Technical note

Perfect order and its role in highly efficient liquid chromatography on micro pillar array columns (µPAC™)

Part 1: Permeability

Introduction

As stated by Giddings in the 1960's, resolution and analysis time are two important factors for any given chromatographic separation. The ultimate goal is to maximize resolution while shortening the analysis time. The quest for higher resolution has driven manufacturers to produce columns with ever smaller particle diameters. By using smaller particles, the path length of the diffusion process is shortened and mass transfer kinetics are improved. Peaks will elute sharper (low plate heights (H)), providing better overall efficiencies or plate counts (N) (eq. 1). The length of a column (L) is another key factor determining its efficiency.

(1) $\mathrm{N} = \frac{L}{H}$

The trend of downsizing particle diameter however comes at a price: increased column backpressures. Since the pressure drop is inversely proportional to the square of the particle diameter (eq.2), reducing the particle diameter by 50% will increase the pressure drop by a factor of 4. Yet, technological advances in liquid chromatography instrumentation have enabled the use of sub-2 µm particle columns with lengths up to 75 cm. Further gain in overall efficiency by increasing column length and decreasing particle diameter is very unlikely to happen due to practical considerations.

(2) $\Delta P \sim \frac{F.\eta.L}{A.d_p^2}$

By introducing perfect order in chromatographic support structures, PharmaFluidics µPAC™ cartridges enable reducing peak dispersion to an absolute minimum while simultaneously generating much lower pressure drops as compared to classical 'packed bed' liquid chromatography columns. The latter allowing extraordinary column lengths, this then translates into previously unmatched high plate counts (eq.1). For example, a µPAC™ cartridge with a separation lengths of 200 cm produces up to 300.000 plates (k'=2) and can easily be operated in a wide range of flow rates (ranging from 50 to 1500 nl/min) at pressures well below current instrumentation limits.

Comparison of column pressure to conventional packed bed nano LC columns

Column pressure as a function of flow rate was compared for three commercial packed bed nano LC columns and a 200 cm long µPAC™ cartridge. All three nano LC columns were 25 cm in length, 75 µm in diameter and packed with sub-2 µm porous particles, which can currently be considered as state-of-the-art. The pressure was monitored for flow rates in the range of 50 to 300 nl/min using a mixture (7:3) of water and acetonitrile at 30°C column temperature.

Under these conditions and using current commercially available nano-(U)HPLC instrumentation with a pressure limit of 800 bar, it was simply not feasible, nor recommended, to operate the nano LC columns at flow rates higher than 400

nl/min. On the contrary, the pressures measured on a 200 cm µPAC™ cartridge were approximately 10 times lower than those observed on the state-of-the-art nano LC columns (fig. 1). µPAC™ cartridges could therefore be operated at flow rates up to 1500 nl/min, still only generating a pressure drop of approximately 300 bar. Whereas maximal chromatographic efficiency is obtained at 200 nl/min (fig. 2), operation at higher flow rates can be advantageous when higher throughput and shorter duty cycles are required.

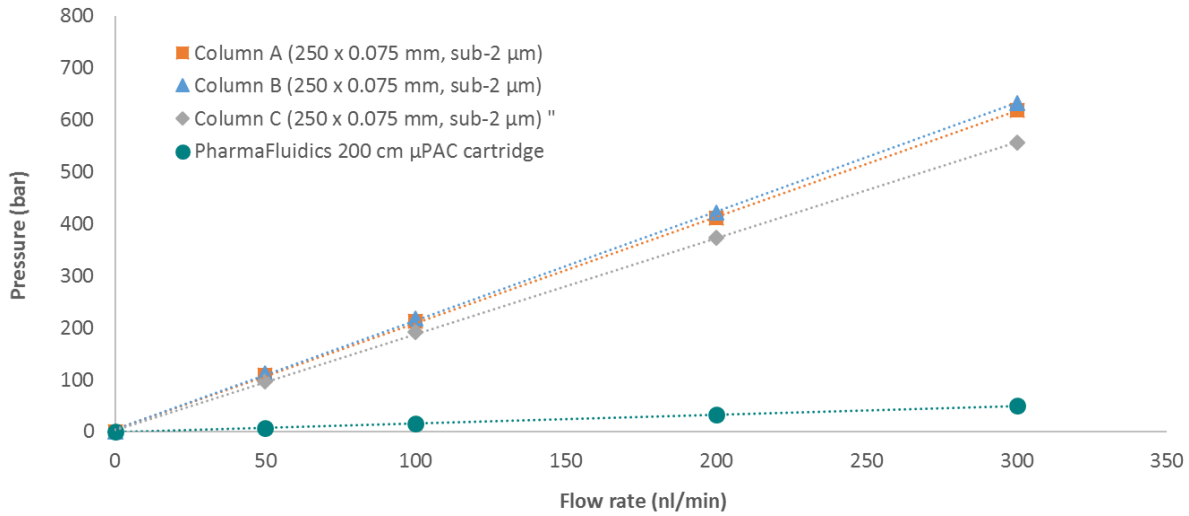

Figure 1: Comparison of column pressure for 3 commercially available nano LC columns (250 x 0.075 mm, sub-2 µm particles) and a 200 cm long µPAC™ cartridge. Mobile phase: water /acetonitrile (7:3); temperature 30°C; flow rate 50 to 300 nl/min.

Comparison of column efficiency to state-of-the-art packed bed nano LC columns

Secondly, a mixture of uracil and octanophenone was used to evaluate the efficiencies that can be achieved on both types of columns. 4 nl of sample (100 ppm each) was injected under isocratic conditions at 30°C and detected at 260 nm wavelength using a 3 nl volume UV-VIS flow cell. For all columns the water/acetonitrile composition of the mobile phase was carefully adjusted to obtain a separation with a k' value of approximately 2 for octanophenone. Efficiencies (N) at different flowrates were then calculated according to equation 3:

$$(3) \quad N = 5{,}54.\left(\frac{t_R}{w_{50\%}}\right)$$

An increase in plate count up to 6 times compared to the commercial columns could be achieved by operating the 200 cm µPAC™ cartridge at flow rates typically used in nano LC separations (100 to 300 nl/min). Furthermore, the flow rate versatility of the µPAC™ cartridges allows straightforward operation at increased flow rates. This with higher corresponding plate counts than observed for commercial nano LC columns operated at their optimal mobile phase velocity or flow rate (300 nl/min).

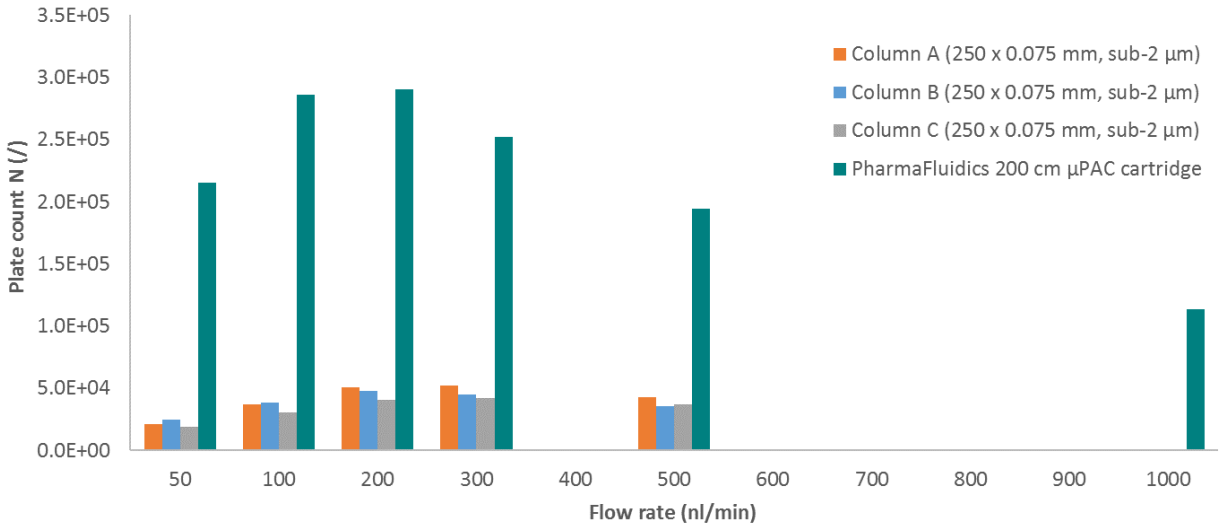

Figure 2: Comparison of column efficiency for 3 commercially available nano LC columns (250 x 0.075 mm, sub-2 µm µm particles) and a 200 cm long µPAC™ cartridge. Sample: 100 ppm uracil, 100 ppm octanophenone; 4 nl injection volume; Mobile phase: water /acetonitrile; temperature 30°C; flow rate 50 to 1000 nl/min.

Conclusion

- With the currently available nano LC pressures, conventional packed bed columns have reached a limit in terms of particle size reduction or column length increase.
- µPAC™ cartridges inherently have much lower backpressure. Hence, cartridges with lengths up to 200 cm can be operated at conventional pressures below 400 bar.
- Efficiencies up to 300.000 theoretical plates can be obtained.
- 200 cm long µPAC™ cartridges can be run at flow rates up to 1.5 µl/min and are rated up to 350 bar.