

Optimizing chromatographic performance with small particle packed columns

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Abstract

The work presented in this poster illustrates how to select the best column geometry, particle size and operating conditions to develop fast, robust and sensitive separations. The importance of optimizing the LC system and method when operating with sub-2 µm particle packed columns is demonstrated.

Introduction

Efficient chromatographic peaks are important because these enhance separation, resolution, peak capacity and method sensitivity.

The separating power of a chromatographic column can be described by the height equivalent to a theoretical plate (H), which varies with the linear velocity (u) of the mobile phase as it passes through the column, the particle size (d_p) and the diffusion coefficient in the mobile phase (D_m). This relationship is described by equation 1:

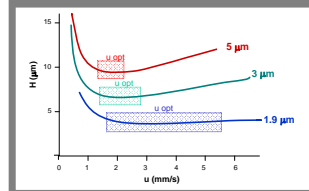
$$\text{Equation 1: } H = A d_p + B D_m / u + C (d_p^2 / D_m) u$$

The lower the plate height, the higher the separating power of the column. There is an optimum linear velocity for which H is at a minimum and the plate number, or efficiency, N, is at a maximum. The plate height is related to the efficiency by equation 2:

$$\text{Equation 2: } H = L / N$$

The equations show that efficiency is inversely proportional to particle size. The van Deemter curve (Figure 1) illustrates how the limitations in column efficiency at higher linear velocities can be overcome through the use of smaller particles. As particle size is reduced, the optimum mobile phase velocity (u) increases and the curve becomes flatter. Therefore columns packed with smaller particles can be operated over a wider range of linear velocity while maintaining high efficiencies. This has the resultant effect of enabling the use of higher flow rates to decrease analysis time without compromising performance. In addition, smaller particles allow for reduction in column length whilst maintaining efficiency, thus providing further gains in speed of analysis at high flow rates without exceeding the pressure limits of standard HPLC systems.

FIGURE 1. Representation of van Deemter curves for 5, 3 and 1.9 µm particles, indicating the optimum linear velocity range (u opt).



However, in order to maximize the resolution, speed, and sensitivity benefits of small particle packed columns, the LC system needs to be optimized, i.e., all system components for the assay should also be considered. System volume (connecting tubing ID and length, injection volume, flow cell volume in UV) must be minimized, detector time constant and sampling rate need to be carefully selected, and when running fast gradients, pump dwell volume must be minimal.

Materials & Methods

- Columns - Hypersil GOLD™ 5 µm, 100 x 4.6 mm; Hypersil GOLD 5 µm, 100 x 4.6 mm; Hypersil GOLD 5 µm, 200 x 2.1 mm; Hypersil GOLD 5 µm, 50 x 2.1 mm; Hypersil GOLD 1.9 µm, 20 x 2.1 mm; Hypersil GOLD 1.9 µm, 50 x 2.1 mm; Hypersil GOLD 1.9 µm, 200 x 2.1 mm (Thermo Scientific, Bellefonte, PA).
- Instrumentation - Finnigan™ Surveyor™; Accela™ (Thermo Scientific, San Jose, CA)
- Experimental conditions (mobile phase compositions, gradients, flow rates, solutes, temperature, injection volumes) are specified on each figure.

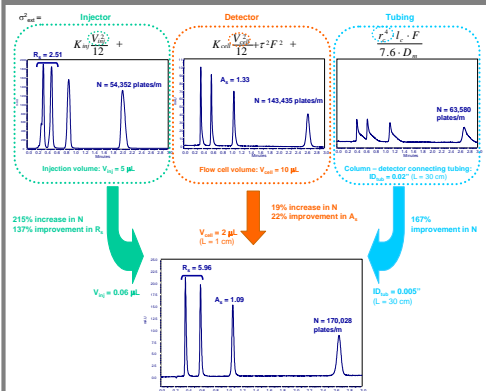
Results

I) System optimization

Minimizing dispersion

Band broadening effects which occur in the fluid path of the HPLC instrument are volumetric effects, each contributes a variance (σ²) to the width of the chromatographic band, and are additive. Extra column band broadening (σ²_{ec}), which has a detrimental effect on chromatographic performance, is more significant when working with short narrow bore columns, that have a small volume, and with sub-2 µm particle packed columns that produce very narrow peaks, at optimum performance. It is, therefore, critical to minimize dispersion when working with these columns. In Figure 2 the effects of injection volume, detector flow cell volume and column to detector connecting tubing ID, on chromatographic performance are illustrated.

FIGURE 2. Contributions of injection volume (σ²_{inj}), detector flow cell volume (σ²_{det}) and connecting tubing ID (σ²_{ct}) to extra column band broadening (σ²_{ec}). Minimizing these effects greatly improves performance.



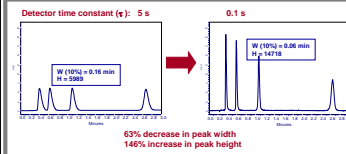
Columns: Hypersil GOLD 1.9 µm, 50x2.1 mm
Mobile phase: H₂O/ACN (50/50 v/v) 1% formic acid
Flow rate: 0.5 mL/min
Temperature: 25 °C
Detection: UV@254 nm
HPLC system: Finnigan Surveyor

Analyses: 1. Cortisone
2. 11-hydroxyprogesterone
3. 17-hydroxyprogesterone
4. Progesterone

K = constant
V_{inj} = injection volume
V_{det} = detector flow cell volume
F = flow rate
r = detector time constant
F = flow rate
r = flow rate
L = tubing radius
L = tubing length
D_m = diffusion coefficient in the mobile phase

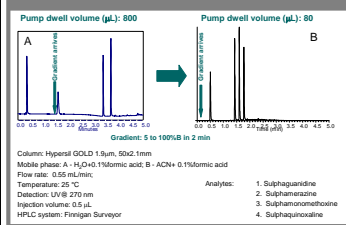
Optimizing detector time constant setting

FIGURE 3. Effect of detector time constant on peak width (W) and height (H).



Pump dwell volume

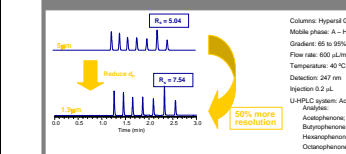
FIGURE 4. Effect of pump dwell volume during fast gradient elution.



II) Method optimization

HPLC method transfer to sub-2 µm particle packed columns brings significant improvements in sensitivity, speed and resolution. This generally involves method scaling down to shorter and narrower columns, and therefore adjustment of the operating conditions to ensure that the chromatographic profile is equivalent or superior.

FIGURE 5. Increasing resolution by reducing particle size.



The detector time constant also has an impact on the chromatographic performance. Typically this is set to 1 or 2 seconds. In Figure 3, 5s and 0.1s are compared. If the detector responses is too slow, as on the left chromatogram, the peaks are artificially wide. By correctly adjusting the detector time constant a gain in peak height and therefore analysis sensitivity is achieved.

The pump dwell volume is extremely important when running high speed applications using ballistic gradients. It affects the time it takes for the gradient to reach the head of the column.

In Figure 4 the same gradient was run on a pump with a 800 µL dwell volume, and a pump with a 80 µL dwell volume. The chromatograms are very different: for chromatogram A, it was necessary to introduce an isocratic hold at the end of the 2 minutes gradient to allow elution of the analytes.

FIGURE 6. Maximizing sensitivity (peak height, H) by manipulating column length (L), column internal diameter (ID), particle size (d_p) and flow rate (F).

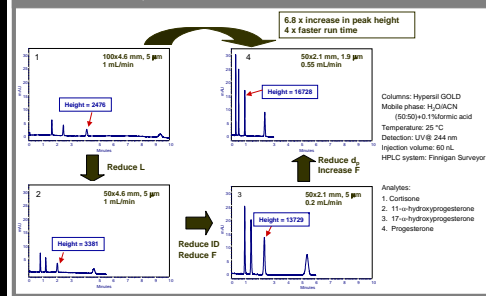
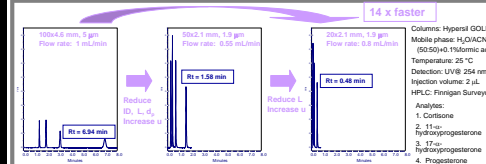


FIGURE 7. Reducing run time by changing column length (L), column internal diameter (ID), particle size (d_p) and linear velocity (u).



Conclusions

- Assay cycle time can be significantly reduced, and resolution and sensitivity improved using short columns packed with 1.9 µm particles, on conventional HPLC systems. In order to get the best performance from these particles, it is necessary to operate at the optimum flow rate and to optimize the whole HPLC system.
 - minimize extra column band broadening (in the tubing, injector, detector)
 - adjust detector setting for narrow chromatographic peaks
 - select the correct pump to perform fast gradients
- Improvements of 7 fold in peak height, 14 fold in speed and 50% higher resolution are demonstrated when using 1.9 µm Hypersil GOLD columns.

Additional Information

For additional information, please browse our Chromatography Resource Centre which can be accessed from: www.thermo.com/columns.

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