Technical Note 20755

Comparison of Solid Core HPLC Column Performance: Effect of Particle Diameter

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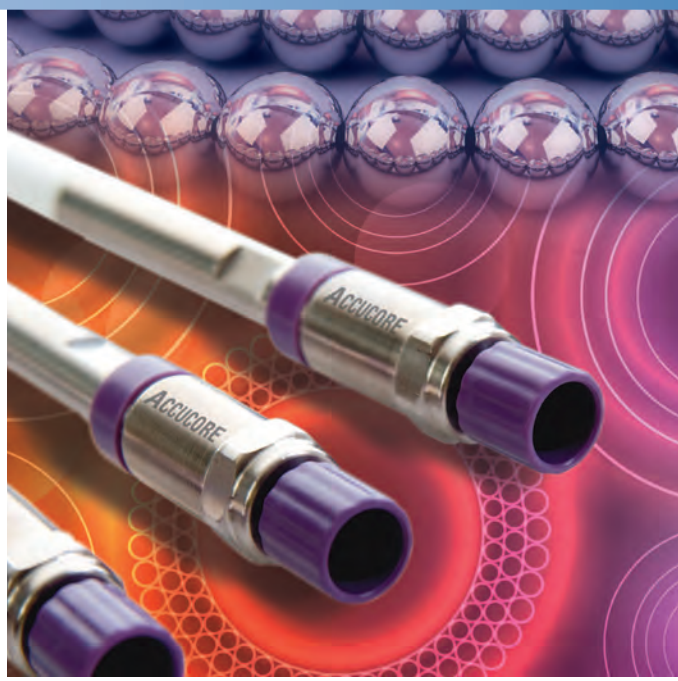
Key Words
Solid core, fused core, superficially porous, pressure, efficiency, impedance

Abstract
In this technical note, the chromatographic performance of solid core 4 µm and 2.6 µm particle-packed columns is compared. Parameters compared are column pressure, efficiency, and impedance.



Introduction

The use of partially porous particles is gaining momentum as they provide higher efficiency than fully porous particles of equivalent particle size. Initially introduced with a particle size in the range 2–3 µm, they are now commercially available in a range of particle sizes, from sub-2 to 5 µm. This gives the chromatographer the flexibility of being able to select the most appropriate particle size for each specific assay; however, it may not be clear what the most suitable particle size is. This technical note partially addresses this gap in information by providing advice on what particle size to select under which experimental conditions. We compare the performance of the Thermo Scientific™ Accucore™ XL 4 µm and Accucore 2.6 µm particle packed columns.

Accucore HPLC columns are based on Core Enhanced Technology™, which features solid core materials with a very tight particle size distribution and advanced bonding technology to functionalize the surface. The particles in the Accucore stationary phases can be described as a solid silica core surrounded by a porous outer layer. The very tight particle size distribution of these materials results in columns with high permeability. Therefore, for the same nominal pressure, Accucore provides better separations than fully porous materials.

Equation 1, known as the Blake-Kozeny equation, shows the dependency of the pressure drop across the column on a variety of experimental parameters under laminar flow conditions. It can be seen that the pressure is directly proportional to the column length, flow rate, and mobile phase viscosity and inversely proportional to the square of the particle size diameter and the square of the column internal diameter. The interstitial porosity (the spaces between the particles that are accessible by the mobile phase) has a more complicated relationship to the pressure. There are other operating parameters that will have an impact on the overall system pressure. Some of these are the inner diameter and length of the connecting tubing in the LC system, the detector set-up parameters, such as flow cell volume in UV, or the inner diameter and length of the capillary components in ESI or APCI sources in LC/MS.

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Equation 1

$$\Delta P = a \frac{(1-\varepsilon_i)^2}{\varepsilon_i^3} \frac{F\,L\,\eta}{d_c^2\,d_p^2}$$

where ΔP – pressure drop across the column
a – constant (dependent on packing, normal values in the range 150 -300 [1,2])
ε_i – interstitial porosity of the packed bed
F – flow rate through the column
L – length of the column
η – kinematic viscosity of the mobile phase
d_p – particle diameter
d_c – column internal diameter

The conventional approach to compare the chromatographic performance of columns is to plot a HETP - height equivalent to a theoretical plate as a function of mobile phase flow rate or linear velocity, often referred to as a van Deemter plot. This approach does have limitations, since it does not account for analysis time or pressure restrictions of the chromatographic system. Kinetic plots [3] are an alternative method of plotting the same experimental data but allowing other parameters such as pressure to be incorporated, and therefore allow us to infer the these performance limits of the tested chromatographic materials. There are a variety of ways in which this data can be presented and all of these plots are referred to as kinetic plots. In one of the most useful forms of these plots a term called impedance is used. Impedance (Equation 2) is a term that defines the resistance a compound is subjected to as it moves down the column relative to the performance of that column. This term gives a true measure of the performance of the column as it incorporates efficiency, time, and pressure, which are critical practical considerations of a chromatographic separation.

Equation 2

$$E = \frac{\Delta P\,t_0}{\eta\,N^2}$$

where E – impedance
ΔP – pressure drop across the column
η – kinematic viscosity of mobile phase
N – efficiency
t_0 – column dead time

Pressure comparison

Figure 1 shows how the column backpressure of the Accucore XL 4 µm column compares with that of the Accucore 2.6 µm column. On average, across the flow rate range tested, the pressure measured on the Accucore 2.6 µm column is 2.2 times higher. At 1 mL/min flow rate the pressures measured are 94 and 202 bar for the 4 and 2.6 µm columns, respectively.

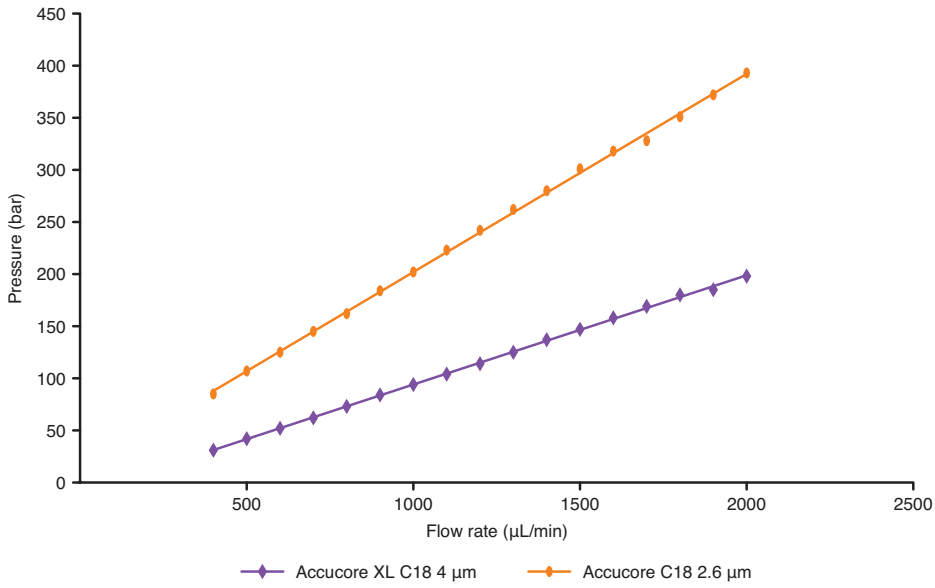

Figure 1: Comparison of column pressure for Accucore XL 4 µm and Accucore 2.6 µm columns

All columns 150 × 4.6 mm; test conditions: water / acetonitrile (50:50 v/v) mobile phase, 30° C column temperature

Efficiency comparison

Figure 2 compares the efficiency of the Accucore XL 4 µm column with that of the Accucore 2.6 µm column using a van Deemter plot. On average (across the flow rate range tested) Accucore 2.6 µm gives 27% higher efficiency than the Accucore XL 4 µm column, and the improvement in efficiency increases as the linear velocity increases.

The curves for both columns are very flat, and therefore a wide range of linear velocities (or mobile phase flow rates) can be used without losing chromatographic performance. The flattest regions of the van Deemter curve correspond to a mobile phase flow rate range of 0.9 to 1.4 mL/min for the Accucore XL 4 µm column and 1.2 to 1.8 mL/min for the Accucore 2.6 µm column.

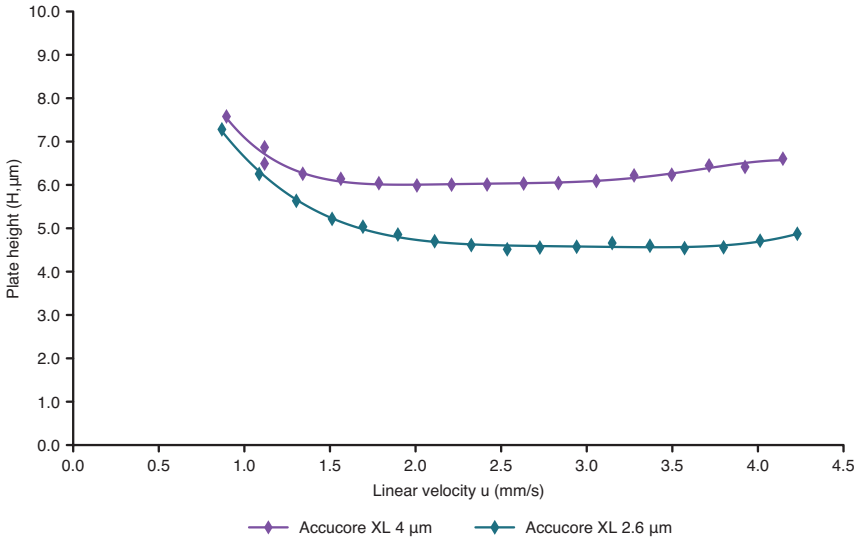

Figure 2: Efficiency comparison using van Deemter plots for Accucore XL 4 µm and Accucore 2.6 µm columns

All columns 150 × 4.6 mm; test conditions: water / acetonitrile (50:50 v/v) mobile phase, 30 °C column temperature, test probes: phenetole and theophylline (t_0 marker)

Impedance comparison

Impedance is a term that gives a true measure of the performance of the column as it incorporates efficiency, time, and pressure, which are critical parameters for chromatographers. Lower impedance values indicate faster chromatography and generation of narrower peaks at lower backpressures. The solid core particles, tight control of particle diameter, and automated packing processes used in Accucore HPLC columns contribute to low impedances.

On average (across the flow rate range tested) the Accucore 2.6 µm column provides 20% more efficiency per unit time than the Accucore XL 4 µm column (Figure 3). In terms of overall performance of both 4 and 2.6 µm materials, the Accucore 2.6 µm column demonstrates 37% lower impedance (Figure 4).

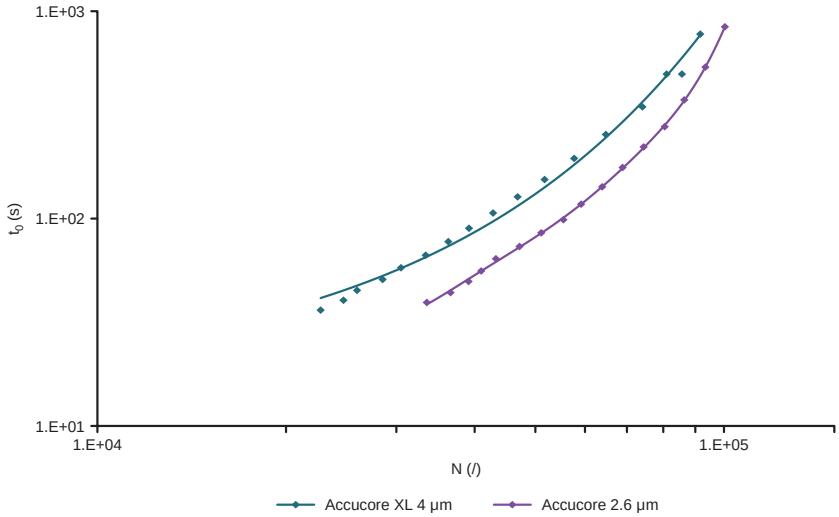

Figure 3: Performance comparison of Accucore XL 4 µm and Accucore 2.6 µm columns using kinetic plots: efficiency per unit time

All columns 150 × 4.6 mm; test conditions: water / acetonitrile (50:50 v/v) mobile phase, 30 °C column temperature, test probes: phenetole and theophylline (t_0 marker)

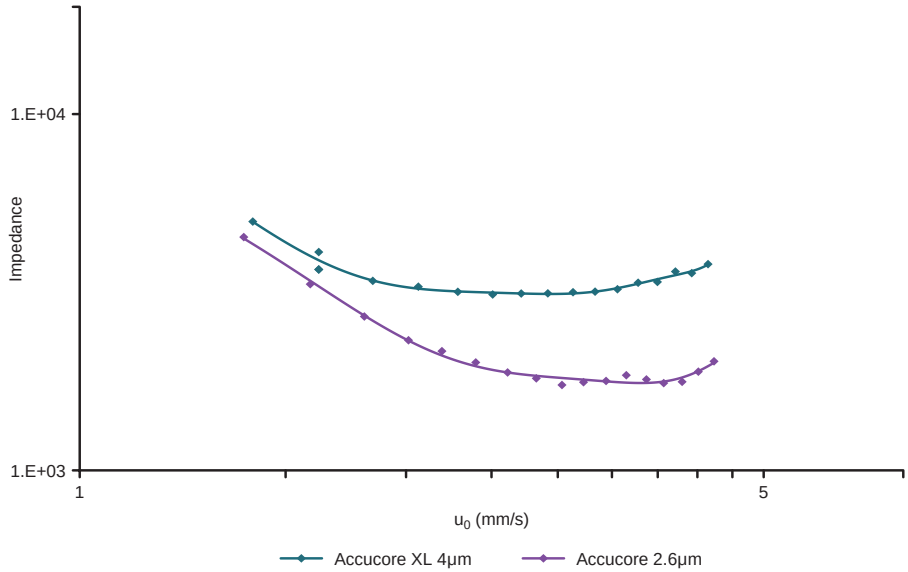

Figure 4: Performance comparison of Accucore XL 4 µm and Accucore 2.6 µm columns using kinetic plots: column impedance (E) relative to linear velocity (u)

All columns 150 × 4.6 mm; test conditions: water / acetonitrile (50:50 v/v) mobile phase, 30 °C column temperature, test probes: phenetole and theophylline (t_0 marker)

Conclusion

Comparison of the Accucore 2.6 µm and Accucore XL 4 µm solid core columns shows that:
- The backpressure of the Accucore 2.6 µm column is 2.2 times higher.
- The Accucore 2.6 µm column is 20% more efficient per unit time.
- The Accucore 2.6 µm column has 37% lower impedance.

The choice between these two solid core materials should be based on the assay goals and the equipment available. The Accucore XL 4 µm columns dramatically improve separation efficiency, and therefore resolution and sensitivity over those obtained with conventional fully porous 5 and 3 µm particle packed columns, without the need to make changes to the operating parameters or system configuration [4]. As demonstrated above, the Accucore 2.6 µm columns provide even higher efficiency and lower impedance, but often system dead volume and operating parameters have to be optimized to get the best possible performance out of these columns [5]. Additionally, when operating at the higher linear velocities, a 600 bar pressure limit LC system may be required.

Therefore, Accucore XL 4 µm columns should be used when:
- There is large dead volume in the system.
- The maximum operating pressure of the pumps is 400 bar.
- The same method as used with a fully porous particle packed column must be maintained.

In contrast, Accucore 2.6 µm columns should be used when even higher efficiency is required and:
- The dead volume of the system is minimal (<100 µL).
- The maximum operating pressure of the pumps is greater than 400 bar.
- The method can be optimized.

References

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