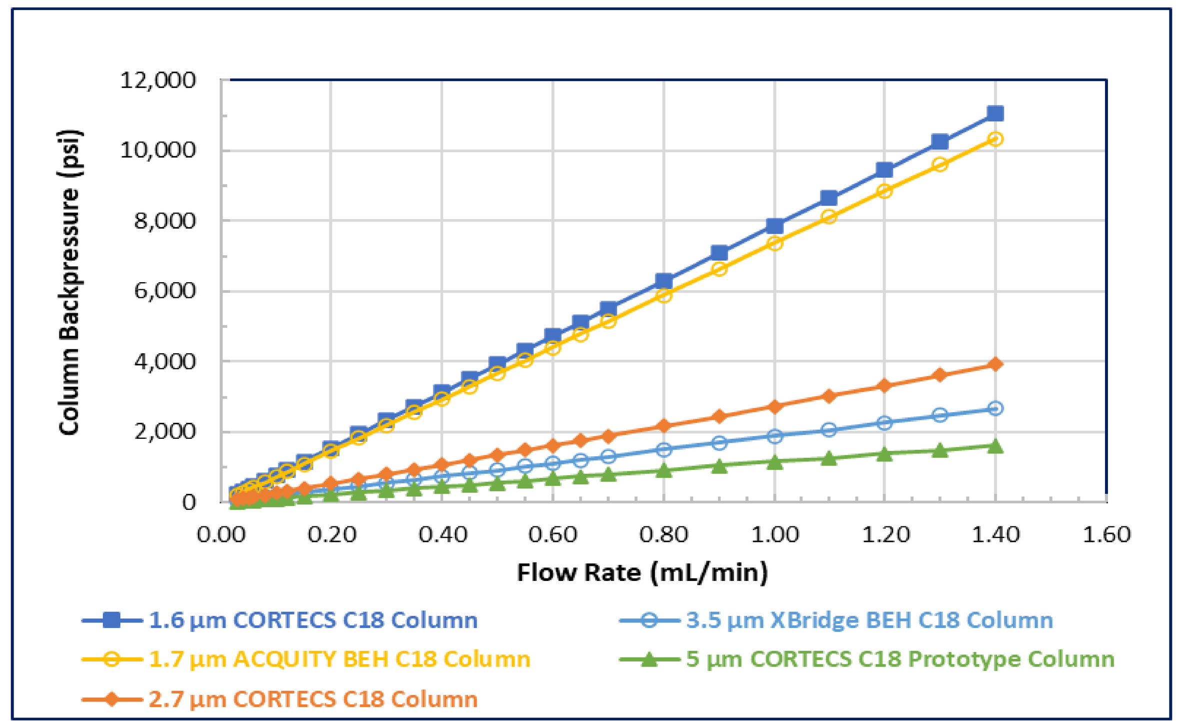

Figure 1: Comparison of column backpressure vs flow rate of various columns and particles sizes including fully porous XBridge BEH C_{18} Columns and solid-core CORTECS C_{18} Columns.

Columns with smaller particle sizes such as the 1.7 µm ACQUITY BEH C_{18} Material and the 1.6 µm CORTECS C_{18} Material may have operating back-pressures much higher than allowed on HPLC systems. The 2.7 µm CORTECS C_{18} Column operates under the allowed 6000 psi backpressure but this may not be the case for longer columns. Knowing HPLC methods may require longer columns, an HPLC user may be limited to a 3.5 µm fully porous column which runs at a lower backpressure of around 2500 psi at 1.4 mL/min, in comparison to the 2.7 µm CORTECS C_{18} Column. However, the 5 µm CORTECS C_{18} Prototype Column operates at an even lower backpressure of 1800 psi which is 28% lower than the 3.5 µm XBridge BEH C_{18} Column. This significant drop in backpressure ensures the longevity of the HPLC system without compromising efficiency as high backpressure can strain system components such as pumps, check valves and tubing.

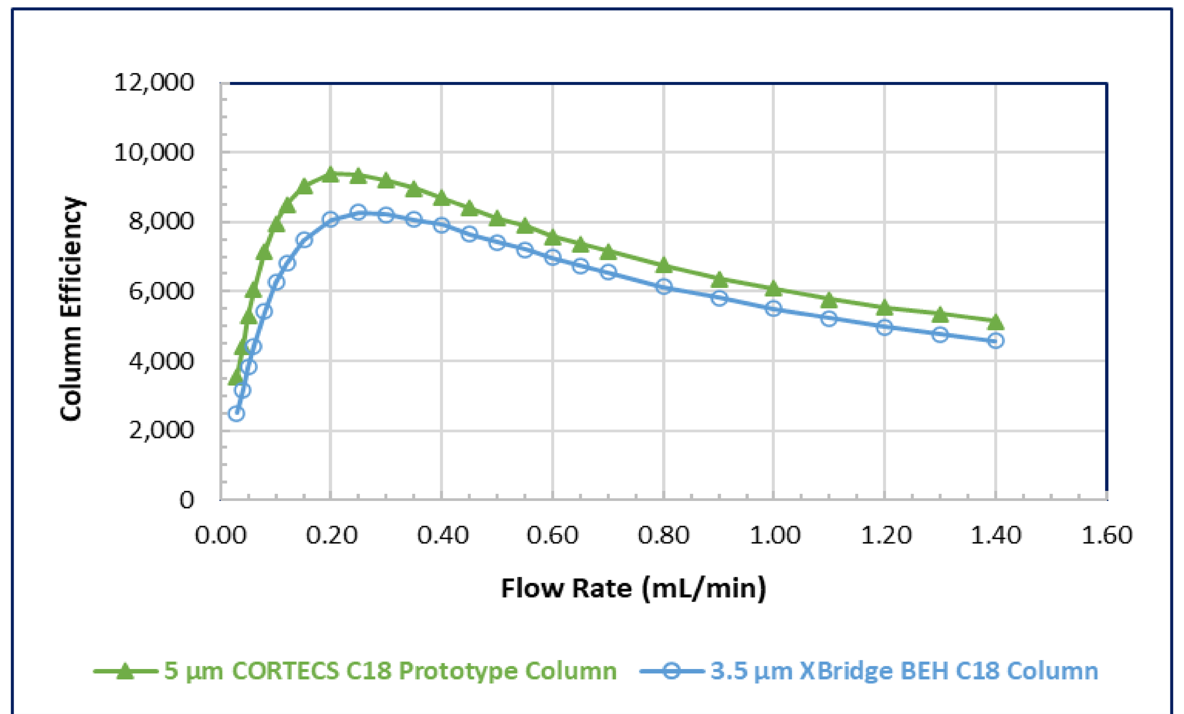

Figure 2: Comparison of column efficiency vs flow rate for the 3.5 µm XBridge BEH C_{18} Column and 5 µm CORTECS C_{18} Prototype Column.

Column efficiency, typically expressed as the count of theoretical plates (N), gauges a column's capacity to generate narrow peaks. The more efficient the column, the narrower the peaks it yields. Figure 2 illustrates a graph comparing column efficiency vs flow rate for two different columns: the 5 µm CORTECS C_{18} Prototype Column and a 3.5 µm XBridge BEH C_{18} Column. Interestingly, the 5 um CORTECS C_{18} Prototype Column, despite its larger particle size, exhibits an optimum efficiency that is 14% greater than that of the 3.5 um XBridge BEH C18 Column. Therefore, the 5 um CORTECS C_{18} Prototype Column outperforms the 3.5 um XBridge BEH C_{18} Column in terms of both efficiency and reduced backpressure.

STUDY 2: SCALABILITY

An isocratic separation of 6 different probes was performed using 35/65 acetonitrile/15.4 mM ammonium formate pH 3.00 at a flow rate of 0.25 mL/min. CORTECS Columns and CORTECS Prototype Columns of different particle sizes were tested using these conditions. Three columns packed with solid-core particles of various particle sizes from two different vendors were also tested under the same conditions, shown in Figure 3.

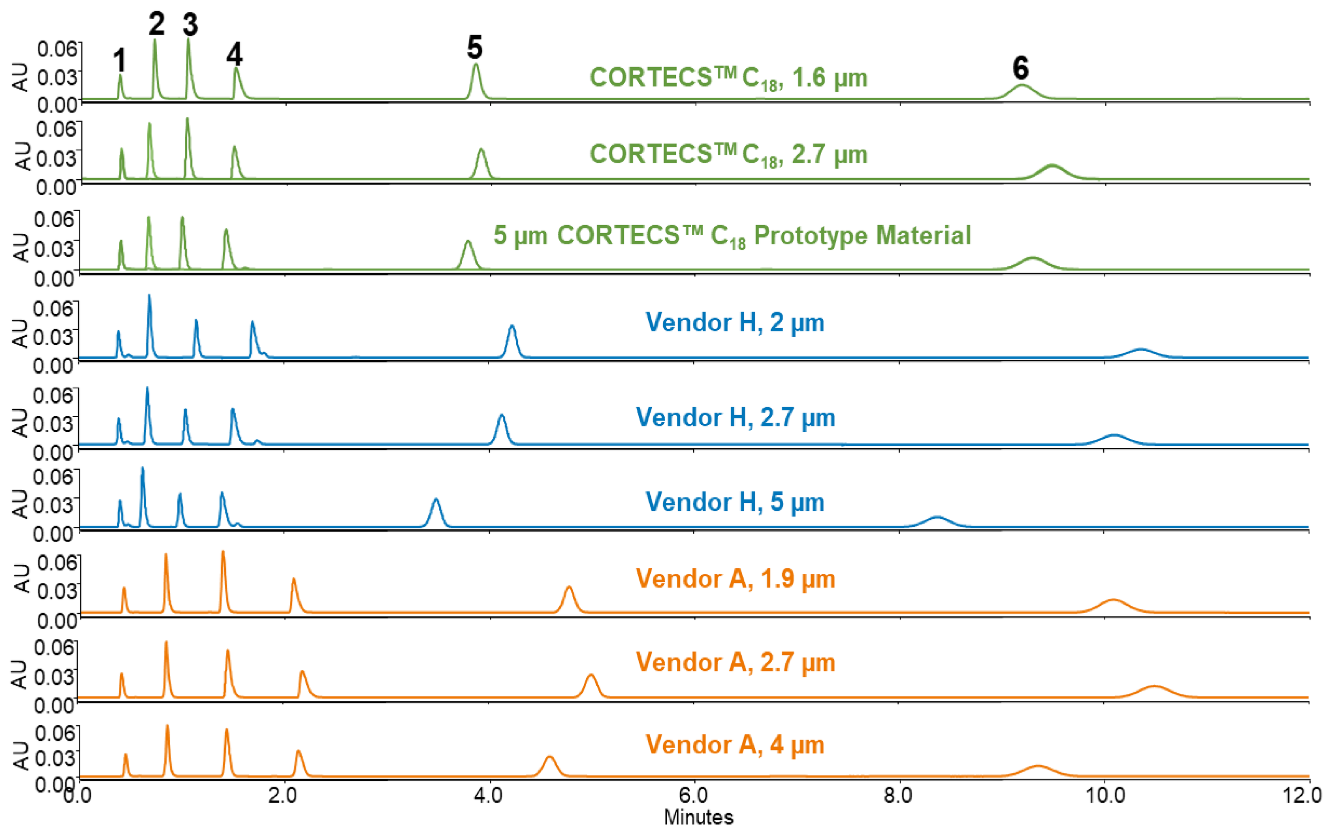

Figure 3: Chromatograms of CORTECS C_{18} Columns of various particle size compared to Vendor H and Vendor A columns.
1) uracil
2) pyrenesulfonic acid
3) promethazine
4) amitriptyline
5) butylparaben
6) naphthalene.

Scalability is essential for HPLC users for several reasons. For example, methods developed on columns with smaller particles sizes (e.g.,sub-2 µm) are often optimized for speed and efficiency however, these columns are not always suitable for routine quality control (QC) procedures due to their higher operating pressures. Therefore, it is imperative to ensure columns are scalable across particle sizes to handle these processes.

Figure 3 highlights the importance of scalability across particle sizes. The CORTECS Columns are all scalable as the retention times are similar across all three particle sizes. However Vendor H and Vendor A columns do not provide the same scalability because the retention times are significantly shifted in the larger particle size columns (5 and 4 µm). For Vendor H and A, the lower retention times in the larger particle size could be due to differences such as inconsistent rho value across particle sizes (the ratio between the solid-core diameter and the diameter of the entire particle). Rho value has a significant impact on retention and thus scalability because as the rho value becomes closer to 1 the outer layer of porous material becomes thinner leading to lower surface area and thus lower retention. CORTECS Columns and 5 µm CORTECS Prototype Columns were developed to meet the requirements of HPLC users who seek a product that not only decreases backpressure and improves efficiency but also allows for scalability across LC systems.

CONCLUSION

- **5 µm CORTECS Prototype Columns have 28% lower back pressure in comparison to 3.5 µm XBridge BEH C_{18} Columns.**
- **5 µm CORTECS Prototype Columns have 14% higher optimal efficiency in comparison to 3.5 µm XBridge BEH C_{18} Columns.**
- **CORTECS Columns are scalable across all 3 particle sizes shown.**
- **In comparison, Vendor H and A columns are not scalable across all 3 particle sizes due to the significantly lower retention in the 4 and 5 µm particle size columns, shown in Figure 3.**

References
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