Selection of Column Length and Particle Size for High Resolution, Fast LC and LC/MS

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Abstract

In this presentation a comparison is made between the performance of columns packed with 1.9, 2.4 and 5 µm. In particular the efficiency, resolution, run time and column backpressure are compared, as these give a good basis for both column performance and also applicability of the column to the various instrumentation that is currently available.

Introduction

The majority of HPLC separations are still performed on silica-based columns packed with 3 or 5 µm diameter particles. These particle sizes offer a good combination of separation efficiency, relatively low backpressure and column lifetime. The use of smaller particles has advantages in terms of efficiency and speed of analysis, due to a lower eddy diffusion within the column. Columns packed with smaller particles can be run at high flow rates to improve sample turnaround, but without sacrificing efficiency and therefore separation performance. However this improved performance comes at the cost of an increase in the back pressure across the column.

Equation 1 shows the relationship that exists between the backpressure across a column and some of the key parameters, including flow rate, column length and particle size. It can be seen that reducing the particle size has a substantial effect on the backpressure because of the inverse square relationship that exists.

Equation 1. $$\Delta P = \frac{\phi \nu \eta l}{d_p^2}$$

l – length of column, φ – packing efficiency term,
υ – linear flow rate of mobile phase, η – viscosity of mobile phase,
ΔP – back pressure across column

In this instance specialized HPLC equipment may be required to handle pressures above 400 bar, however, short columns packed with small particles often provide a combination of high resolving power and the added advantage of not exceeding the pressure limits of conventional HPLC systems. The work presented in this poster illustrates how to select the best particle size (2.4 or 1.9 µm), column length, and operating conditions to develop fast, robust and sensitive separations.

Materials & Methods

Instrumentation:
Thermo Scientific Accela 600 HPLC system
Columns:
Thermo Scientific Hypersil GOLD 5 µm, 100 x 2.1 mm; Hypersil GOLD™ 2.4 µm, 100 x 2.1 mm; Hypersil GOLD 1.9 µm, 100 x 2.1 mm; Hypersil GOLD 2.4 µm, 150 x 2.1 mm; Hypersil GOLD 2.4 µm, 50 x 2.1 mm.
Test method 1:
Mobile phase – H_2O / ACN (50:50); Temperature: 30° C; Flow rate: as indicated in figures
Detection: UV at 254nm (0.1s rise time, 20 Hz)
Injection volume: 1 µL
Test mixture: 1. Theophylline, 2. p-Nitroaniline, 3. Methyl benzoate; 4. Phenetole, 5. o-Xylene (peak 5 used for efficiency)
Test method 2:
Mobile phase – H_2O / ACN (50:50) + 0.1%formic acid; Temperature: 30° C; Flow rate: as indicated in figures
Detection: UV at 254nm (0.1s rise time, 20 Hz)
Injection volume: 1 µL
Test mixture: 1. Methyl paraben; 2. Ethyl paraben; 3. Propyl paraben; 4. Butyl paraben.

Results

Figures 1 compares the performance of columns packed with 3 different particle sizes at different flow rates; the higher efficiency of the smaller particles is obtained at the cost of higher column backpressures as demonstrated in Figures 2 and 3.

FIGURE 1. Efficiency (plates /meter) as a function of flow rate for 5, 2.4 and 1.9 µm particle sizes (test method 1).

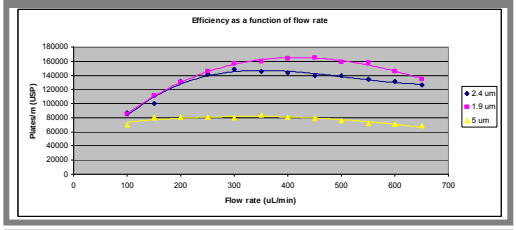


FIGURE 2. Effect of flow rate on column backpressure for columns packed with 5, 2.4 and 1.9 µm particles (test method 1).

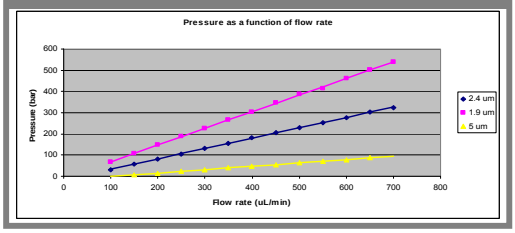
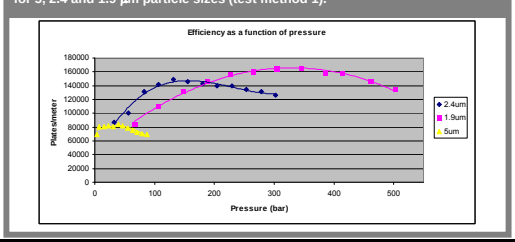
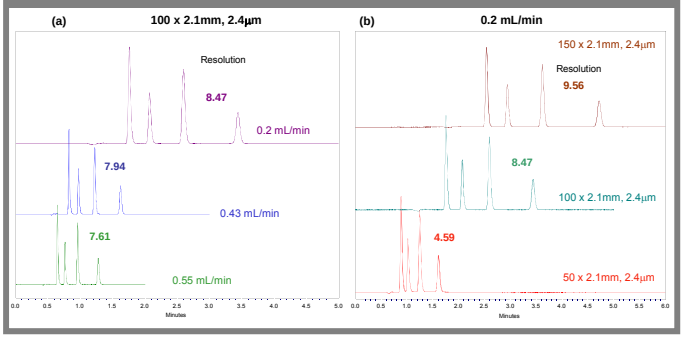


FIGURE 3. Efficiency (plates per meter) as a function of column backpressure for 5, 2.4 and 1.9 µm particle sizes (test method 1).



Figures 4a illustrates the changes in run time and resolution of the last two peaks for a 100 x 2.1mm column packed with 2.4 µm particles when run at different flow rates. The effect on resolution and run time of changing column length, with constant flow rate (0.2 mL/min), is also illustrated in Figure 4b.

FIGURE 4. Comparison of the chromatographic resolution and run time of Hypersil GOLD 2.4µm 2.1mm ID columns when flow rate (a) and column length (b) are changed (test method 2).



The backpressure across 50, 100 and 150 mm length columns packed with Hypersil GOLD 2.4 µm (all 2.1mm in internal diameter), as a function of flow rate, is shown in Figure 5.

The separation efficiency that a column can deliver is determined by the particle size and column length. Figure 6 illustrates the number of plates that can be obtained from columns packed with 5 and 2.4 µm of various lengths.

FIGURE 5. Pressure versus column length for 2.4 µm packed columns (test method 2).

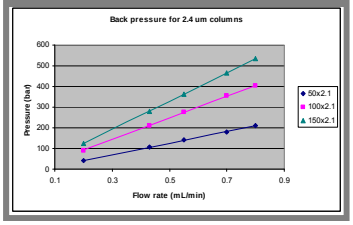


FIGURE 6. Comparison of the number of plates obtained with 5 and 2.4 µm particles per unit of column length (test method 2).

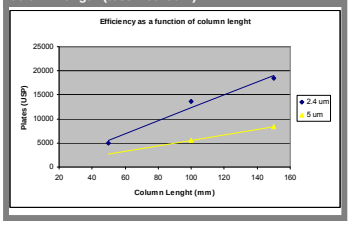
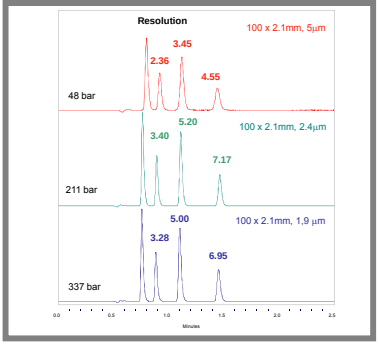


Figure 7 compares the separation (resolution) on Hypersil GOLD 5, 2.4 and 1.9 µm (100 x 2.1mm length) at constant flow rate.

FIGURE 7. Comparison of the separation performance of Hypersil GOLD 5, 2.4 and 1.9 µm columns (test method 2, flow rate 0.430mL/min).



Conclusions

- The separation efficiency, resolution and run time of columns packed with Hypersil GOLD 1.9, 2.4 and 5 µm were compared in this work;
- The backpressure of columns packed with Hypersil GOLD 1.9, 2.4 and 5 µm was compared in this work;
- The higher efficiencies obtained with the smaller particles is at the expense of higher backpressures;
- Short columns packed with small particles generally provide enough resolution for many separations and these can be used in conventional HPLC systems.

For additional information, please visit our Chromatography Resource Centre which can be found at: www.thermoscientific.com/chromatography

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