Technical note

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

Part 2: Performance in gradient separations

Introduction

As discussed in part one of this technical note, the perfect order of µPAC™ stationary phases results in reduced peak dispersion and low column backpressure. It was shown that a µPAC™ cartridge with a separation length of 200 cm produces up to 300.000 plates (k'=2) and can be operated in a wide range of flow rates (up to 1500 nl/min) while keeping the pressure under control (< 350 bar). In this respect, µPACs™ considerably outperform conventional nano LC columns.

Plate counts obtained under isocratic conditions are indeed a good measure for column efficiency. However, isocratic separations suffer from several disadvantages such as poor resolution and increased peak widths. Gradient chromatography, on the other hand, does not have these problems. Being more practical and versatile (high selectivity and resolution in adjustable analysis times), the majority of modern (complex) chromatography is being dictated by this technique. Positioning the performance of µPACs™ under gradient conditions is therefore equally important and will be the scope of this note. Plate count (N), the figure of merit under isocratic conditions (see part 1), thereby being replaced by the so called 'peak capacity'. Peak capacity, a measure for the number of peaks that can be resolved in a specific retention window (running between the first and the i^{th} component of the sample), is commonly defined as follows:

$$(1)\quad n_c = 1 + \frac{t_i - t_1}{(\sum_1^i 4.\sigma_i)/i} = 1 + \frac{t_i - t_1}{\bar{w}_{13\%}}$$

Analysis time and resolution again play a crucial role (see part 1) in gradient separations. As was the case under isocratic conditions, µPACs™ profit once more from their low dispersion and backpressure. For example, a twofold increase in peak capacity for a mix of alkylphenones can easily be obtained compared to conventional nano LC columns. Furthermore, as the range of applicable flowrates is highly versatile (due to the low backpressures), peak capacities and analysis times on µPACs™ can be tuned by simply adjusting the flowrates of separation.

Comparison of µPAC™ with commercial nano LC columns: peak capacities at optimal flow rates

Three commercial packed bed nano LC columns (250 x 0.075 mm, sub-2 µm particles) and a 200 cm long µPAC™ cartridge are compared by injecting a mixture of eight alkylphenones and uracil (100 ppm each). Such compounds are often used to evaluate reversed phase HPLC column performance because of their reasonable retention times and easy separation with a water/acetonitrile mobile phase. 4 nl of the sample was injected and subsequently separated using variable linear gradients (2-78% acetonitrile) at an optimal flow rate of 250 nl/min. Peak capacities were calculated using equation (1).

Comparing chromatograms obtained under a specific gradient, alkylphenones clearly elute much sharper on µPAC™ cartridges (Fig.1). Average peak widths are reduced twofold compared to the conventional nano LC columns (Table 1). This reduction in peak width results in a higher peak capacity (Eq.1). For the separations shown in Figure 1, peak capacity is on average 1.7 times larger for the µPAC™ cartridge.

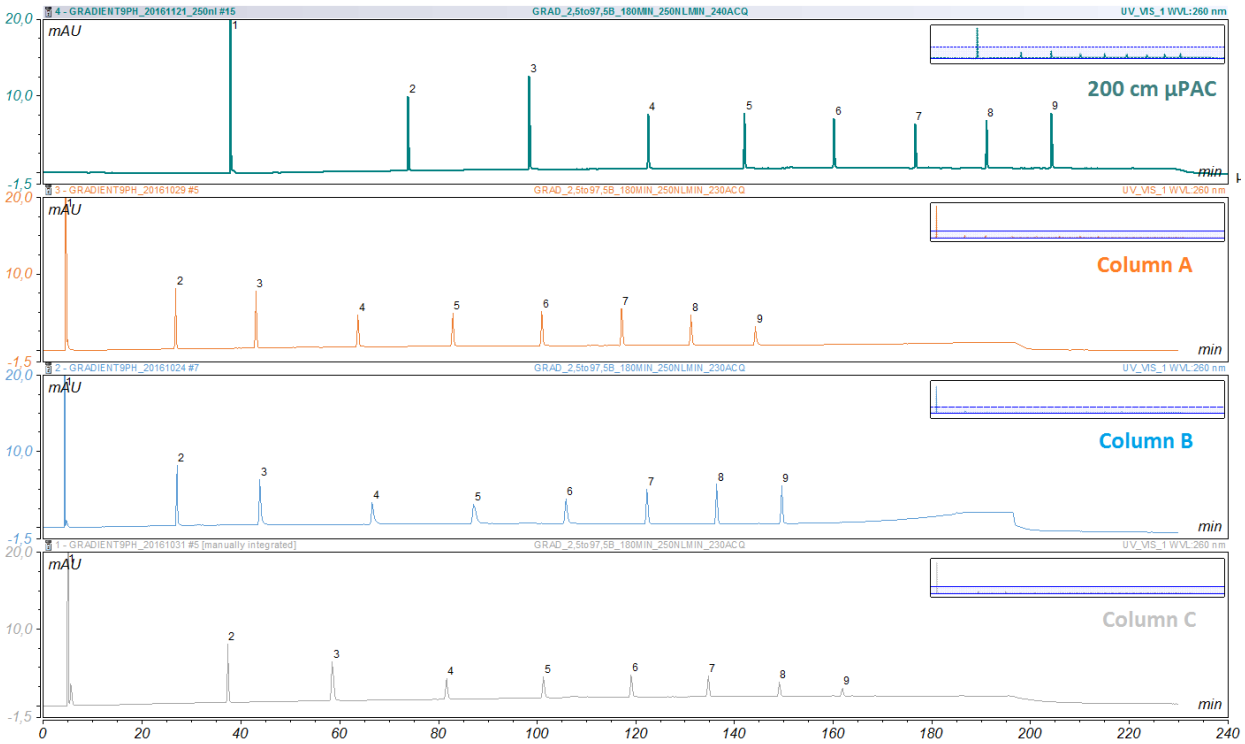

Figure 1: Chromatograms for 180 min gradients obtained on three commercially available nano LC columns (250 x 0.075 mm, sub-2 µm particles) and a 200 cm long µPAC™ cartridge. Sample: uracil (1), acetanilide (2), acetophenone (3), propriophenone (4), butyrophenone (5), valerophenone (6), hexanophenone (7), heptanophenone (8), octanophenone (9) (100 ppm each); gradient: 2-78% acetonitrile in 180 min; temperature 30°C; flow rate 250 nl/min; UV-VIS detection: 260 nm.

		Peak width [min]			
		µPAC™	**Column A**	**Column B**	**Column C**
1	Uracil = t_0	0,11	0,05	0,05	0,05
2	Acetanelide	0,27	0,33	0,33	0,37
3	Acetophenone	0,32	0,44	0,55	0,57
4	Propiophenone	0,34	0,53	0,72	0,68
5	Butyrophenone	0,35	0,56	0,95	0,70
6	Valerophenone	0,35	0,55	0,84	0,69
7	Hexaphenone	0,35	0,54	0,67	0,68
8	Heptaphenone	0,34	0,54	0,60	0,64
9	Octaphenone	0,34	0,55	0,57	0,67
	Average	0,31	0,45	0,58	0,56

Table 1: Accompanying peak widths for chromatograms depicted in Figure 1. Peak widths are measured at 10% peak height.

Running these separations at different gradient times, it becomes clear that varying the gradient time hardly improves the separation resolution for the conventional nano LC columns (Fig. 2). No significant increase in peak capacity is observed when gradient times are extended beyond 60 minutes. This is a direct consequence of peak widths increasing rapidly for longer separations. Peaks remain much narrower on µPAC™ cartridges, even for long gradient times. Accordingly, peak capacities far above those of the nano LC columns can be obtained (Fig. 2). Capacity values are plotted against t_i, the elution time of the final component. This representation enables a true comparison between columns as the complete time frame of a separation is included.

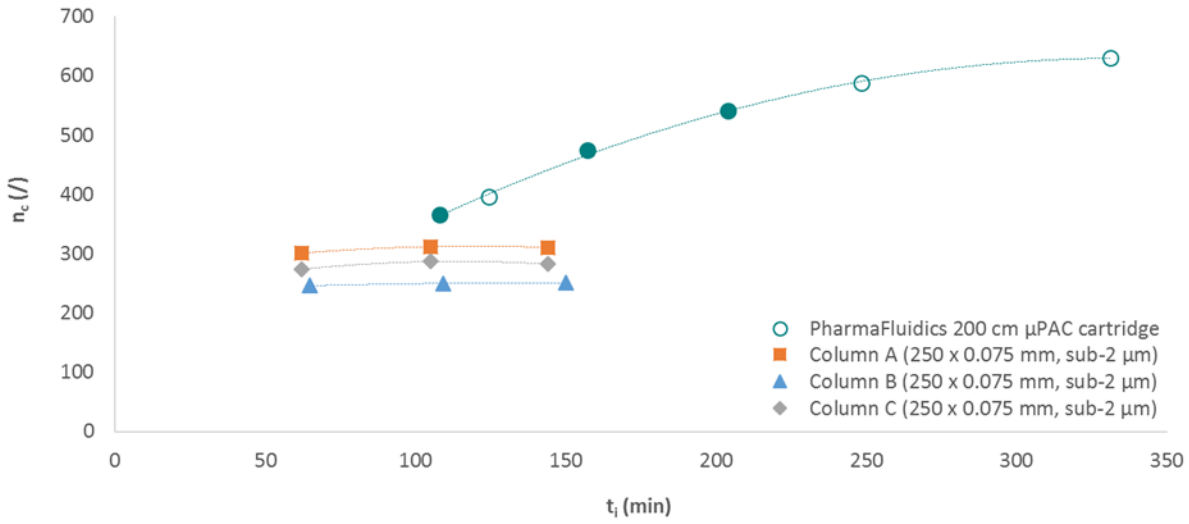

Figure 2: Comparison of peak capacities (n_c) for three commercially available nano LC columns (250 x 0.075 mm, sub-2 µm particles) and a 200 cm long µPAC™ cartridge. Sample: uracil (1), acetanilide (2), acetophenone (3), propriophenone (4), butyrophenone (5), valerophenone (6), hexanophenone (7), heptanophenone (8), octanophenone (9) (100 ppm each); gradient: 2-78% acetonitrile in 60, 120 and 180 min gradient time; temperature 30°C; flow rate: 250 nl/min; UV-VIS detection: 260 nm. *Filled dots for the µPAC™ curve represent the same gradients as those run on the commercial columns (60,120 and 180 min). Unfilled dots represent gradient times that were added to complete the curve (gradient times: 80, 240 and 360 minutes)*

Employing the flowrate versatility on µPAC™ cartridges: n_c at flow rates of 1000,500 and 250 nl/min

Run at their optimal flow rate (250 nl/min) µPACs™ produce very high peak capacities for long gradient times. Yet, for shorter gradients nano LC columns seem to outperform a µPAC™ (Fig. 3). This effect is caused by the high t_i values observed on 200 cm long µPACs™ (equivalent gradients are located at higher t_i). A substantial part of these elution times however is void time which can be significantly reduced at elevated flow rates (Table 2). µPACs™ allow this transition to be made due to low backpressures, whereas for nano LC columns this is not feasible. The resulting performance again surpasses the conventional nano LC columns and increases competitiveness of µPACs™ when analysis time is limited. For example, the time required to outperform commercial nano LC columns is reduced from 80 to nearly 30 minutes by operating at fourfold the optimal flow rate (indicated in Fig. 3).

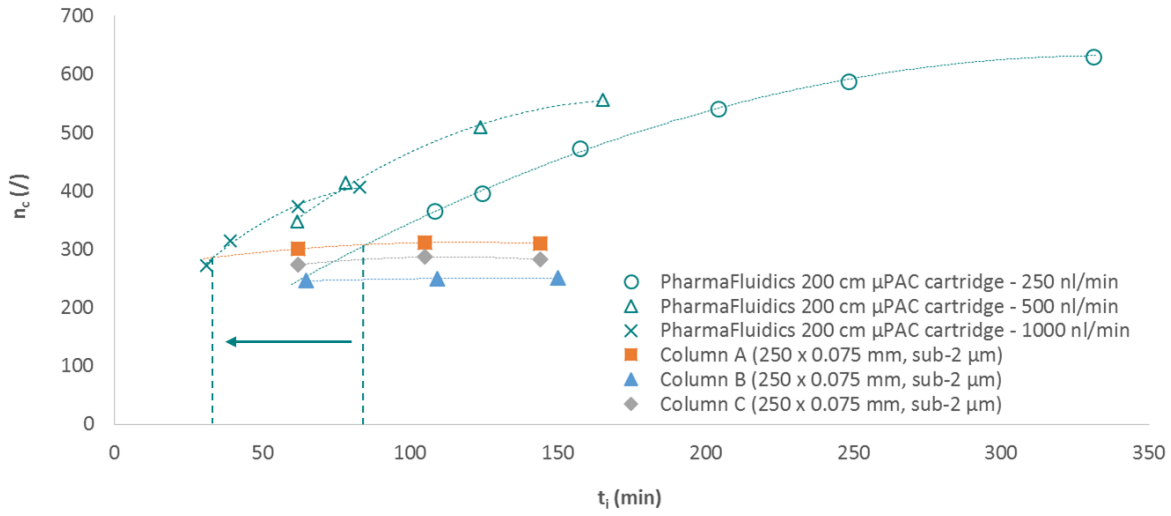

Figure 3: Comparison of peak capacity (n_c) for three commercially available nano LC columns (250 x 0.075 mm, sub-2 µm particles) and a 200 cm long µPAC™ cartridge. Sample: uracil (1), acetanilide (2), acetophenone (3), propriophenone (4), butyrophenone (5), valerophenone (6), hexanophenone (7), heptanophenone (8), octanophenone (9) (100 ppm each); gradient: 2-78% acetonitrile in a range of gradient times; temperature 30°C; flow rate 250 nl/min for conventional nano LC columns; flow rates 250, 500 and 1000 nl/min for µPAC™ cartridge. UV-VIS detection: 260 nm. *Dashed vertical lines indicate where the µPAC™ cartridge, run at 250 (right) and 1000 nl/min (left), is being outperformed by the best nano LC columns (intersect between curve of column A and the µPAC™ curves).*

	Flowrate [nl/min]	Void time [min]	backpressure [bar]
200 cm µPAC™	1000	9,5	250
	500	19	110
	250	38	62
Column A	250	4,5	480
Column B	250	4,3	490
Column C	250	4,8	440

Table 2: Observed backpressures (at start of the gradients) and void times for three commercially available nano LC columns (250 x 0.075 mm, sub-2 µm particles) and a 200 cm long µPAC™ cartridge.

Conclusions

- For conventional nano (U)HPLC columns, no adequate gain in separation resolution is observed when extending gradient times from 60 to 120 or 180 min. The gain that is obtained by expanding the elution window is practically completely neutralized by the rapidly increasing peak widths.
- Peak widths remain narrow on µPAC™ cartridges, even for long gradient times.
- The highest resolution can be obtained for low flow rates (250 nl/min). Peak capacities two times those on conventional nano LC columns can be obtained.
- The low backpressure of µPACs™ allows operation at elevated flow rates, where shorter gradient times can be deployed in order to have superior resolution, even for shorter analysis times.