Use of Small Particles in Ultra High Pressure Liquid Chromatography

L. Pereira[1], C. Blythe[1], R. Sherant[2], H. Ritchie[1]   [1]Thermo Electron Corporation, Runcorn, UK,   [2]Thermo Electron Corporation, Bellefonte, PA

Abstract

The work presented in this poster demonstrates how 1.9 μm particles facilitate higher resolution, higher sensitivity and faster analyses. The gains in resolution through manipulation of selectivity (column chemistry) are also illustrated.

Introduction

The aim of a chromatographic separation is to obtain good resolution in a short analysis time, i.e., resolution needs to be maximized while minimizing analysis time. Sub 2 μm particles have been developed as a chromatographic media to achieve high resolution of the sample components, fast analyses and high sensitivity.

Resolution (R_s) is proportional to the square root of separation efficiency (N), as described by equation 1 which expresses resolution as a function of capacity factor (k), selectivity (α), and efficiency (N). Efficiency is, in turn, inversely proportional to the diameter of particle size (d_p). Unlike 5 and 3 μm particles, sub 2 μm particle packed columns can be operated over a wide flow rate range without loss of efficiency. The trade-off of using columns packed with such small particles and of operating at high flow rates is that the pressure drop (P) across the column increases. Equation 2 shows the dependency of P on particle size and flow rate. To operate under these conditions specialized equipment is required.

Equation 1.

$$R_s = \frac{1}{4}\,\frac{(\alpha-1)}{\alpha}\,\sqrt{N}\,\frac{k}{1+k}$$

α - selectivity, N – efficiency, k – retention factor

Equation 2.

$$P \cong \frac{250\,L\,\eta\,F}{d_p^2\,d_c^2}$$

L - column length (cm), η - viscosity (cP), F - flow rate (mL/min)
d_p – particle diameter (μm), d_c – column internal diameter (cm)

TABLE 1. Operational parameters for N = 10000.

d_p (μm)	L (mm)	t_0 (s)	N / t_0	ΔP (bar)
12	300	720	14	17
8	200	320	31	39
5	125	125	80	100
3	75	45	222	278
1.9	48	18	556	692
1.8	45	16	625	772
1.7	43	15	667	865
1	25	5	2000	2500

As illustrated in Table 1, when particle size (d_p) is reduced, column length (L) can also be reduced by the same factor, whilst separation efficiency (N) remains constant and run time is reduced. The plate generation rate (N/t_0, efficiency / dead time) increases dramatically as particle size is reduced; the limiting factor is pressure drop across the column (ΔP).

A very effective way to increase the resolution of two chromatographic bands is to increase the selectivity factor (α) by manipulating column chemistry or mobile phase composition. 1.9 μm Hypersil GOLD™ columns are available in three chemistries, a C18 selectivity, a polar endcapped C18 and a perfluorinated phenyl that allow more flexibility when developing high resolution, fast methods. 1.9μm particle size Hypersil GOLD phases are based on very high purity silica using a new technique for processing particles with a very narrow size dispersion. This technology, combined with the latest in silica bonding and endcapping processes, enables the manufacturing of stationary phases which provide very symmetrical peak shapes, speed and resolution.

A process for packing 1.9 μm particles into robust and reproducible columns has also been developed and optimized. Packing bed homogeneity at higher pressures has to be ensured to maintain efficiency, and column hardware and end-fittings had to be re-designed to withstand high operating pressures.

Methods

- Columns - Hypersil GOLD 12 μm, 200 x 2.1 mm; Hypersil GOLD 8 μm, 200 x 2.1 mm; Hypersil GOLD 5 μm, 200 x 2.1 mm; Hypersil GOLD 3 μm, 200 x 2.1 mm; Hypersil GOLD 1.9 μm, 200 x 2.1 mm; Hypersil GOLD 1.9 μm, 100 x 2.1 mm; Hypersil GOLD 1.9 μm, 50 x 2.1 mm; Hypersil GOLD aQ™ 1.9 μm, 50 x 2.1 mm; Hypersil GOLD PFP 1.9 μm, 50 x 2.1 mm (Thermo Electron Corporation, Bellefonte, PA).
- Instrumentation - Finnigan™ Surveyor™; Finnigan LCQ™ Deca (Thermo Electron Corporation, San Jose, CA)
- U-HPLC system: Accela™ (Thermo Electron Corporation, San Jose, CA)
- Mobile phase compositions, gradients, flow rates, solutes, temperatures and detection details are specified on each figure.
- Method transfer to smaller column geometries packed with smaller particles (Figure 4A to C), was performed using the following equations for flow rate and gradient time adjustment:

a) Adjust flow rate (keep reduced linear velocity constant between original and new method)

$F_2 = F_1 \times (d_{c2}^2 / d_{c1}^2) \times (d_{p1} / d_{p2})$

F_1 - original flow rate; F_2 - new flow rate (mL/min)
d_{c1} - original column; d_{c2} - new column ID (mm)
d_{p1} – original particle size; d_{p2} – new particle size (μm)

b) Keep initial and final mobile phase composition, adjust gradient time

$t_{g2} = t_{g1} \times (V_{02} / V_{01}) \times (F_1 / F_2)$

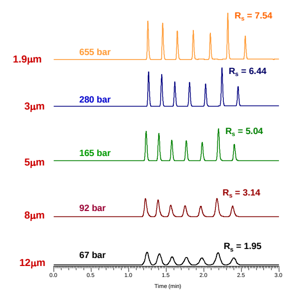
t_{g1} – gradient time in original method; t_{g2} - gradient time in new method (min)
V_{01} – original column volume; V_{02} – new column volume (mL)
F_1 - original flow rate; F_2 - new flow rate (mL/min)

$V_0 = 0.68 \times \pi \times r^2 \times L$ V_0 – column void volume (mL); L – column length (cm); r – column radius (cm)

Resolution and sensitivity

Figure 1 demonstrates the effect of particle size on the resolution of a mixture of 7 phenones, the half height resolution of the two last eluting components is annotated. The Y-axes of the five chromatograms have been normalized to illustrate the gain in sensitivity when more efficient peaks are obtained.

FIGURE 1. Resolution (R_s) and sensitivity are increased as particle size is reduced.



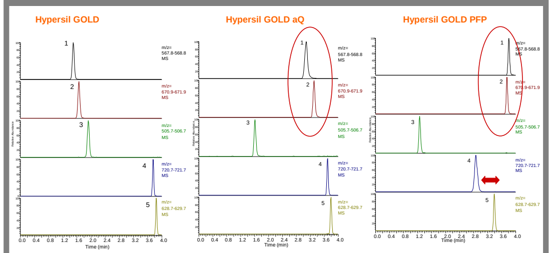
Columns: Hypersil GOLD 200 x 2.1 mm
Mobile phase: A - H_2O; B - MeCN
Gradient: 65 to 95 %B in 1.5 min, hold for 1.5 min
Flow rate: 600 μL/min
Temperature: 40 ºC
Detection: 247 nm (0.1s rise time)
Injection volume: 0.2 μL
U-HPLC System: Accela

Analytes:
Acetophenone
Propiophenone
Butyrophenone
Valerophenone
Hexanophenone
Heptanophenone
Octanophenone

Selectivity

C18 like selectivity (Hypersil GOLD), polar endcapped C18 (Hypersil GOLD aQ) and perfluorinated (Hypersil GOLD PFP) columns were used to separate a mixture of protease inhibitors (anti-HIV drugs) and a mixture of aromatic amines (Figures 2 and 3 respectively). In the first instance, several changes in elution order are observed with both the aQ and the PFP phases. For the aromatic amines, the elution order of the o-toluidine and 4,4-oxydianiline on the PFP is reversed.

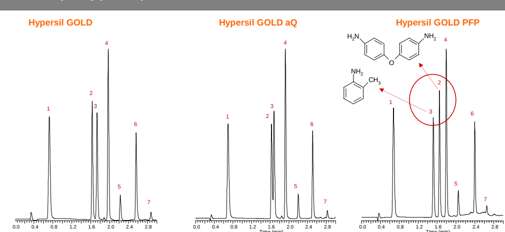
FIGURE 2. Effect of column chemistry (C18 selectivity, polar endcapped C18 and pentafluorophenyl) on separation of protease inhibitors.



Columns: Hypersil GOLD 1.9 μm, 50 x 2.1 mm
Mobile phase: H_2O + 0.1% Formic Acid / ACN + 0.1% Formic Acid
Gradient: 0 – 2.2 mins @ 35% B, then to 100 % B @ 4 mins
Flow rate: 0.5 mL/min
Temperature: 30 °C
Detection: ESI +ve, scan 450 – 750amu (above mass chromatograms for $[M+H]^+$)
Injection Volume: 0.5 μL

Analytes: 1. Amprenavir
2. Nelfinavir
3. Saquinavir
4. Ritonavir
5. Lopinavir

FIGURE 3. Effect of column chemistry (C18 selectivity, polar endcapped C18 and pentafluorophenyl) on separation of aromatic amines.



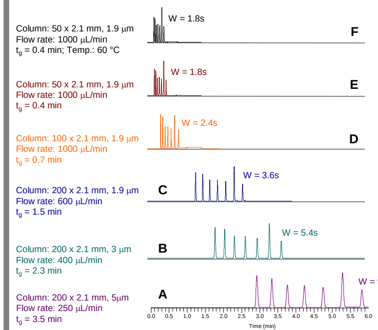
Columns: Hypersil GOLD 1.9 μm, 50 x 2.1 mm
Mobile phase: 25mM NH_4OAc pH 5 / ACN
Gradient: 10 – 100% B in 3 mins
Flow rate: 0.5 mL/min
Temperature: 40 °C
Detection: UV@ 254 nm (2 μL flow cell)
Injection Volume: 0.5 μL

Analytes: 1. 2,4-diaminotoluene
2. 4,4-oxydianiline
3. o-toluidine
4. 2-methoxy-5-methylaniline
5. 2,4,5-trimethylaniline
6. 4,4-methylene-bis(2-chloroaniline)
7. Impurity from analyte No. 6

Speed

A separation on a 200 x 2.1mm, 5 μm column was transferred to shorter columns packed with smaller particles to reduce analysis time (Figure 4). The 1.9 μm particles facilitate a decrease in run time from 6 to 0.5 minutes, whilst maintaining baseline resolution of the seven phenones.

FIGURE 4. Effect of column geometry, particle size and operating conditions on run time and peak width (at baseline).



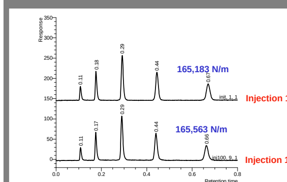
Columns: Hypersil GOLD
Mobile phase: A - H_2O; B - MeCN
Gradient: 65 to 95%B in t_g
Temperature: 40 ºC
Detection: 247 nm (0.1s rise time)
Injection volume: 0.2 μL
U-HPLC System: Accela

Analytes:
Acetophenone
Propiophenone
Butyrophenone
Valerophenone
Hexanophenone
Heptanophenone
Octanophenone

Column robustness and reproducibility

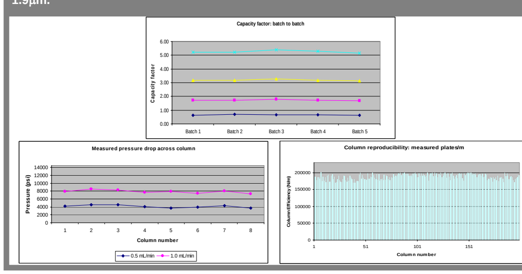
The stability of 1.9 μm Hypersil GOLD columns at high pressures is shown in Figure 5. After 100 injections of a standard mixture at 11,500 psi the change in efficiency is 0.22%. Figure 6 shows the batch-to-batch reproducibility of 5 batches (determined using the capacity factor of a standard solute) and column-to-column reproducibility determined using the column efficiency (of 200 columns) and pressure drop across the column (8 columns).

FIGURE 5. Hypersil GOLD 1.9 μm, 50 x 2.1 mm column stability at 11,500 psi.



Column: Hypersil GOLD 1.9 μm, 50 x 2.1 mm
Mobile phase: H_2O / ACN (50:50)
Flow rate: 1.2 mL/min
Pressure: 11,500 psi (790 bar)
Detection: UV at 254nm

FIGURE 6. Batch-to-batch and column-to-column reproducibility for Hypersil GOLD 1.9μm.



Conclusions

The work presented in this poster demonstrates that:

- 1.9 μm Hypersil GOLD columns provide very efficient peaks for high resolution, sensitive and fast analysis; 12-fold reduction in analysis time can be achieved
- 1.9 μm Hypersil GOLD column chemistries provide selectivity differences to facilitate method development under generic mobile phase conditions
- The manufacturing process for 1.9 μm Hypersil GOLD columns is reproducible and the columns are stable when operated at high pressures

Additional Information

For additional information, please browse our website: www.thermo.com/columns.

PO20328_E0606

Thermo ELECTRON CORPORATION