Comparison of the Chromatographic Resolution of Solid Core 4 µm and Fully Porous 3 µm and 5 µm Columns

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Key Words
Solid core, fused core, superficially porous, resolution, efficiency, productivity, Core Enhanced Technology

Abstract
In this technical note, the chromatographic resolution of solid core 4 µm particle packed columns is compared with that of fully porous 5 and 3 µm particle packed columns.

Introduction

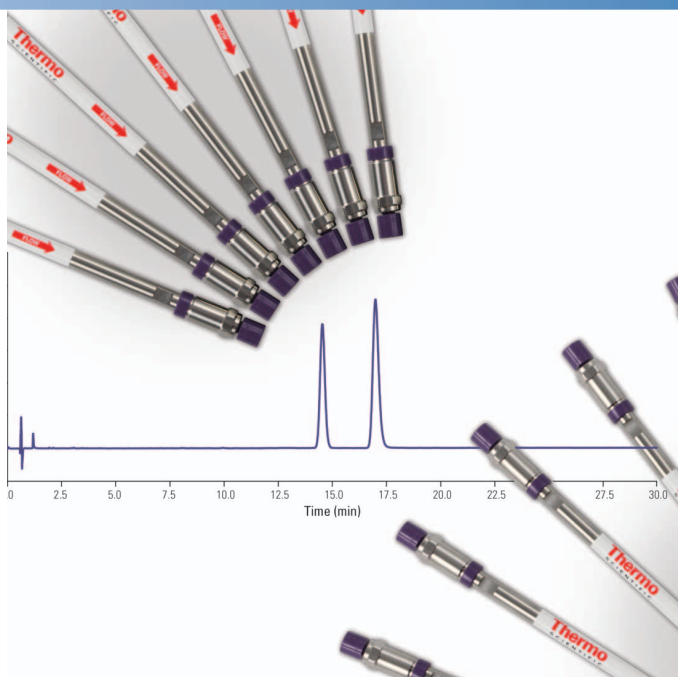
The primary goal of developing a chromatographic separation is to resolve a mixture of analytes. From the general resolution equation (Equation 1), it is evident there are three parameters that control resolution, namely efficiency (N), selectivity (α), and retention (k') factors. Selectivity and retention factor are analyte-dependent and can be improved by changing the column chemistry, mobile phase composition, or temperature. The third parameter, efficiency, is analyte-independent. Therefore, columns that provide improved efficiency have a wider chromatographic applicability.

Equation 1.

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

Chromatographic efficiency, and therefore resolution, can be increased by use of a longer column, however this results in a longer analysis time. The favored methods of increasing chromatographic efficiency are a reduction in the particle size or a change to solid core particles, which for the same particle size produce sharper, more efficient peaks and hence better separations than fully porous materials. An advantage of converting from fully porous to solid core materials of a similar particle size for improved efficiency is that users of conventional HPLC methods in regulated environments can change the column format without the need of a full revalidation under the current regulatory guidelines.

Using a solid core 4 µm particle packed in conventional column dimensions, significant improvements in the assay performance can be achieved without the need to make changes to the operating parameters or system



configuration. Based on Core Enhanced Technology™ using 4 µm solid core particles, Thermo Scientific™ Accucore™ XL HPLC columns allow users of conventional HPLC methods to obtain performance far beyond that of columns packed with 5 µm or even 3 µm fully porous particles. Very high peak efficiencies using standard HPLC instrumentation and conditions allow for increased peak resolution for the same stationary phase chemistry. Additionally, the lower volume of mobile phase in columns packed with solid core particles (i.e., lower v_0 or t_0) combined with a higher optimal linear velocity results in improved productivity.

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Resolution Comparison in Isocratic Mobile Phase Conditions

Figure 1 illustrates the separation of six fat soluble vitamins under isocratic mobile phase conditions. On the Accucore XL C18 4 µm HPLC column, the resolution is greater than or equal to 2.5 for all compounds. Resolution of the critical pair (vitamin D2 and D3) increased by 30% to 2.5 when using the Accucore XL C18 4 µm HPLC column compared to 1.92 for the 5 µm fully porous C18 column. This is a result of the improved efficiency provided by the Accucore XL HPLC column, which is visually evident from the peak widths in the chromatograms. Table 1 shows that the Accucore XL HPLC column almost doubles the efficiency in all cases compared to the fully porous material. This was achieved under the same chromatographic conditions, with no changes in system configuration and with only a small backpressure increase (from 47 bar with the 5 µm fully porous column to 62 bar for the 4 µm Accucore XL HPLC column).

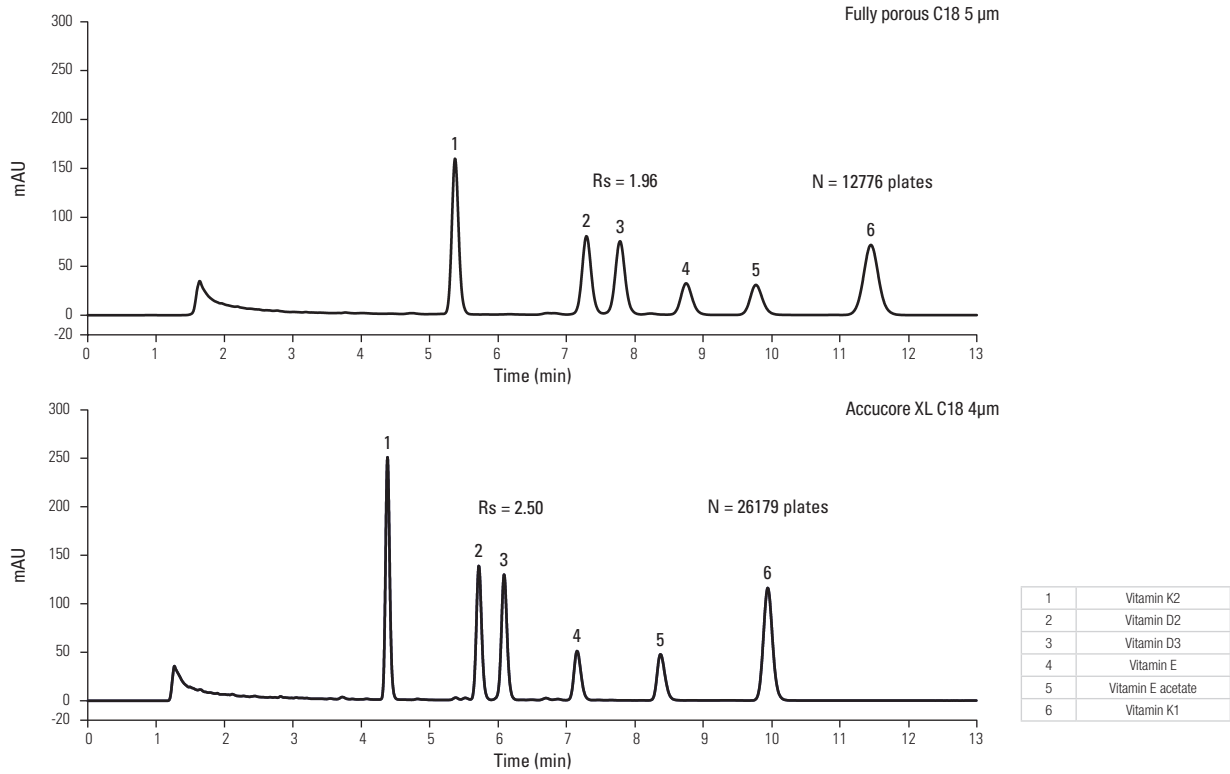


Figure 1: Comparison of the resolution of a critical pair (peaks 2 and 3) on 5 µm fully porous C18 and 4 µm Accucore XL C18 HPLC columns. Experimental conditions: columns – 150 x 4.6 mm; mobile phase – acetonitrile / methanol (80:20 v/v); flow rate – 1 mL/min; column temperature – 30 °C; UV detection - 280 nm; injection volume – 5 µL

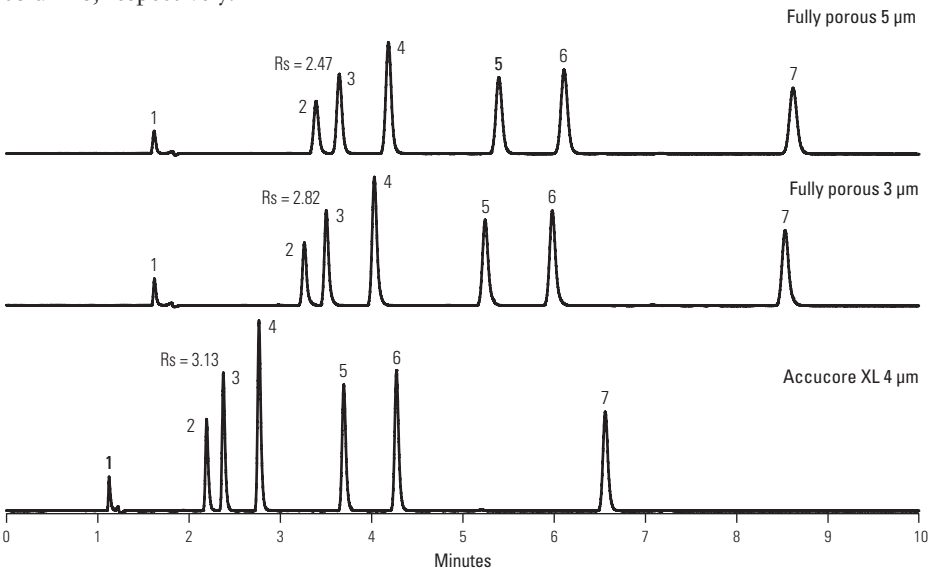
	Efficiency (USP)	
	Accucore XL 4 µm	Fully porous 5 µm
Vitamin K2	23826	13599
Vitamin D2	25566	13963
Vitamin D3	25710	13985
Vitamin E	22788	13288
Vitamin E acetate	24568	13880
Vitamin K1	26179	12776

Table 1: Efficiency values for the six fat soluble vitamins

Resolution Comparison in Gradient Mobile Phase Conditions

In Figure 2 the resolution of the Accucore XL 4 µm HPLC column is compared to that of the fully porous 5 and 3 µm materials, using gradient mobile phase conditions, and maintaining all other experimental conditions for the 3 columns. The higher efficiencies of the solid core 4 µm column results in improved resolution: 27% and 11% higher resolution of the critical pair over the fully porous 5 and 3 µm columns, respectively. When using gradient mobile phase conditions, efficiency cannot be used as a measure of column performance; instead, peak width or peak capacity are generally used. In Figure 3 the peak capacities of the 3 columns are compared. The Accucore XL 4 µm HPLC column shows 66% and 44% higher peak capacity than the fully porous 5 and 3 µm columns, respectively.

An even more significant improvement in resolution with an Accucore XL C8 4 µm HPLC column is demonstrated in Figure 4 for the analysis of 7 catechins. Under the conditions adopted for this analysis, resolution of greater than 2.6 was achieved for all catechins on the Accucore XL C8 4 µm HPLC column. On the fully porous C8 column, resolution between the critical pair (peaks 4 and 5) was only 1.17, which more than doubled with the Accucore XL C8 4 µm HPLC column. The peak widths improved on average by 34% when changing from the 5 µm fully porous to the Accucore XL C8 4 µm HPLC column (Table 2). This was achieved under the same chromatographic conditions, with no changes in system configuration and with a small backpressure increase, from 182 bar with the 5 µm fully porous column to 241 bar for the Accucore XL C8 4 µm column.



1	Uracil
2	Tebuthiuron
3	Metoxuron
4	Monuron
5	Chlorotoluron
6	Diuron
7	Linuron

Figure 2: Comparison of the resolution of a critical pair (peaks 2 and 3) on fully porous 5 and 3 µm and Accucore XL 4 µm HPLC columns. Experimental conditions: columns – C18, 150 x 4.6 mm; mobile phase – water and acetonitrile; gradient – 35% to 60% acetonitrile in 10 min; flow rate – 1 mL/min; column temperature – 30 °C; UV detection - 247 nm; injection volume – 5 µL

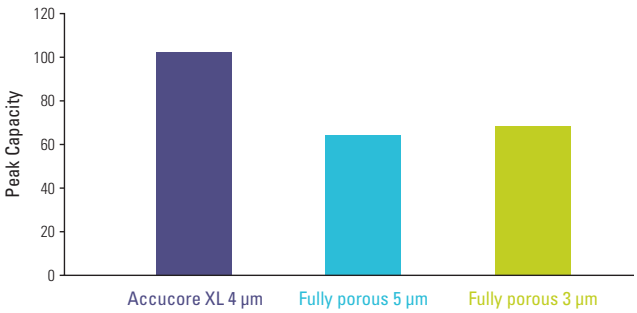


Figure 3: Peak capacity comparison for the herbicides method

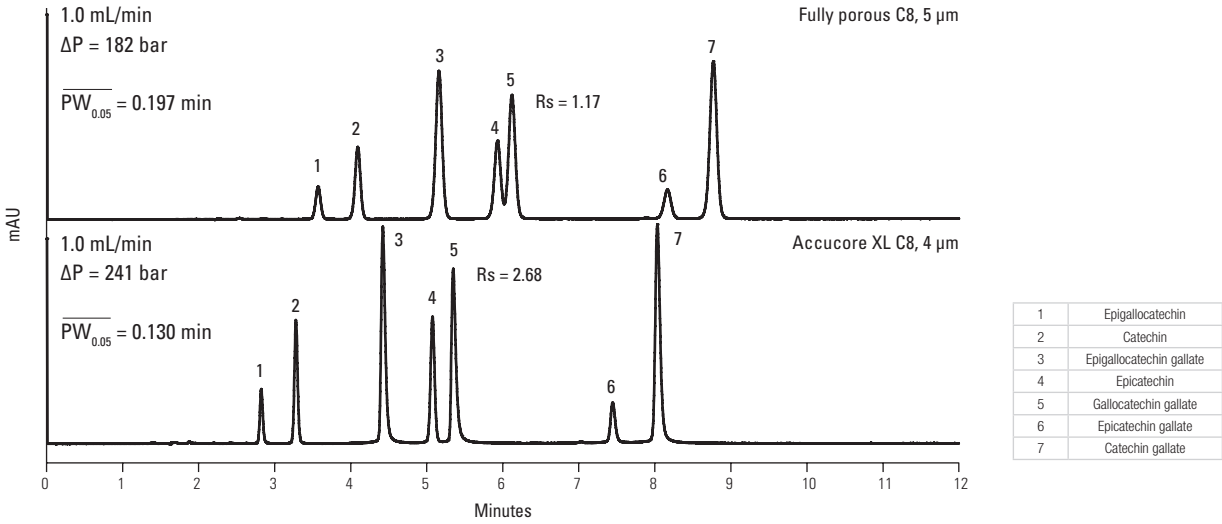


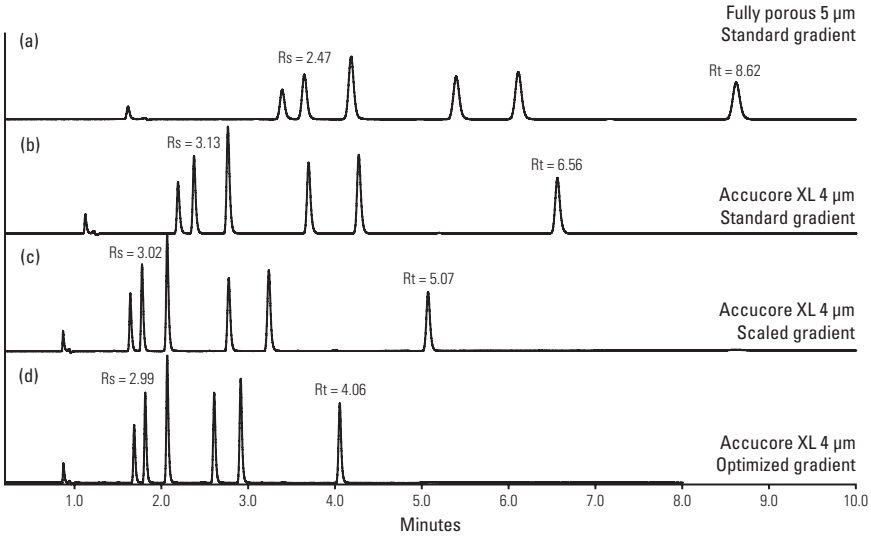
Figure 4. Comparison of the resolution of a critical pair (peaks 4 and 5) on 5 µm fully porous C8 and 4 µm Accucore XL C8 HPLC columns. Experimental conditions: columns – 150 x 4.6 mm; mobile phase A – water + 0.1% formic acid; mobile phase B – methanol + 0.1% formic acid; gradient: 20% to 50% in 15 min; flow rate – 1 mL/min; column temperature – 25 °C; UV detection – 280 nm; injection volume – 5 µL

Productivity Comparison

The high efficiencies of the Accucore XL 4 µm HPLC column over a wide flow rate range can be used to reduce analysis time by optimizing flow rate and gradient conditions. The van Deemter curve for the Accucore XL 4 µm HPLC column is very flat at high flow rates [1]. Therefore, a wide range of or flow rates can be used without losing chromatographic performance. In Figure 5, a reduction of run time by half is demonstrated (Figures 5a and 5d), simultaneously improving the resolution of the critical pair when using the Accucore XL HPLC column compared to the fully porous 5 µm column. When running the fully porous 5 µm and the Accucore XL 4 µm HPLC column under the same conditions, the retention time of the last eluting peak reduces from 8.62 to 6.56 min, respectively (Figures 5a and 5b), which can be attributed to the lower column volume of the solid core material and lower carbon load of the C18 phase. From the van Deemter curve for a 4.6 mm ID Accucore XL 4 µm HPLC column, the flow rate that provides the highest efficiency is 1.3 mL/min [1]. When increasing the flow rate, the gradient time needs to be adjusted to keep the same gradient through the column. The original 10 min gradient was reduced to 7.5 min (scaled gradient), which enabled a reduction in the retention time of the last peak from 8.62 to 5.07 min (Figures 5a and 5c). The resolution of the critical pair is still >3 under the scaled gradient conditions. Optimizing the gradient by making it faster (4 min) enables a reduction in analysis time to just over 4 min (Figure 5d).

	Peak width (5% height)	
	Accucore XL 4 µm	Fully porous 5 µm
Epigallocatechin	0.094	0.157
Catechin	0.101	0.173
Epigallocatechin gallate	0.132	0.201
Epicatechin	0.124	Partial co-elution
Gallocatechin gallate	0.149	
Epicatechin gallate	0.151	0.228
Catechin gallate	0.158	0.227

Table 2: Peak widths for the seven catechins



1	Uracil
2	Tebuthiuron
3	Metoxuron
4	Monuron
5	Chlorotoluron
6	Diuron
7	Linuron

Figure 5: Productivity comparison for fully porous 5 µm and Accucore XL 4 µm HPLC columns. Experimental conditions: columns – C18, 150 x 4.6 mm; mobile phase – water and acetonitrile; standard gradient – 35% to 60% acetonitrile in 10 min and flow rate – 1 mL/min; scaled gradient: 35% to 60% acetonitrile in 7.5 min and flow rate – 1.3 mL/min; optimized gradient: 35% to 60% acetonitrile in 4.5 min and flow rate – 1.3 mL/min; column temperature – 30 °C; UV detection – 247 nm; injection volume – 5 µL

Conclusion

- The Accucore XL 4 µm HPLC columns produce significant resolution improvements over fully porous 3 µm and 5 µm columns with no changes to methodology or HPLC system configuration.
- The solid core 4 µm particles in Accucore XL HPLC columns provide significant improvements over fully porous 5 µm and 3 µm particles in terms of separation efficiency and resolution.
- The flat nature of the van Deemter curves on the Accucore XL 4 µm HPLC columns enable reduction in analysis time by optimization of flow rate and gradient conditions.

References

[1] Thermo Scientific Technical Note 20641: Solid Core 4 µm HPLC Column Comparison to Fully Porous 3 µm and 5 µm Columns: Efficiency and Pressure.

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