Technical Note 20542

What Pressure to Expect from the Thermo Scientific Accucore HPLC Columns?

Luisa Pereira, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words

Fast LC, Pressure, Solid Core Particles, Sub-2 µm Particles, Core Enhanced Technology

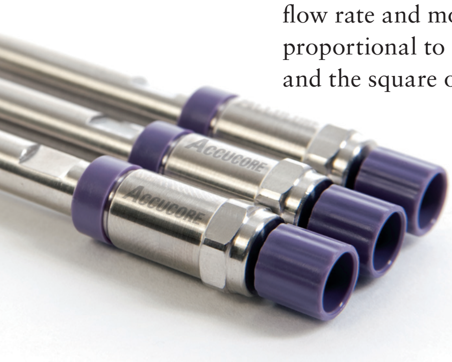
Abstract

This technical note discusses the effect that column length, internal diameter (ID), particle size, and mobile phase flow rate and viscosity have on the operating pressure in HPLC. Comparison data on the measured pressure for solid core and fully porous particle packed columns (5, 3 and sub-2 µm) is shown.

Introduction

Most stationary phases currently used for fast HPLC have a fully porous particle support, with diameters in the sub-2 µm region. The small particle diameter improves the separation kinetics and therefore efficiency, but at the expense of operating backpressure. Additionally, sub-2 µm particle packed columns are generally run at high linear velocities as these produce higher efficiencies. Consequently, the HPLC equipment has to be able to operate at pressures in excess of the conventional 400 bar, unless very short column lengths (<50 mm) are used. Partially porous particles, with a diameter between 2 and 3 µm, provide similar performance to sub-2 µm particles at significantly lower column backpressures. The Thermo Scientific™ Accucore™ HPLC column range uses Core Enhanced Technology™ to produce a 2.6 µm solid-core material with a very tight particle size distribution. This results in columns with high permeability, and therefore "bar for bar" Accucore columns produce improved separations when compared to fully porous materials.

Equation 1 shows the dependency of pressure drop across the column on particle size and flow rate, discussed above. Pressure is directly proportional to 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.



Equation 1: $$\Delta P = 236 \frac{(1-\varepsilon_i)^2}{\varepsilon_i^3} \frac{F\,L\,\eta}{d_c^2\, d_p^2}$$

Where:

ΔP – pressure drop across the column
ε_i – interstitial porosity of the packed bed
F – flow rate through the column
L – length of the column
η – viscosity of the mobile phase
d_p – particle diameter
d_c – column internal diameter

Other operating parameters that will have an impact on pressure are the ID and length of the connecting tubing in the LC system, detector set-up parameters such as flow cell volume in UV or the ID and length of the capillary components in ESI or APCI sources in LC/MS. Pressure can be a useful symptom when troubleshooting LC systems.

Thermo
SCIENTIFIC

Comparison of Column Pressure for Accucore 2.6 µm and Fully Porous 5, 3 and Sub-2 µm

From equation 1, it is clear that reducing the particle size in the column significantly increases the observed pressure drop across the column. The data in Figure 1 was generated on 100 × 2.1 mm columns, using a mixture (1:1) of acetonitrile and water, at 30 °C column temperature and running flow rates in the range 0.1 to 1 mL/min. The pressure measured on the Accucore 2.6 µm column is approximately half of that on the sub-2 µm particle packed column and double that of the 3 µm particle packed column. Based on particle size only, and given that the pressure drop across the column is inversely proportional to the square of the particle diameter, the ratio between the measured pressure on the sub-2 µm and Accucore columns should be 1.9, and between the 3 µm and Accucore should be 1.3. However, column permeability also depends on the interstitial porosity (as indicated in equation 1) and this parameter accounts for the observed differences in measured pressure ratios versus those predicted based only on particle size.

Chromatographic systems that have the conventional pressure limit of 400 bar will reduce the effective flow rate range that can be used on a column packed with small particles. On standard HPLC systems, sub-2 µm particle packed columns can only be run at reduced flow rates, often below the flow rate that provides the best performance. However, the Accucore column used in this comparison can be operated at 800 µL/min, double the optimal flow rate, before it experiences the same issues.

Effect of Column Length and Column ID on Pressure

Pressure is directly proportional to column length. The data in Figure 2 was obtained when Accucore columns with length of 30, 50, 100, 150 and 200 mm were run at 400 µL/min with a mobile phase of water/acetonitrile (1:1) and the measured pressure matches well with the predicted values.

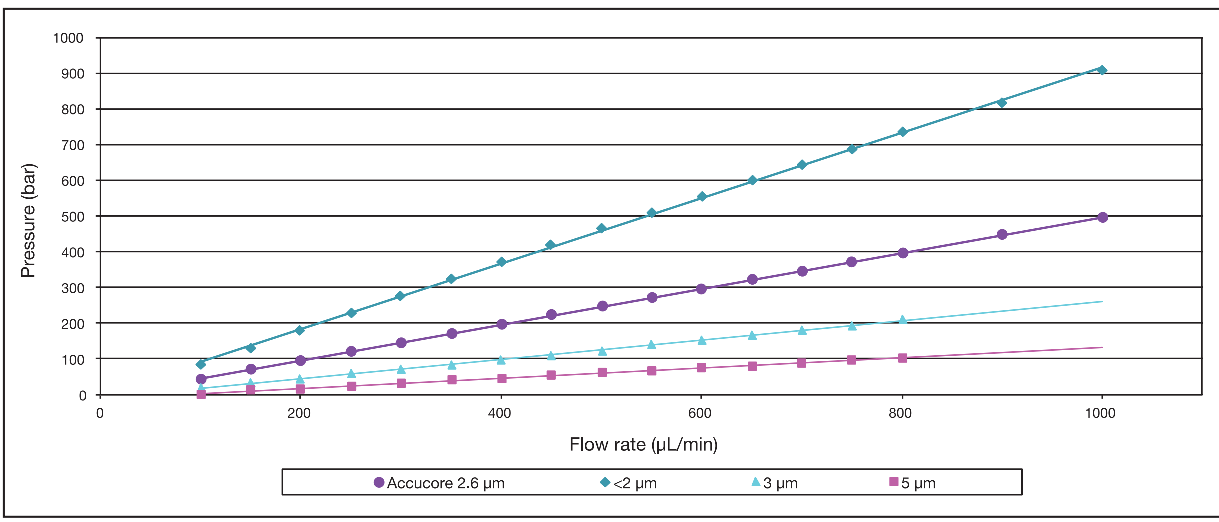


Figure 1: Comparison of column pressure for Accucore 2.6 µm and fully porous 5, 3 and sub-2 µm. Columns: 100 × 2.1 mm; mobile phase: water/acetonitrile (1:1); temperature: 30 °C; flow rate: 0.1 to 1.0 mL/min.

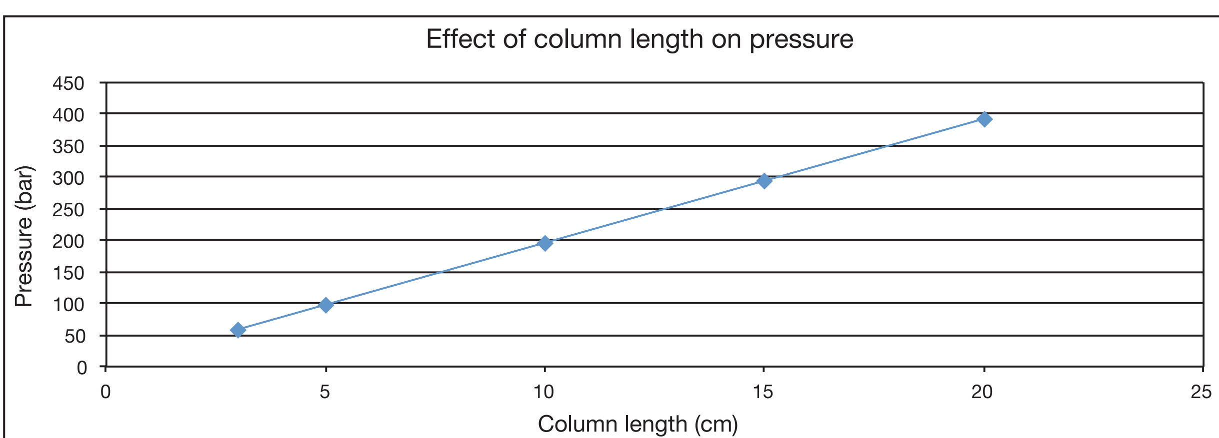


Figure 2: Pressure drop across Accucore 2.6 µm columns of different lengths, at a flow rate of 400 µL/min, mobile phase of water/acetonitrile (1:1) and temperature of 30 °C.

From Equation 1, pressure is inversely proportional to the square of the column ID and therefore decreasing the column ID results in a significant increase in pressure. In practical terms however, if the column is run at a typical linear velocity, the pressure measured will also be typical for that system set-up. Mobile phase linear velocity is the flow rate normalized for the column cross-section.

For instance, if a method is transferred from a 4.6 to a 2.1 mm ID column, all other operating parameters kept unchanged, and the 2 columns are run at the same linear velocity, then the measured pressure drop across both columns will be the same.

Effect of Mobile Phase Viscosity on Pressure

Column operating pressure is affected by the mobile phase composition. Viscosity is a property of each solvent, which varies with temperature. The proportion the solvent is mixed with other mobile phase components and the operating temperature will determine the mobile phase viscosity. The pressure drop across the column itself will also have an effect on the viscosity since it affects the effective column temperature. Figure 3 shows how water viscosity varies with the addition of acetonitrile or methanol. Water/methanol mixtures are more viscous than water/acetonitrile mixtures and therefore using methanol as the organic modifier in reversed-phase LC produces higher pressure drops across the column. When mobile phase gradients are used the mobile phase composition and therefore the viscosity changes during the run, which results in a change of pressure during the chromatographic run.

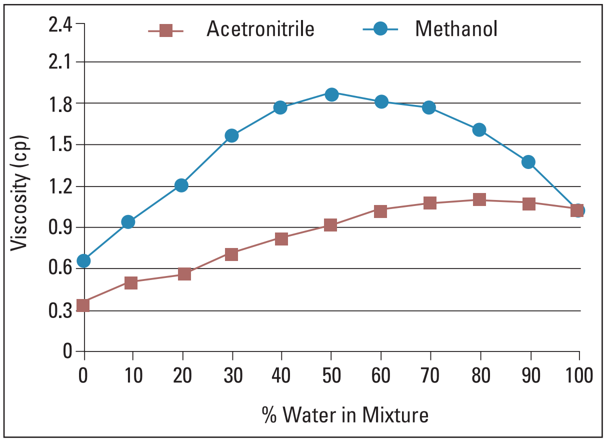


Figure 3: Mobile phase viscosity changes with the composition. Water/methanol mixtures can be up to 80% more viscous than water/acetonitrile mixtures.

Conclusion

- Pressure is dependent on column length, ID, and particle size
- Pressure is dependent on mobile phase flow rate and viscosity
- Accucore 2.6 µm particle packed columns show approximately half of the pressure of a sub-2 µm fully porous particle packed column and approximately double that of a 3 µm fully porous particle packed column
- Accucore columns can be run at high flow rates on conventional HPLC equipment and are rated to 600 bar

For more information, visit our website at **www.thermoscientific.com/crc**

USA and Canada +1 800 332 3331
Australia 1300 735 292 (free call domestic)
China +86 21 68654588
+86 10 84193588
+86 20 83145199
800 810 5118
France +33 (0)1 60 92 48 34
Germany +49 (0) 2423 9431 20 or 21
India +91 22 6742 9494
+91 27 1766 2352
Japan +81 3 5826 1615
United Kingdom +44 (0) 1928 534 110
New Zealand 0800 933 966 (free call domestic)
All Other Enquiries +44 (0) 1928 534 050

Technical Support
North America +1 800 332 3331
Outside North America +44 (0) 1928 534 440

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

TN20542_E 10/12M