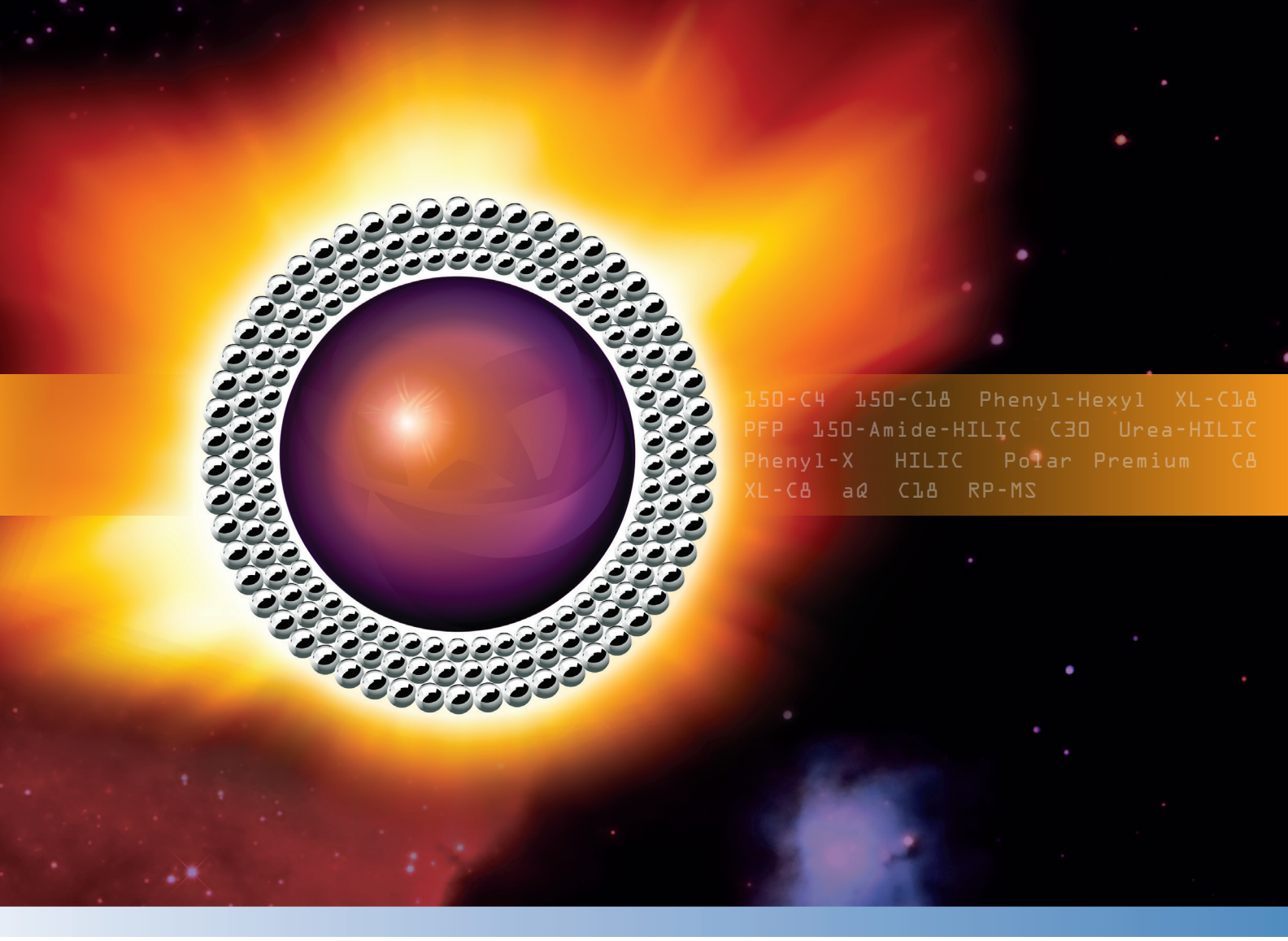

Solid Core

Technical Compendium

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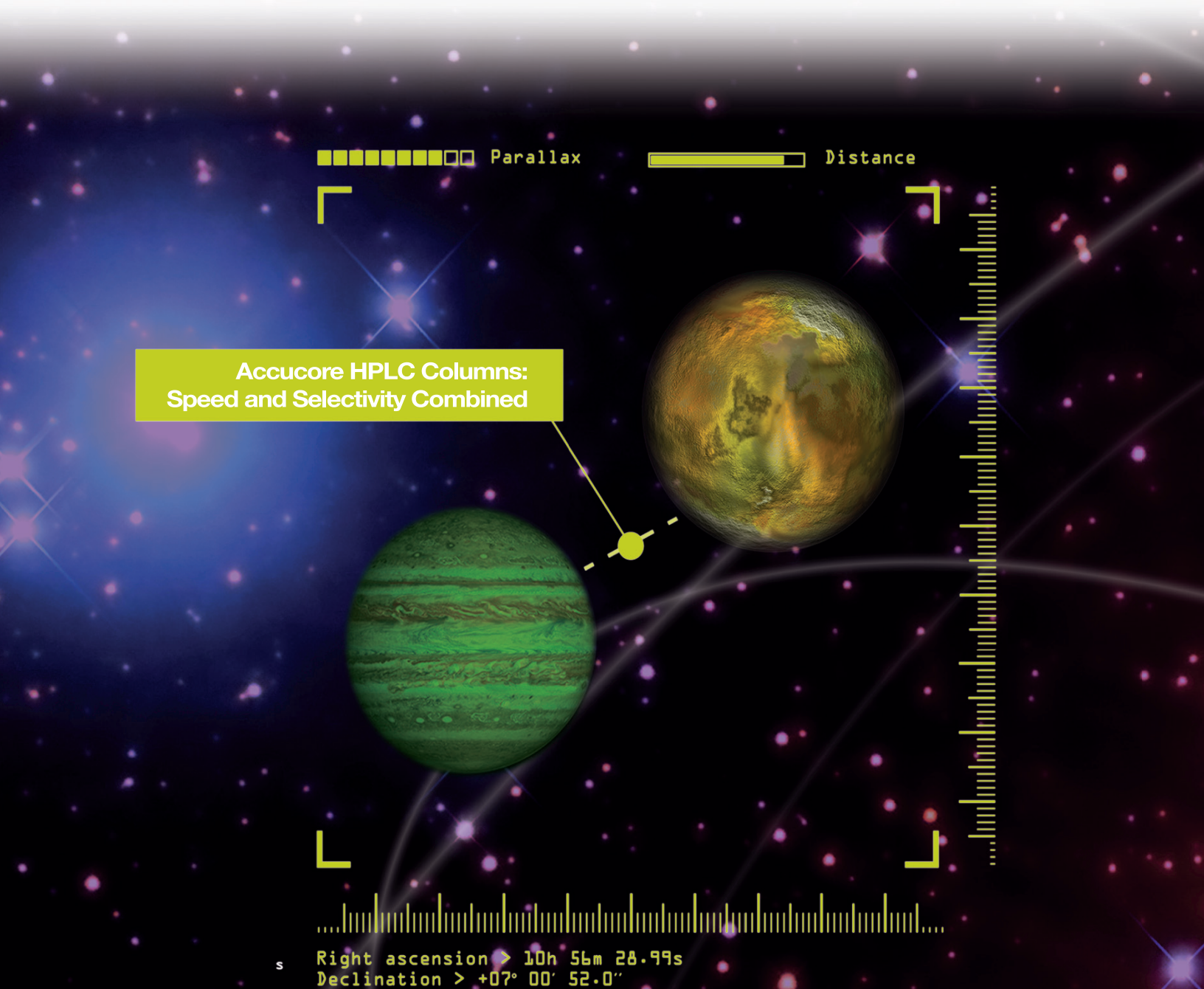

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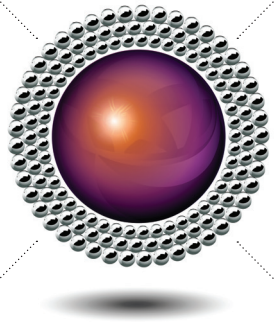
The key components of Core Enhanced Technology

Solid Core Particles

With a solid central core and porous outer layer, these particles generate high speed, high resolution separations without excessive backpressure

Automated Packing Process

Enhanced automated procedures ensure that all columns are packed with the highest quality



Tight Control of Particle Diameter

Enhanced selection process keeps particle size distribution to a minimum and produces high efficiency columns

Advanced Bonding Technology

Optimized phase bonding creates a series of high coverage, robust phases

Accucore HPLC Columns

- *Rugged and reproducible 2.6 µm solid core particles*
- *Fast separations with superb resolution*
- *Low backpressures*

Accucore HPLC Columns for Biomolecules

- *150 Å pore size solid core particles for fast biomolecule separations*
- *Superb resolution at low backpressures*
- *Exceptionally rugged analytical and nano scale columns*

Accucore XL HPLC Columns

- *4 µm solid core particles for all users*
- *Same system, same method, better results*
- *Robust, fast and easy to use*

An Overview of Core Enhanced Technology for Fast, High Efficiency HPLC

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The chromatographic material described herein uses core enhanced technology to produce columns that offer fast and high efficiency separations at pressures compatible with conventional HPLC equipment. The particles in these new stationary phases are not fully porous but rather have a solid silica core surrounded by a porous outer layer. The very tight particle size distribution results in columns with high permeability, and therefore 'bar for bar' this solid-core material gives higher performance separations than fully porous materials. This paper gives an overview of the fundamentals of the dispersion process in chromatography and applies it to the use of solid-core particles in the separation mechanism, illustrating the benefits of this type of particle in fast, high efficiency separations. Column selection based on selectivity, method transfer and the advantages that this technology has to offer in terms of column robustness are also reported.

In the past decade there has been continuous drive to develop chromatographic stationary phases to perform fast HPLC separations, as sample throughput can be increased and therefore cost per sample reduced. The theory of chromatography predicts that the efficiency of a LC separation increases with decreasing particle size. As such, most columns currently used for fast HPLC are packed with particles in the sub-2µm internal diameter region. The small particle diameter improves the separation kinetics and therefore efficiency, but at the expense of increased operating backpressure. A two-fold reduction in particle size (d_p) doubles efficiency (N is proportional to $1/d_p$), and produces therefore a 40% fold increase in resolution (resolution is proportional to the square root of N). However, it also results in a four-fold increase in pressure drop across the column as pressure is inversely proportional the square of d_p. Additionally, sub-2µm particle packed columns are generally run at high linear velocities as these produce higher efficiencies; consequently the HPLC equipment has to be able to operate at pressures in excess of the conventional 400 bar, unless very short column lengths (< 50mm) are used. While a number of manufacturers produce such HPLC equipment, for laboratories that do not have the financial luxury of being able to purchase new instrumentation these columns are not an option.

Manufacturers typically provide sub-2µm particles in a fully porous format. The use of partially porous particles, with a diameter between 2 and 3µm, is starting to gain momentum, as these provide similar performance to sub-2µm particles at significantly lower column backpressures. Pellicular particles of large diameters have been around since the 1960's [1], but it was Jack Kirkland who in 2000 developed 5 µm particles that had a 0.25µm thick porous layer and 30 nm pores for the separation of large molecules [2]. The idea behind this development was to take advantage of the smaller diffusion distance of the molecule in the particle, as macromolecules have low diffusivity. Further developments of the technology have allowed the manufacture of solid-core particles of sub-3µm total diameter. The Thermo Scientific Accucore uses Core Enhanced Technology to produce a 2.6µm solid-core material with very tight particle size distribution and advanced bonding technology to functionalise the surface. The particles in the new Accucore™ stationary phases can be described as a solid silica core surrounded by a porous outer layer. The very tight particle size distribution of this material results in columns with high permeability, and therefore for the same nominal pressure Accucore gives better separations than fully porous materials. The solid-core and the well defined porous outer layer provides shorter diffusion paths into the stationary phase compared with those in fully porous particles, which reduces band broadening and therefore improves separation efficiency. Additionally, the better packing facilitated by the tight particle size distribution reduces differences in the radial diffusion path in the liquid mobile phase.

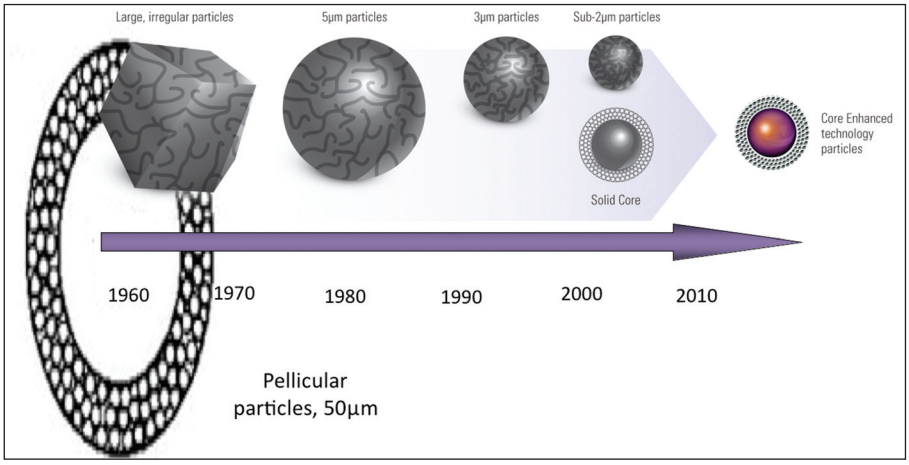


Figure 1: Particle evolution: packing materials have changed from large pellicular particles via smaller totally porous particles to spherical particles with diameters of less than 2µm, to 2.6µm solid-core particles

Theory of solid-core particles

The general resolution equation relates the separation power of the chromatographic support to its efficiency, selectivity and retention capacity, which are dependant on particle size and quality of the packing, bonded phase chemistry and surface area respectively. Efficiency is solute independent (i.e. is an inherent function of the physical properties of the column), whereas retention factor and selectivity are not.

Equation 1

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

R_S – resolution
N – efficiency
α – selectivity factor
k' – retention factor

Equation 2

$$HETP = A + \frac{B}{\mu} + C_m\mu + C_s\mu$$

HETP – height equivalent to a theoretical plate

μ - Linear velocity of mobile phase

A – Eddy diffusion constant

B – Longitudinal diffusion constant

C_m – Resistance to mass transfer in the mobile phase

C_s - Resistance to mass transfer in the stationary phase

The height equivalent to a theoretical plate (HETP) is generally used as a measure of efficiency when comparing columns. HETP is related to linear velocity through the column via the van Deemter equation. In this equation A, B and C (both components) are constants that describe contributions to band broadening through Eddy diffusion, longitudinal diffusion and resistance to mass transfer respectively. Peak or band broadening is the consequence of several mass transfer processes that occur as the analyte molecules migrate down the column. The A-term, Eddy diffusion, is dependent on particle size and the homogeneity of the packed bed. Smaller particles reduce the A-term and therefore improve efficiency. The average particle size distribution of a spherical chromatographic medium is generally defined through the ratio d90/10;

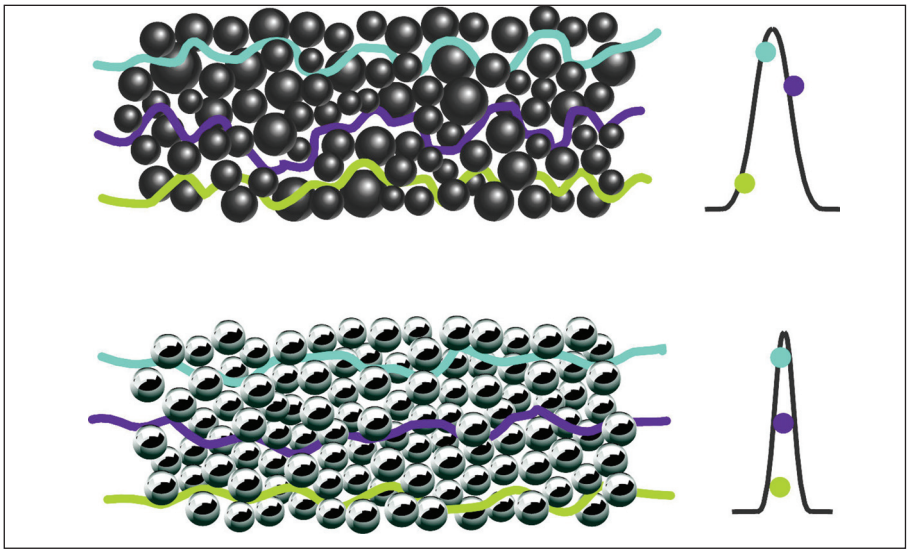


Figure 2: Representation of the effect of average particle size distribution (D90/10) on the packed bed homogeneity and band broadening through Eddy diffusion. Top - D90/10 ~ 1.5; Bottom - D90/10 ~ 1.1.

the closer this value is to 1 the less spread there is on the average diameter of the particles. The Accucore material has a d90/10 of 1.12 whereas most fully porous particles have a d90/10 around 1.50. The schematic on Figure 2 illustrates the effect of the average particle size distribution on the homogeneity of the chromatographic packed bed.

Whereas the A-term is independent of the linear velocity of the mobile phase through the column, the C-term, resistance to mass transfer, is proportional to it and therefore an important consideration when working with fast separations. The C-term has two contributors:

- resistance to the mass transfer in the stationary phase Cs
- resistance to the mass transfer in the mobile phase Cm.

The first occurs when the analyte molecule diffuses in and out of the pores of the stationary phase particle. With solid-core particles the diffusional path of the analytes is limited by the depth of the outer porous layer, and therefore analytes do not have the propensity to have greater diffusional lengths within the more limited pore structure of the solid-core material. This results in less band broadening and more efficient peaks. The resistance to mass transfer in the mobile phase is caused by the fact that the liquid is flowing in the channels between particles and analytes have to diffuse through the liquid to reach the stationary phase. This effect is equivalent to the longitudinal diffusion, however whereas with the longitudinal diffusion increasing the flow reduces the band broadening, increasing the flow will have an adverse effect on the homogeneity of the flow in a radial direction. Analytes that are in the centre of the flow will have a longer diffusional path to the particle than analytes that are at the edge nearer to the particle. Better packing and smaller particles result in a more uniform diffusional path in the liquid mobile phase.

From the discussion above we may expect solid-core particle packed columns to be more efficient than fully porous particle packed columns of the same average particle diameter. Both the A and C-terms are reduced, and therefore H is reduced which equates to higher efficiencies. It would also be expected that the drop off in efficiency that is seen with increasing flow rates will be less with solid-core material than with fully porous material due to a lesser contribution form the resistance to mass transfer terms. The next section will investigate the experimental findings found when comparing porous and solid-core particles.

Benefits of solid-core particles

Figure 3 compares the experimentally determined separation efficiency (measured as HETP) of fully porous 5 and 3 and sub-2μm with that of the solid-core Accucore 2.6 μm material. The van Deemter curves have a very definite minimum HETP, which is where minimal band broadening occurs, and therefore a very definite maximum in term of chromatographic efficiency. This means that for a chromatographic support there is a maximum chromatographic efficiency delivered at a very definitive flow through the column. Deviation from that flow will severely impact chromatographic efficiency which in turn may compromise assay performance. As the particle size is decreased, HETP becomes smaller and therefore the chromatographic efficiency increases; also, for smaller particles the flow rate that provides the best efficiency

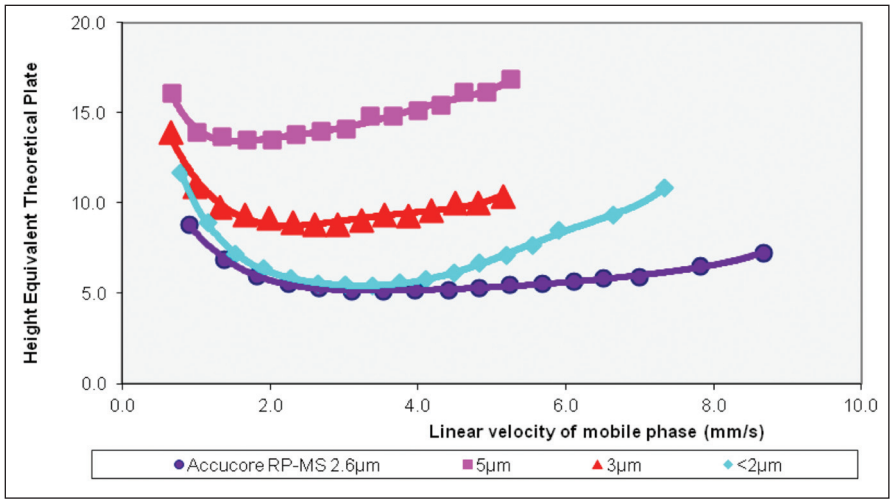


Figure 3: Efficiency comparison using Van Deemter plots for Accucore 2.6µm and fully porous 5, 3 and sub-2µm.

is shifted to a higher value and the curve becomes flatter, which indicates that a wider flow rate range can be used without losing performance. For instance, for porous 5µm particles the best linear velocity is less that 2mm/s and when the linear velocity increases the efficiency drops quickly, whereas for solid-core 2.6µm particles the linear velocity that provides the best efficiency is around 3.5mm/s (which corresponds to 400µL/min for a 2.1mm id column) and there is a wide range of flow rates that can be used. The highest efficiency and lowest rate of efficiency loss with linear velocity is observed for the solid-core material.

Figure 4 shows the column backpressure measured for the same set of experiments. Reducing the particle size increases the observed back pressure and for the data shown in Figure 4 it can be seen that for chromatographic systems that have a pressure limit of 400 bar this will reduce the effective flow rate range that can be used on a column. In this example the data was generated on a 100 x 2.1mm column using a mixture of acetonitrile and water, where the optimum flow rate is approximately 400µL/min. Clearly the use of a sub 2µm material will limit the use of many standard HPLC systems where the maximum operating pressure is 400 bar. However, the solid-core material is able to operate at 800µL/min, double the flow rate before it experiences the same issues.

This van Deemeter equation graphical representation has limitations as it allows us to understand the effect of band broadening on the efficiency and how that varies with linear velocity of the mobile phase but it does not account for analysis time or pressure restrictions of the chromatographic system, or in other words, it does not account for the flow resistance or the permeability of the column. Kinetic plots [3] are an alternative method of plotting the same data (HETP and linear velocity values) which takes into account the permeability of the columns, which is a measure of column length, mobile phase viscosity, and maximum pressure drop across the column, and therefore allow us to infer the kinetic performance limits of the tested chromatographic materials. The linear velocity, conventionally plotted on the x-axis in the van Deemeter plot, is transformed into the pressure drop limited plate number. Using a maximum pressure drop for the system, any experimental set of data of HETP- linear velocity obtained in a column with arbitrary length and pressure drop can be transformed into a projected efficiency (N)-t0 representing the plate number and t0-time, which could be obtained if the same chromatographic support was used in a column that was long enough to provide the maximum allowed inlet pressure for the given linear velocity.

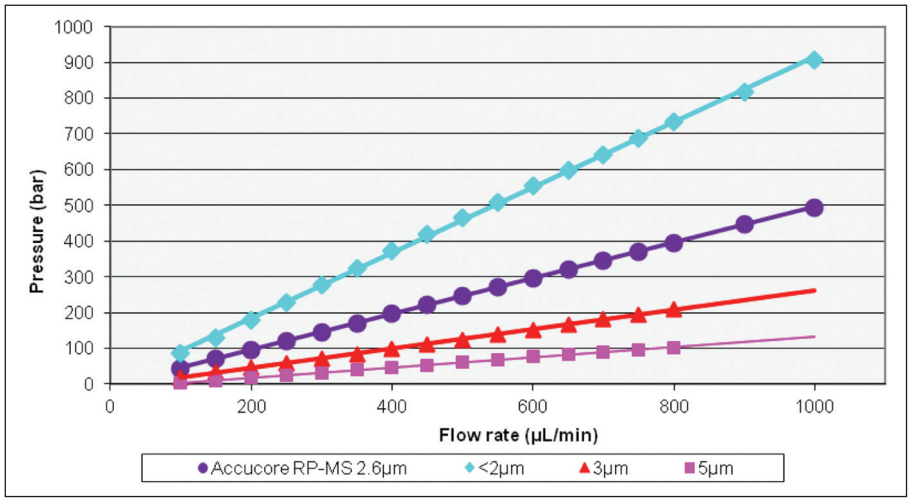


Figure 4: Comparison of column pressure for Accucore 2.6 µm and fully porous 5 and 3 and sub-2µm (100x2.1 mm columns, mobile phase: water/acetonitrile (1:1), temperature 30°C).

The mathematics underlying the kinetic plot method is very simple and is based on three 'classical' chromatographic equations (Equations 3 to 5). Kinetic plots are ideally suited to compare the performance of differently shaped or sized LC supports.

Equation 3

$$L = NH$$

L – column length
N – efficiency
H – HETP

Equation 4

$$t_0 = \frac{L}{\mu}$$

µ - Linear velocity of mobile phase
t_0 – dead time of the chromatographic system

Equation 5

$$\mu = \frac{\Delta P K_V}{\eta L}$$

ΔP – pressure drop
K_V – column permeability
η - mobile phase viscosity

Impedance

Kinetic plots can take different forms, and some of the simpler forms are displayed in Figure 5 (a) and (b). These compare the column efficiency per unit time (a) and column efficiency per unit length (b), for the fully porous 5, 3 and sub-2µm and solid-core 2.6µm particles. The Accucore 2.6µm material is the most efficient per unit length of column and the most efficient per unit time, with the fully porous sub-2µm performing similarly. Figures 5 (c) and (d)

show the relationship between impedance and mobile phase linear velocity or impedance and efficiency. Impedance (Equation 6) is a term that defines the resistance a compound is subjected to as it moves down the column relative to the performance of that column. This term gives a true measure of the performance of the column as it incorporates efficiency, time and pressure, which are critical practical considerations of a chromatographic separation. Impedance is often plotted in a reversed N – axis to mimic the van Deemter plot (Figure 5d). From the four materials in this study, the solid-core 2.6µm HPLC columns provide the highest efficiency with the lowest impedance. Of particular interest is the comparison of the impedance of sub-2µm fully porous and the solid-core materials. These show similar values of efficiency (as demonstrated in Figure 3), however the impedance is directly proportional to the pressure drop across the column. Solid-core particle packed columns show a pressure drop that can be half or even less that of a fully porous sub-2µm particle packed column, of the same length and internal diameter, when run under identical conditions. Therefore, solid-core particles are favoured because for the same nominal pressure they provide the highest efficiency. The kinetic plots in Figure 5 were plotted using a template downloaded from reference [4].

Equation 6

E – impedance

$$E = \frac{\Delta P t}{\eta N^2}$$

ΔP – pressure drop
η - kinematic viscosity of mobile phase
N - efficiency

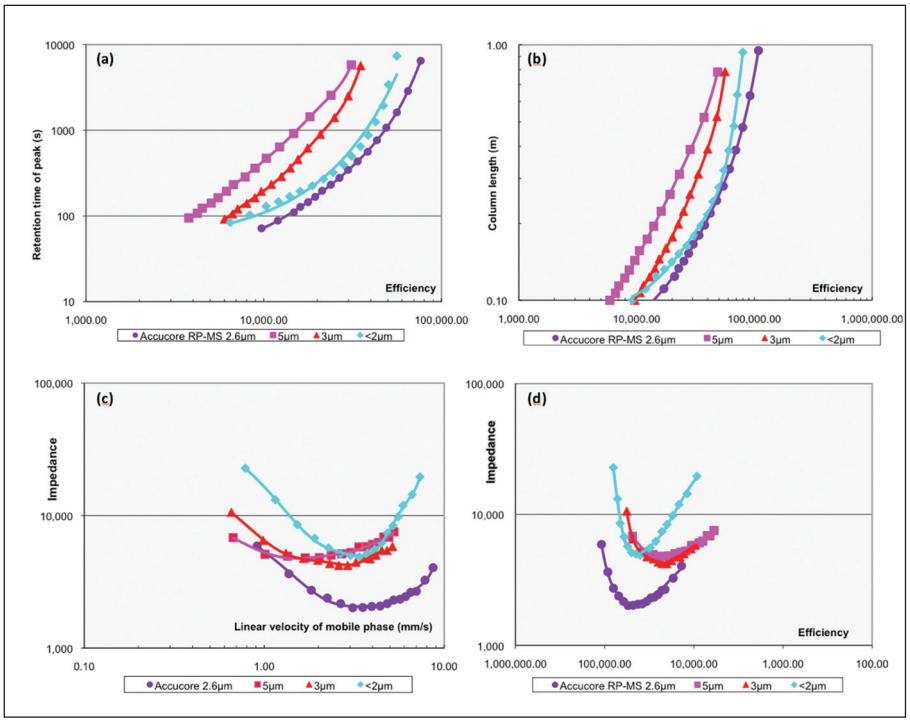


Figure 5: Performance comparison of Accucore 2.6µm and fully porous 5, 3 and sub-2µm using kinetic plots: (a) efficiency per unit time, (b) efficiency per column unit length, (c) impedance relative to mobile phase linear velocity, (d) impedance relative to efficiency.

Peak capacity, resolution and sensitivity

In isocratic separations efficiency, measured as plate height or number of theoretical plates, is used as a measure of chromatographic performance. However, this concept is not applicable to gradient elution. An alternative measure of separation efficiency is peak capacity, which is a concept first introduced by Giddings [5]. Peak capacity (Pc) represents the maximum number of components that can be chromatographically separated with unit resolution (Rs ~1) within a given time window (Δt) under a given set of experimental conditions. For a linear solvent strength gradient, the peak capacity, Pc, is given by Equation 7 [6].

Equation 7

$$P_c = 1 + \frac{\Delta t}{4\sigma_t R_s}$$

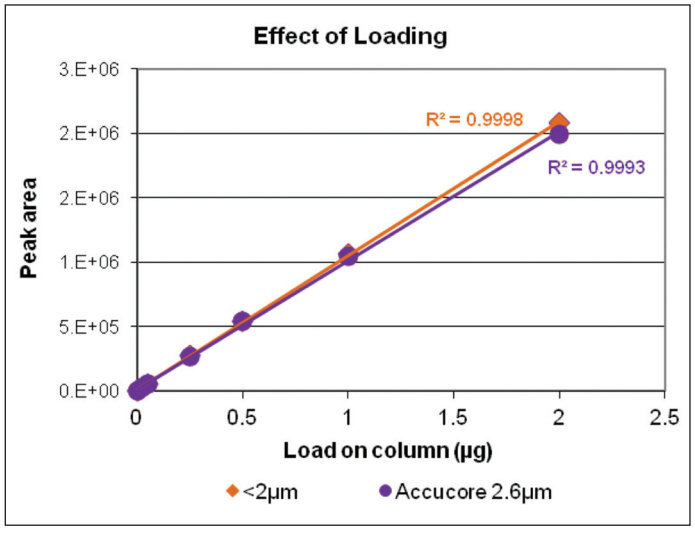
Δt – analysis time for which the peak capacity is calculated

σt – standard deviation of a peak
R_s – resolution

Equation 8

$$P_c = 1 + \frac{t_g}{w}$$

t_g – gradient time
W – peak width at baseline



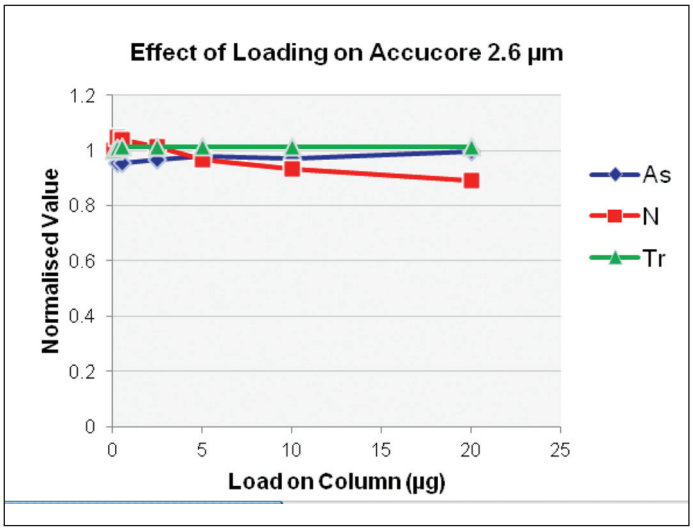


Figure 6: Effect of column loading on chromatographic parameters. (a) Comparison of loading 2µg on solid-core 2.6µm and fully porous sub-2µm; (b) Effect of loading on peak asymmetry (As), retention time (Tr) and efficiency (N) on the solid-core column.

	5µm	3µm	<2µm	Accucore 2.6µm
Resolution critical pair	2.57	3.26	4.39	3.87
Peak capacity	32	43	51	51
Signal-to-noise ratio	122	152	211	228
Pressure (bar)	31	67	268	133

Table 1. Comparison of the peak capacity, resolution of a critical pair, signal-to-noise ratio and column backpressure for a mixture of phenones on fully porous 5, 3, <2µm and solid-core 2.6µm particle packed 100x2.1mm columns.

However, in practice the peak capacity is generally calculated using gradient time and baseline peak width, and assuming constant peak width during the gradient run (Equation 8, [7]). The calculated peak capacities for a mixture of seven phenones (Table 1) are similar for the fully porous sub-2µm and solid-core materials and approximately 50% higher than that on the fully porous 5µm material, for the same column dimensions, run under the same conditions. As discussed above, solid-core materials suffer less diffusional effects (C- terms of the van Deemeter equation) and are more homogeneously packed (A- term of the van Deemeter equation) than fully porous materials of similar particle size. Therefore, peak widths are greatly reduced and peak capacities increased for this type of column packing material. The observed high peak capacity of the fully porous sub-2µm can be attributed to the small particle size (A- term of the van Deemter equation). Narrower chromatographic peak widths have other advantages, such as improved resolution and improved sensitivity. In practice, resolution is calculated by dividing the distance (in minutes) between peaks by the average width of those peaks (Equation 9). From Table 1 it is evident that the fully porous sub-2µm and the Accucore 2.6µm columns provide the highest resolution for the closely eluting compounds in the phenone mixture, 4.39 and 3.87 respectively. When analytes are eluted from the column in narrow chromatographic bands, or in other words in low volume peaks, the sensitivity of the analysis in increased as the solute mass is concentrated into a smaller volume. Table 1 also compares the signal-to-noise ratios (S/N) obtained under the same chromatographic conditions for 4 columns of the same dimensions, packed with 5, 3, sub-2µm fully porous and 2.6 µm solid-core particles. The highest S/N is observed for the latter material. This is particularly important in trace analysis, where a narrow peak is more likely to "appear" above the baseline noise.

Equation 9

$$R_s = \frac{2(t_2 - t_1)}{(w_1 - w_2)}$$

$\mathbf{R_s}$ – Resolution between a pair of peaks

$\mathbf{t_x}$ – retention time of peak x

$\mathbf{w_x}$- peak x width at baseline

Loading

Despite the low surface area characteristic of solid-core materials, the performance of Accucore is comparable to that of fully porous materials for the same sample loading. On Figure 6, the loading on Accucore is compared with that on a sub-2µm material. The plot of peak area as a function of the amount of solute loaded on the column (Figure 6a) shows a linear relationship for both the solid-core 2.6µm and the sub-2µm columns, with a high correlation coefficient (0.999) for both, which is indicative of no overload. Monitoring of peak asymmetry, efficiency and retention time at the peak apex as the loading on the solid-core column was increased revealed no significant change of the normalised values of asymmetry efficiency and retention time as a function of load on the column, Figure 6b. If the columns were mass overloaded there would be a loss of peak asymmetry and efficiency and a decrease in the retention time at the peak apex.

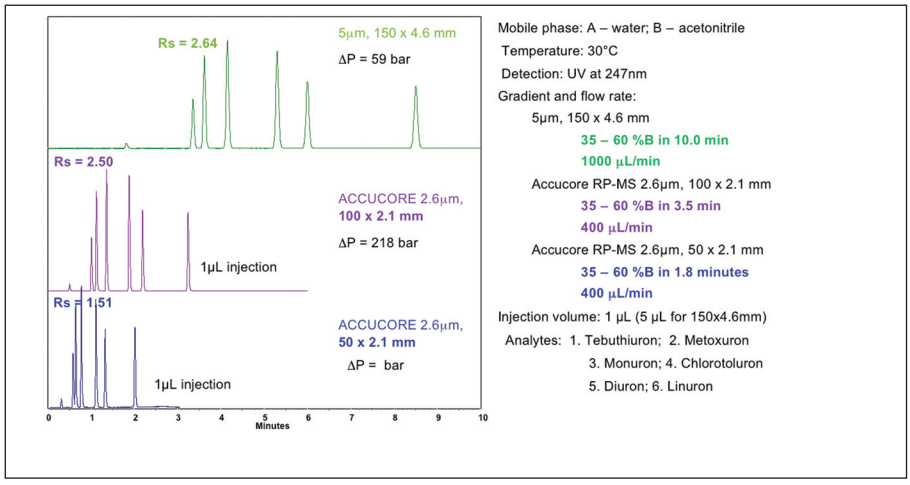


Figure 7. Example of method transfer from fully porous 5µm 150x4.6mm column to Accucore 2.6µm, 100x2.1 ID mm column (similar column chemistry)

Working with solid-core particle packed columns

Method transfer from 5 µm fully porous columns

There are several reasons for scaling down a method from a conventional 4.6mm ID column packed with fully porous 5 or 3µm particles to short, narrow-bore columns packed with fully porous sub-2µm or solid-core particles. As discussed above, fully porous sub-2µm and solid-core particles facilitate improvements in resolving power, sensitivity and peak capacity. Furthermore, reducing the column internal diameter also facilitates sensitivity improvements and shorter columns can often deliver the required resolution. Figure 3 demonstrated that columns packed with fully porous sub-2µm or solid-core particles are run at high linear velocities to achieve their optimal performance compared to equivalent columns packed with larger particles, therefore providing faster run times and increased sample throughput. The faster separations reduce the quantity of mobile phase per run compared with separations of the same efficiency with longer columns of larger particles. This has cost implications in terms of solvent consumption and also waste disposal and therefore significant savings can be achieved by scaling down methods. When transferring methods to fast LC, several approaches can be taken, depending on the analytical needs. If column dimensions are maintained and only particle size is reduced then an improvement in efficiency and, therefore, resolution, sensitivity and peak capacity is obtained. A second, more common approach is to reduce not only particle size but also column dimensions, which has the benefit of reducing analysis time.

In Figure 7, a gradient method run on a fully porous 5µm, 150 x 4.6mm ID column is

transferred to a Accucore 2.6µm, 100 x 2.1mm ID column, taking into account the difference in column volume and the optimal flow rate for the solid-core 2.6µm, 2.1mm ID column (from the van Deemter plot), specifically, the flow rate change takes into consideration the columns internal diameter and particle size. The gradient is scaled ensuring that the number of column volumes is kept constant. Injection volume is also scaled down proportionally to the reduction in column volume [8]. For the example in Figure 7, resolution of the critical pair is maintained (2.64 and 2.50), whilst reducing analysis time from 17 to 6 minutes (including column re-equilibration) and solvent consumption from 17mL to 2.4mL per run. Analysis time can be further halved by reducing the solid-core column length to 50 mm, which still provides baseline resolution of the critical pair (1.51).

Selectivity

The primary goal of developing a chromatographic separation is to resolve a mixture of analytes. So far in this paper the discussion has been focused on efficiency and the benefits this parameter can bring to the assay. However, from the general resolution equation it is evident that the selectivity parameter has the greatest impact on resolution. Selectivity can be changed by modification of the mobile phase composition, column chemistry or temperature.

Accucore columns are available in a series of chemistries to provide a wide range of selectivities for method development; these are:

- Optimised alkyl chain (RP-MS)
- C18
- Polar endcapped C18 (aQ)
- Phenyl-Hexyl
- Pentafluorophenyl (PFP)
- unbonded silica for HILIC.

To fully characterise the surface chemistry of the reversed-phase materials, a series of diagnostic chromatographic tests were used (based on those developed by Tanaka [9]). These tests characterise analyte/stationary phase interactions and combine probes to measure hydrophobicity, shape selectivity and secondary interactions with bases, acids and chelators. These tests are described here in Table 2-4.

The phase characterisation data obtained from these tests can be summarised in radar plots (Figure 8), which allow visual comparison of the overall selectivity of the different stationary phase chemistries. The hydrophobic retention and selectivity of the C18, RP-MS and aQ are comparable, and significantly higher that those of the PFP and Phenyl-Hexyl phases. The steric selectivity of the aQ phase is slightly higher than that of the C18 or RP-MS phase but considerable lower than that of the PFP phase, which shows the highest steric selectivity. The introduction of fluorine groups into the stationary phase causes significant changes in analyte-stationary phase interactions, which can produce high selectivity for

Parameter	Interaction investigated	Test molecules
HR	Hydrophobic retention is the retention factor of a hydrophobic hydrocarbon, pentylbenzene, which gives a broad measure of hydrophobicity of the ligand and its density.	Pentylbenzene
HS	Hydrophobic selectivity is the selectivity factor between pentylbenzene and butylbenzene and provides a measure of the surface coverage of the phase; these two alkylbenzenes differ by one methylene group and their selectivity is dependent on ligand density.	Butylbenzene Pentylbenzene
SS	Steric selectivity (SS) is the ability of the stationary phase to distinguish between molecules with similar structures and hydrophobicity but different shapes. The selectivity factor between o-terphenyl and triphenylene is indicative of steric selectivity as the former has the ability to twist and bend, while the latter has a fairly rigid structure and will be retained quite differently.	o-Terphenyl Triphenylene
HBC	Hydrogen bonding capacity (HBC) is the selectivity factor between caffeine and phenol, which provides a measure of the number of available silanol groups and the degree of endcapping.	Caffeine Phenol

Table 2. Hydrophobic tests

Parameter	Interaction investigated	Test molecules
IEX2.7	Ion-exchange capacity at pH 2.7 is estimated by the selectivity factor between benzylamine and phenol, at pH 2.7. Tanaka [7] showed that the retention of protonated amines at pH < 3 could be used to get a measure of the ion exchange sites on the silica surface. Silanol groups (Si-OH) are undissociated at pH < 3 and therefore cannot contribute to the retention of protonated amines, but the acidic silanols in the dissociated form (SiO-) can. The latter contribute to the retention of the protonated amines.	Benzylamine Phenol
AI	The capacity factor and tailing factor of chlorocinnamic acid are also measured to test the applicability of the stationary phase acidic interactions.	4-Chlorocinnamic acid

Table 3. Secondary interactions and ion exchange tests at low pH

Parameter	Interaction investigated	Test molecules
IEX7.6	Ion-exchange capacity at pH 7.6 is estimated by the selectivity factor between benzylamine and phenol and is a measure of the total silanol activity on the surface of the silica. At pH > 7 the silanol groups are dissociated and combine with the ion exchange sites to influence the retention of benzylamine.	Benzylamine Phenol
C	Silica surface metal interactions can cause changes in selectivity and peak shape for analytes which are able to chelate. Changes in the capacity factor and tailing factor of quinizarin, which is a chelator, are indicative of secondary metal interactions.	Quinizarin
BA	The presence of dissociated silanols at pH>7 can cause poor peak shapes of protonated basic compounds such as amitriptilyne. Secondary ion exchange and silanolic interactions can cause shifts in retention and asymmetrical peaks. The capacity factor and tailing factor of amitriptyline are indicative of the overall performance of the column.	Amitriptyline

Table 4. Secondary interactions and ion exchange tests at high pH

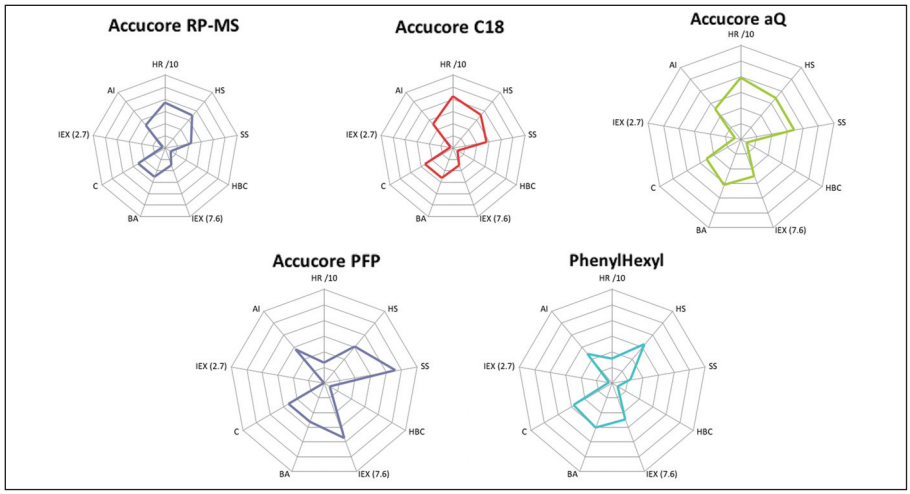


Figure 8: Radar plots for Accucore stationary phases: comparison of the phase selectivities. Tables 2, 3 and 4 for axis labels.

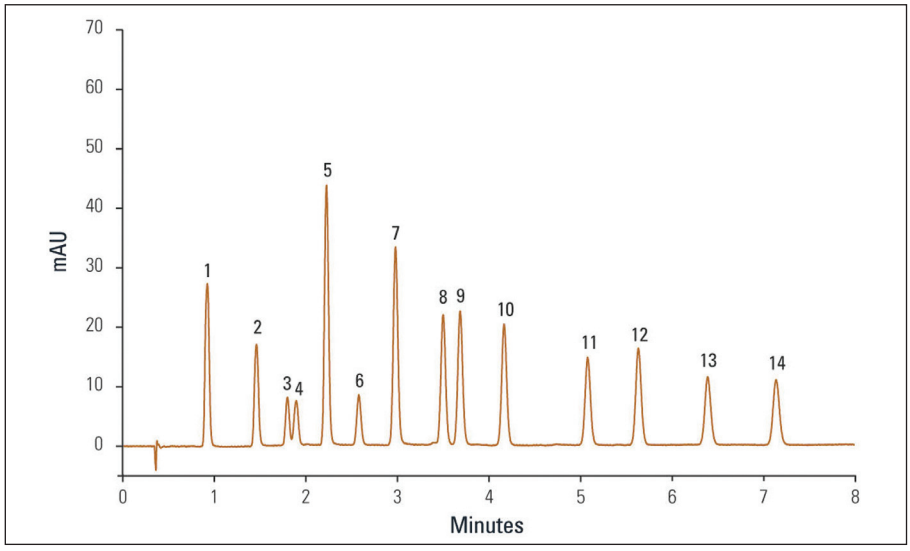


Figure 9: Separation of 14 positional isomers on Accucore PFP. Experimental conditions: Column - Accucore PFP 2.6µm, 50mm x 2.1mm; Mobile phase: A – Water + 0.1% Formic Acid, B – Acetonitrile + 0.1% Formic Acid; Gradient: 15 – 30% B in 7 minutes; Flow rate: 600µL/min; Temperature: 50°C; Detection: UV at 270nm; Injection volume: 2µL. Analytes: 1. 3,4 – Dimethoxyphenol; 2. 2,6 – Dimethoxyphenol; 3. 2,6 – Difluorophenol; 4. 3,5 – Dimethoxyphenol; 5. 2,4 – Difluorophenol; 6. 2,3 – Difluorophenol; 7. 3,4 – Difluorophenol; 8. 3,5 – Dimethylphenol; 9. 2,6 – Dimethylphenol; 10. 2,6 – Dichlorophenol; 11. 4 – Chloro-3-Methylphenol; 12. 4 – Chloro-2-Methylphenol; 13. 3,4 – Dichlorophenol; 14. 3,5 – Dichlorophenol.

positional isomers of halogenated compounds (Figure 9). The Phenyl-Hexyl phase offers a mixed mode separation mechanism, with the C6 chain responsible for hydrophobic interactions and the phenyl ring responsible for π-π interactions. The HILIC stationary phase provides an approach for the retention of very polar compounds via a retention mechanism that involves partitioning, hydrogen bonding and weak electrostatic interactions [10]. For an example HILIC separation, see Figure 10.

System considerations

One of the great advantages of solid-core particle packed columns is that the backpressures produced often allow the use of standard HPLC instrumentation. However, the LC system needs to be optimised in order to produce efficient chromatography. In particular, system volumes (connecting tubing ID and length, injection volume, flow cell volume in UV) must be minimised, detector time constant and sampling rate need to be carefully selected, and when running fast gradients pump delay volume needs to be minimal. Failure to consider the parameters may result in loss of the efficiency gained by using the solid-core particles [11].

Band broadening, which has a detrimental effect on the chromatographic performance, can be caused by high sample volume, it can occur in the tubing connecting the column to injector and detector and in the detector flow cell. These band broadening effects

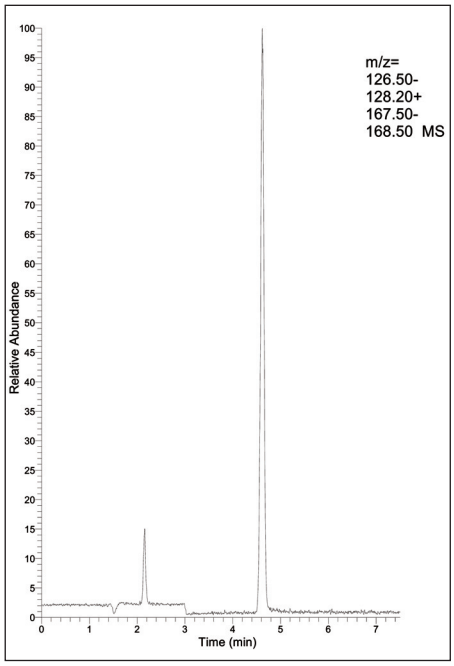


Figure 10. Separation of melamine and cyanuric acid on Accucore HILIC. Experimental conditions: Column Accucore HILIC 2.6µm, 150mm x 4.6mm; Mobile phase: 90:10 (v/v) Acetonitrile:50mM Ammonium Acetate, pH 5; Flow rate: 1mL/min; Temperature: 40°C; Detection: MS at m/z 127, 128, 168 (negative mode 0-3 mins, positive mode 3-10 mins); Injection volume: 5µL; Backpressure: 117 bar; Analytes: Cyanuric Acid: m/z 128.1 (-1) Melamine: m/z 127.1 (+1), 168.1 (+1 with Acetonitrile adduct).

which occur in the fluidic path of the HPLC instrument are volumetric effects. Each contributes an additive variance to the width of the chromatographic band. In general, the extra column band broadening, covering the injection volume, flow cell volume and tubing volume should not exceed 10% of the total band broadening. The extra column effects are more significant for scaled down separations (as column volume decreases) and for less retained peaks which have a lower peak volume. It is therefore critical to minimise extra column dispersion if high efficiency separations are required. In addition to the volumetric effects, the time constant of the detector (response rate) and the scan rate may also contribute to the broadening of the peak, and should be considered. With solid-core particles peaks may be of the order of 1-2 seconds in width. It is important to scan the detector quickly enough to achieve optimum peak definition, otherwise resolution, efficiency and analytical accuracy will be compromised. This is illustrated in Figure 11, which clearly shows a loss of peak height and area when less than ten data points are taken across the width of the peak. For fast gradients it is also important to minimise the pump dwell volume to ensure that there in no delay in

delivering the gradient to the column. For instance for a pump with 800µL dwell volume, running at 400µL/min flow rate, it will take two minutes for the gradient to reach the head of the column. Conversely, a pump with 80µL dwell volume, running at the same flow rate, will deliver the gradient to the head of the column in 0.2 minutes.

Solid-core particle packed columns robustness

The robustness and reproducibility of a chromatographic separation is dependent on the column stability and lifetime but also on operational parameters such as mobile phase pH, temperature and sample cleanliness. Common causes of column instability can be either chemical or physical. For instance, use of extremes of pH in the mobile phase can lead to degradation of the column through chemical attack of the bonded stationary phase or dissolution of the base silica. Another aspect of column stability is the ability of the packed bed to resist pressure changes such those experienced inline sample preparation techniques such as TurboFlow chromatography.

The tight control of the particle size distribution on solid-core materials allows for highly uniform and mechanically stable packed beds which can withstand a very high number of injections. The robustness of the bonded phase will determine the column's stability under different mobile phase pHs and temperature. At low mobile phase pH, the bonded phase can be lost through hydrolysis of the organosilane bond and at high pH the mobile phase can dissolve the silica support resulting in collapse of the stationary phase. The advanced bonding technology used for Accucore columns generates robust bonded phases that are resistant to extremes of pH and also temperature. Figures 12 and 13 demonstrate Accucore C18 column stability at pH 1.8 and 10.5 respectively. Over 30,000 column volumes of mobile phase were run through the column in each instance using a gradient method which is equivalent to 5.5 days of continuous operation. Monitoring of capacity factor of the test mixture components over this period reveals no loss of retention for any of the analytes, which would be expected if bonded phase cleavage had occurred. The pH range for the RP-MS and aQ phases is 2 – 9 and 2 – 8 for the Phenyl-Hexyl, PFP and HILIC phases.

Most LC separations are performed at 25 to 40°C, however, temperature is a useful method development parameter. The use of higher temperatures has advantages: mass transfer is improved because analyte diffusivity is increased, thus the peaks obtained are sharper, which provides better peak height and therefore better signal-to-noise ratio, improving the sensitivity of the analysis. Also at high temperatures, solvent viscosity is lower, which allows the use of higher flow rates to increase speed, without loosing efficiency. One limiting factor is column stability, where thermal degradation of the bonded surface

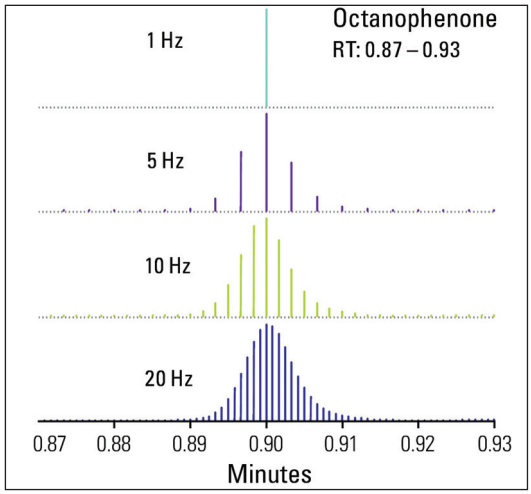


Figure 11: Effect of detector sampling rate on the peak height and peak area.

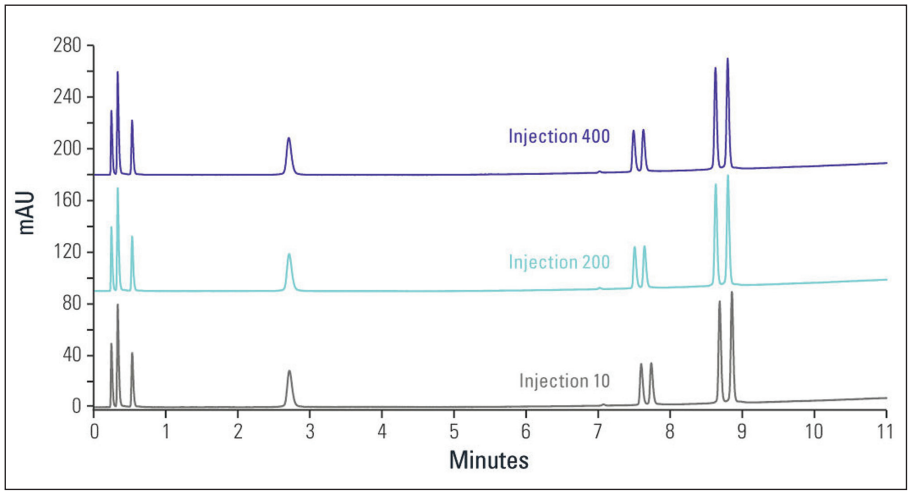


Figure 12: Accucore column stability at pH 1.8. Experimental conditions: Column - Accucore C18 2.6µm, 100 x 2.1mm; Mobile phase: A – Water + 0.1% Trifluoroacetic Acid, B – Methanol + 0.1% Trifluoroacetic Acid; Gradient: 25%B for 0.75 min, then to 100%B by 10 min, hold at 100%B for 2 min, return to 25%B and hold for 5 min for re-equilibration; Flow rate: 400µL/min; Injection volume: 1µL; Temperature: 30°C; Detection: UV at 254nm (0.1s rise time, 20Hz); Order of elution: 1. Uracil (t0), 2. Acetaminophen, 3. p-Hydroxybenzoic acid, 4. O-Hydroxybenzoic acid, 5. Amitriptyline, 6. Nortriptyline, 7. Di-isopropyl phthalate, 8. Di-n-propyl phthalate.

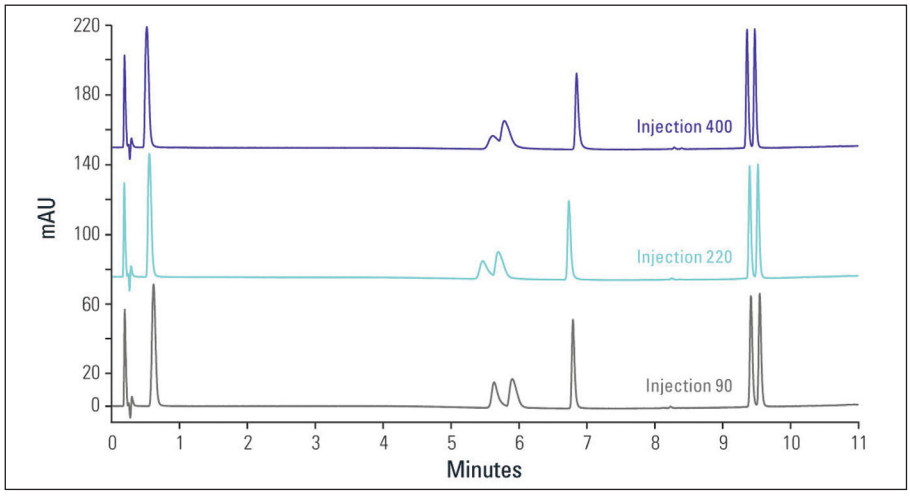


Figure 13: Accucore column stability at pH 10.5. Experimental conditions: Column - Accucore C18 2.6µm, 100 x 2.1m; Mobile phase: A – Water + 0.1% Ammonia, B – Methanol + 0.1% Ammonia; Gradient: 15%B for 1 min, then to 100%B by 8 min, hold at 100%B for 3 min, return to 15%B and hold for 5 min for re-equilibration; Flow rate: 400µL/min; Injection volume: 1µL; Temperature: 30°C; Detection: UV at 254nm (0.1s rise time, 20Hz); Order of elution: 1. Uracil (t0), 2. 4-Chlorocinnamic acid, 3. Procainamide, 4. 4-Pentylbenzoic Acid, 5. N-Acetylprocainamide, 6. Di-isopropyl phthalate, 7. Di-n-propyl phthalate.

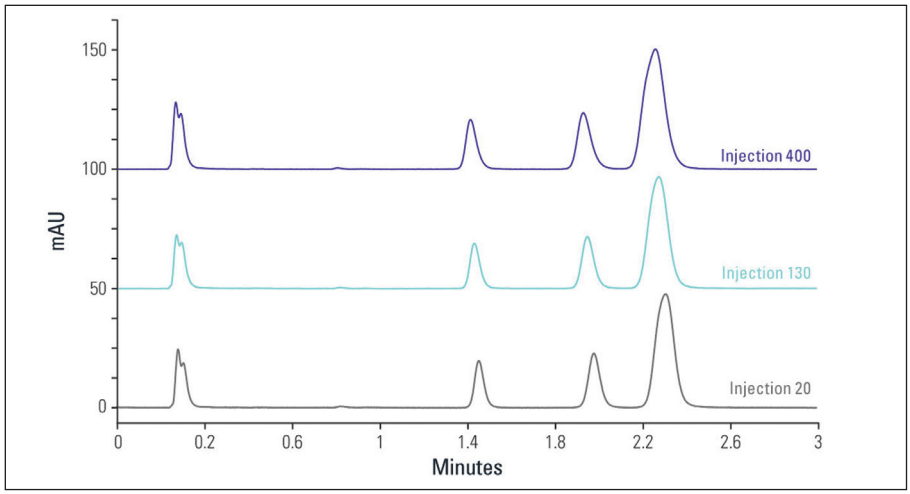


Figure 14: Accucore column stability at 70° C. Experimental Conditions: Column - Accucore C18 2.6µm, 100 x 2.1mm; Mobile phase: 35:65 (v/v) Water/Methanol; Flow rate: 400µL/min; Injection volume: 1.5µL; Temperature: 70°C; Detection: UV at 254nm (0.1s rise time, 20Hz); Order of elution: 1. Theophylline/Caffeine (t0), 2. Phenol, 3. Butylbenzene, 4. o-Terphenyl, 5. Pentylbenzene/Triphenylene.

can occur. Figure 14 demonstrates the stability of the Accucore C18 column at 70°C, where it can be seen that even with 400 injections there is no loss of performance at these elevated temperatures with a water/methanol mobile phase. The stability of these columns at 70°C under more aggressive mobile phase conditions (for instance, pH extremes) has not been tested to date.

Conclusion

The data presented in this article illustrates solid-core chromatographic supports exhibit less band broadening through eddy diffusion and resistance to mass transfer than fully porous chromatographic supports. As a result, solid-core columns exhibit higher efficiency than fully porous columns and a lower rate of efficiency loss with linear velocity. From the columns compared in this study, the Accucore 2.6µm material is the most efficient per unit length of column and the most efficient per unit time, with the fully porous sub-2µm performing similarly. Impedance is a term that defines the resistance a compound has to move down the column relative to the performance of that column. Sub-2µm fully porous and solid-core materials show similar values of efficiency, however the impedance is directly proportional to the pressure drop across the column. Solid-core particle packed columns show a pressure drop that can be half or even less that of a fully porous sub-2µm particle packed columns. Therefore, Accucore columns provide higher efficiency (more resolving power) than fully porous columns for the same nominal pressure (or 'bar for bar') and can, in most cases, be used in conventional HPLC instrumentation. The higher efficiencies of solid-core columns result in reduced peak widths and increased peak capacities. Narrower chromatographic peak widths have advantages such as improved resolution and improved sensitivity (better signal-to-noise ratios), particularly important in trace analysis and impurity profiling.

Column selectivity is still the most effective way of controlling resolution of a chromatographic separation. To make solid-core columns a serious contender in the analytical laboratory, manufacturers need to ensure these materials are available in a range of stationary phase chemistries for method development.

Band broadening in the column is significantly reduced with the solid-core chromatographic supports. However, in order to fully harvest this gain, extra column band broadening needs to be considered and minimised through consideration of system volume and optimisation of detector acquisition parameters. Whereas UHPLC equipment has been designed to have reduced system volume, conventional HPLC equipment often needs some attention when operating with high performance columns such as the solid-core materials.

Columns used in fast, high efficiency separations are often stressed considerably through chemical and physical operating parameters. The ruggedness and durability of these materials needs to equal or exceed that of traditional HPLC columns. The robustness of Accucore columns under pH extremes and elevated temperature was demonstrated in this paper.

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AR20526_E 06/12S

Equivalent Performance to Sub-2 µm with Lower Pressure

With solid core design, tight particle size distribution and uniform packed bed Accucore HPLC columns have broadly equivalent performance to sub-2 µm columns and yet generate only a fraction of the backpressure.

Lower backpressure eliminates the requirement for UHPLC systems with maximum pressure ratings >600 bar. If a UHPLC system is used then the lower backpressure reduces wear on the instrument.

What Pressure to Expect from the Thermo Scientific Accucore HPLC Columns?

Luisa Pereira, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words

Fast LC, Pressure, Solid Core Particles, Sub-2 µm Particles, Core Enhanced Technology

Abstract

This technical note discusses the effect that column length, internal diameter (ID), particle size, and mobile phase flow rate and viscosity have on the operating pressure in HPLC. Comparison data on the measured pressure for solid core and fully porous particle packed columns (5, 3 and sub-2 µm) is shown.

Introduction

Most stationary phases currently used for fast HPLC have a fully porous particle support, with diameters in the sub-2 µm region. The small particle diameter improves the separation kinetics and therefore efficiency, but at the expense of operating backpressure. Additionally, sub-2 µm particle packed columns are generally run at high linear velocities as these produce higher efficiencies. Consequently, the HPLC equipment has to be able to operate at pressures in excess of the conventional 400 bar, unless very short column lengths (<50 mm) are used. Partially porous particles, with a diameter between 2 and 3 µm, provide similar performance to sub-2 µm particles at significantly lower column backpressures. The Thermo Scientific™ Accucore™ HPLC column range uses Core Enhanced Technology™ to produce a 2.6 µm solid-core material with a very tight particle size distribution. This results in columns with high permeability, and therefore "bar for bar" Accucore columns produce improved separations when compared to fully porous materials.

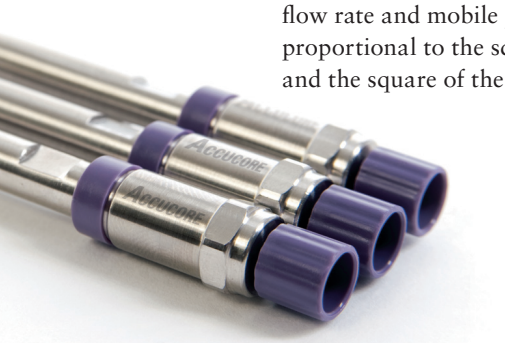
Equation 1 shows the dependency of pressure drop across the column on particle size and flow rate, discussed above. Pressure is directly proportional to column length, flow rate and mobile phase viscosity and inversely proportional to the square of the particle size diameter and the square of the column internal diameter.

Equation 1: $$\Delta P = 236 \frac{(1-\varepsilon_i)^2}{\varepsilon_i^3} \frac{F L \eta}{d_c^2 d_p^2}$$

Where:

ΔP – pressure drop across the column
ε_i – interstitial porosity of the packed bed
F – flow rate through the column
L – length of the column
η – viscosity of the mobile phase
d_p – particle diameter
d_c – column internal diameter

Other operating parameters that will have an impact on pressure are the ID and length of the connecting tubing in the LC system, detector set-up parameters such as flow cell volume in UV or the ID and length of the capillary components in ESI or APCI sources in LC/MS. Pressure can be a useful symptom when troubleshooting LC systems.



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Comparison of Column Pressure for Accucore 2.6 µm and Fully Porous 5, 3 and Sub-2 µm

From equation 1, it is clear that reducing the particle size in the column significantly increases the observed pressure drop across the column. The data in Figure 1 was generated on 100 × 2.1 mm columns, using a mixture (1:1) of acetonitrile and water, at 30 °C column temperature and running flow rates in the range 0.1 to 1 mL/min. The pressure measured on the Accucore 2.6 µm column is approximately half of that on the sub-2 µm particle packed column and double that of the 3 µm particle packed column. Based on particle size only, and given that the pressure drop across the column is inversely proportional to the square of the particle diameter, the ratio between the measured pressure on the sub-2 µm and Accucore columns should be 1.9, and between the 3 µm and Accucore should be 1.3. However, column permeability also depends on the interstitial porosity (as indicated in equation 1) and this parameter accounts for the observed differences in measured pressure ratios versus those predicted based only on particle size.

Chromatographic systems that have the conventional pressure limit of 400 bar will reduce the effective flow rate range that can be used on a column packed with small particles. On standard HPLC systems, sub-2 µm particle packed columns can only be run at reduced flow rates, often below the flow rate that provides the best performance. However, the Accucore column used in this comparison can be operated at 800 µL/min, double the optimal flow rate, before it experiences the same issues.

Effect of Column Length and Column ID on Pressure

Pressure is directly proportional to column length. The data in Figure 2 was obtained when Accucore columns with length of 30, 50, 100, 150 and 200 mm were run at 400 µL/min with a mobile phase of water/acetonitrile (1:1) and the measured pressure matches well with the predicted values.

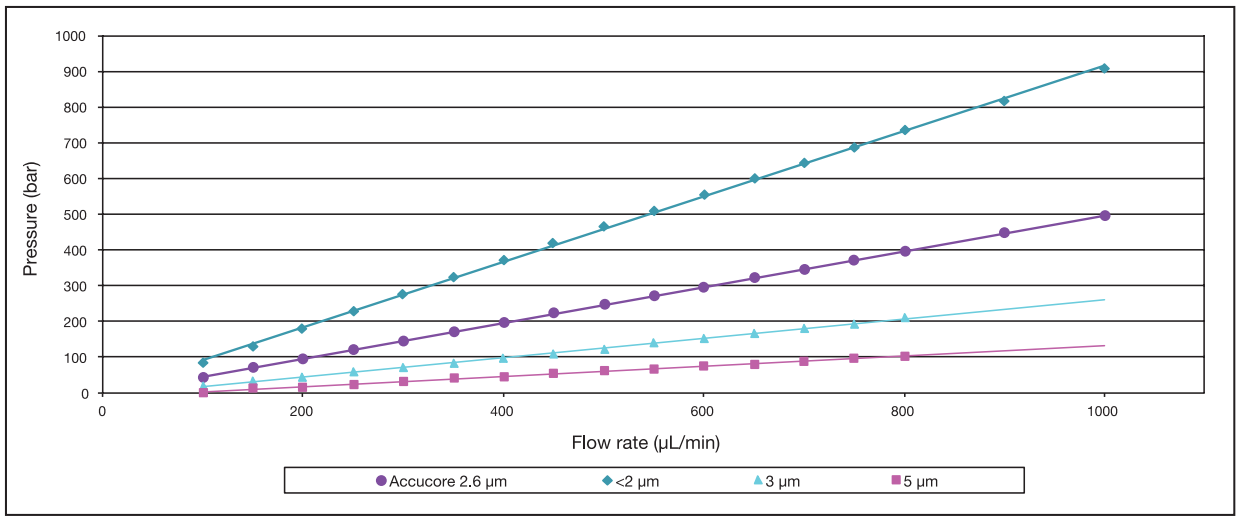

Figure 1: Comparison of column pressure for Accucore 2.6 µm and fully porous 5, 3 and sub-2 µm. Columns: 100 × 2.1 mm; mobile phase: water/acetonitrile (1:1); temperature: 30 °C; flow rate: 0.1 to 1.0 mL/min.

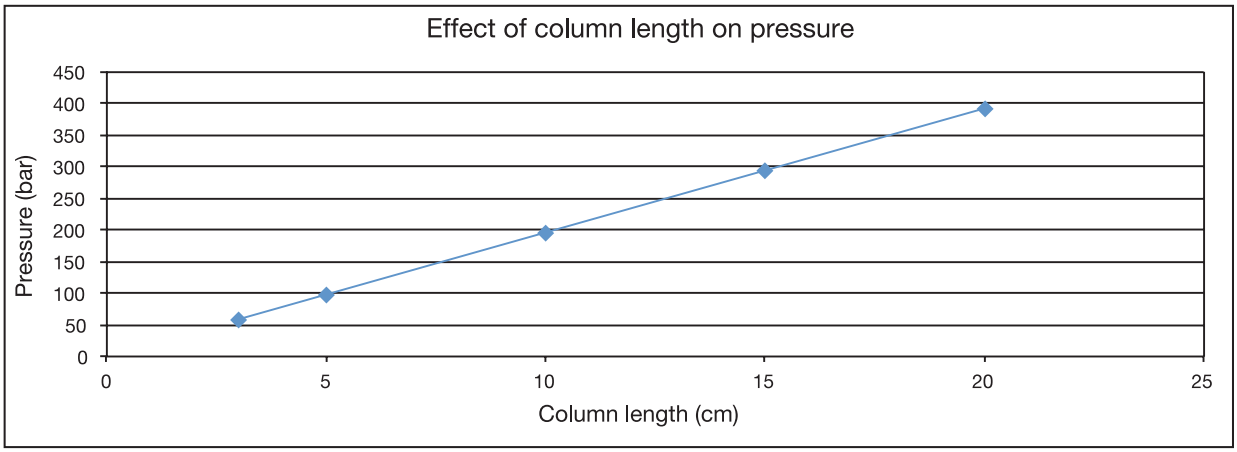

Figure 2: Pressure drop across Accucore 2.6 µm columns of different lengths, at a flow rate of 400 µL/min, mobile phase of water/acetonitrile (1:1) and temperature of 30 °C.

From Equation 1, pressure is inversely proportional to the square of the column ID and therefore decreasing the column ID results in a significant increase in pressure. In practical terms however, if the column is run at a typical linear velocity, the pressure measured will also be typical for that system set-up. Mobile phase linear velocity is the flow rate normalized for the column cross-section.

For instance, if a method is transferred from a 4.6 to a 2.1 mm ID column, all other operating parameters kept unchanged, and the 2 columns are run at the same linear velocity, then the measured pressure drop across both columns will be the same.

Effect of Mobile Phase Viscosity on Pressure

Column operating pressure is affected by the mobile phase composition. Viscosity is a property of each solvent, which varies with temperature. The proportion the solvent is mixed with other mobile phase components and the operating temperature will determine the mobile phase viscosity. The pressure drop across the column itself will also have an effect on the viscosity since it affects the effective column temperature. Figure 3 shows how water viscosity varies with the addition of aceto-nitrile or methanol. Water/methanol mixtures are more viscous than water/acetonitrile mixtures and therefore using methanol as the organic modifier in reversed-phase LC produces higher pressure drops across the column. When mobile phase gradients are used the mobile phase composition and therefore the viscosity changes during the run, which results in a change of pressure during the chromatographic run.

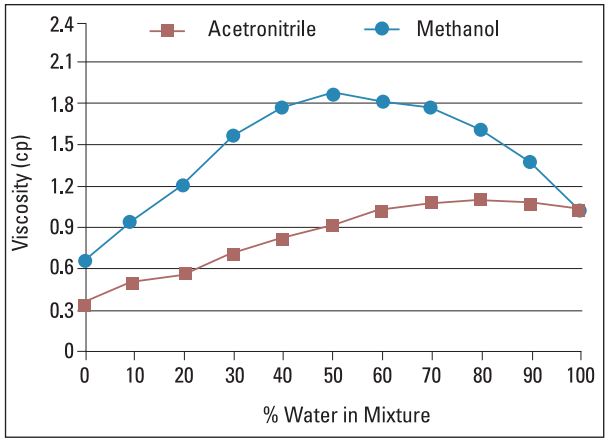

Figure 3: Mobile phase viscosity changes with the composition. Water/methanol mixtures can be up to 80% more viscous than water/acetonitrile mixtures.

Conclusion

- Pressure is dependent on column length, ID, and particle size
- Pressure is dependent on mobile phase flow rate and viscosity
- Accucore 2.6 µm particle packed columns show approximately half of the pressure of a sub-2 µm fully porous particle packed column and approximately double that of a 3 µm fully porous particle packed column
- Accucore columns can be run at high flow rates on conventional HPLC equipment and are rated to 600 bar

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Optimum Selectivity

Accucore based on 2.6 µm particles is available in fourteen different phases to provide an unrivalled range of selectivities.

Each of the bonded phases is manufactured using advanced bonding technology and is characterized using a testing regime based on the Tanaka Tests.

T1: Hydrophobic Interactions

			Parameter	Term
	HR	Hydrophobic Retention	Retention of compounds based on their hydrophobicity	k'
	HS	Hydrophobic Selectivity	Separation of compounds that have similar structure, but differ slightly in hydrophobicity	α
	SS	Steric Selectivity	Separation of compounds that have similar structure, but differ in shape	α
	HBC	Hydrogen Bonding Capacity	Separation related to degree of end capping	α

T2: Secondary Interactions Under Neutral pH

			Parameter	Term
	BA	Base Activity	Peak shape for basic analytes resulting from total silanol activity (all dissociated at pH 7.6)	t_f
	C	Chelation	Peak shapes for chelating analytes resulting from silica metal content	t_f
	IEX(7.6)	Ion Exchange Capacity (pH 7.6)	Separation between basic and neutral compounds resulting from total silanol activity (all dissociated at pH 7.6)	α

T3: Secondary Interactions Under Acidic pH

			Parameter	Term
	AI	Acid Interaction	Interactions resulting in poor peak shape for acidic analytes	t_f
	IEX(2.7)	Ion Exchange Capacity (pH 2.7)	Separation between basic and neutral compounds resulting from acidic silanol activity	α

Technical Note 20788

Comparison of the Reversed-Phase Selectivity of Solid Core HPLC Columns

M. Dolci, L. Pereira, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words

Accucore, solid core, column characterization, selectivity, primary interactions, secondary interactions, Core Enhanced Technology, radar plots

Abstract

The selectivities of Thermo Scientific™ Accucore™ C18 and RP-MS phases are compared to six other solid core reversed-phase stationary phases. The retention properties of the stationary phases were categorized by analyzing primary modes of interaction (hydrophobicity, steric selectivity, and hydrogen bonding) and secondary modes of interaction (ion exchange and chelation).

Introduction

Accucore HPLC columns are based on Core Enhanced Technology™, which features solid core materials with a very tight particle size distribution and advanced bonding technology to functionalize the surface. The particles in the Accucore stationary phases can be described as a solid silica core surrounded by a porous outer layer. The very tight particle size distribution of these materials results in columns with high permeability. Therefore, for the same nominal pressure, Accucore particles provide better separations than fully porous materials.

In this technical note the retention properties of Accucore C18 and Accucore RP-MS (an optimized alkyl chain length bonded phase) materials are compared to other solid core reversed-phase C18 materials. To fully characterize the surface chemistry of the reversed-phase materials, a series of diagnostic chromatographic tests were used (based on those developed by Tanaka [1]). These tests characterize analyte/stationary phase interactions and combine probes to measure hydrophobicity, shape selectivity, hydrogen bonding, and secondary interactions with bases, acids, and chelators. The results from this characterization study will help users to select the best phase for their separations. These tests are described in Tables 1 to 3.

The phase characterization data obtained were summarized in radar plots, which allow visual comparison of the overall selectivity of the different stationary phase chemistries. Radar plots, also known as spider or star charts (because of their appearance), plot the values of each category along a separate axis that starts in the center of the chart and ends on the outer ring.

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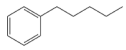
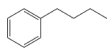
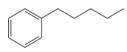
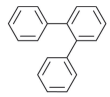
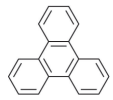
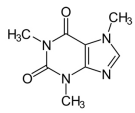
Parameter	Interaction Investigated	Test Molecules
HR	**Hydrophobic retention** is the retention factor of a hydrophobic hydrocarbon, pentylbenzene, which gives a broad measure of retention for a particular molecule. This is based on the available surface area, the ligand, and its density.	Pentylbenzene 
HS	**Hydrophobic selectivity** is the selectivity factor between pentylbenzene and butylbenzene and provides a measure for the selectivity of two molecules based on their hydrophobicity. It is affected by the surface coverage of the phase and the ligand density.	Butylbenzene  Pentylbenzene 
SS	**Steric selectivity** is the ability of the stationary phase to distinguish between molecules with similar structures and hydrophobicity but different shapes. The selectivity factor between o-terphenyl and triphenylene is indicative of steric selectivity, as the former has the ability to twist and bend, while the latter has a fairly rigid structure and will be retained quite differently. More rigid ligands will in general give greater selectivity.	o-Terphenyl  Triphenylene 
HBC	**Hydrogen bonding capacity** is the selectivity factor between caffeine and phenol, which provides a measure of the degree of hydrogen bonding a molecule will experience. Caffeine is a good hydrogen bonder and increasing the number of available silanol groups will increase the retention of the caffeine molecule but not the phenol molecule. This term is affected by the total number of silanols, which in turn is affected by the total surface area and also the degree of endcapping.	Caffeine  Phenol 

Table 1: Hydrophobic tests

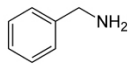
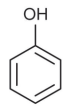
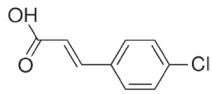
Parameter	Interaction Investigated	Test Molecules
IEX2.7	**Ion-exchange capacity at pH 2.7** is estimated by the selectivity factor between benzylamine and phenol, at pH 2.7. Tanaka [1] showed that the retention of protonated amines at pH < 3 could be used to get a measure of the ion exchange sites on the silica surface. Silanol groups (Si-OH) are undissociated at pH < 3 and therefore cannot contribute to the retention of protonated amines, but the acidic silanols in the dissociated form (SiO^-) can. Acidic silanols can be formed with the addition of impurities and also the different forms (in order of acidity: geminyl, bridged, vicinyl, metallic forms) of the silanols moiety. The more acidic silanols contribute to the retention of the protonated amines.	Benzylamine  Phenol 
AI	The capacity factor and **tailing factor of chlorocinnamic acid** are also measured to test the applicability of the stationary phase towards acidic interactions. These interactions are due to impurities in the substrate material and also to certain ligands that are used.	4-Chlorocinnamic acid 

Table 2: Secondary interactions and ion exchange tests at low pH

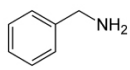
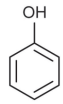
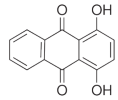
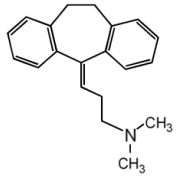
Parameter	Interaction Investigated	Test Molecules
IEX7.6	**Ion-exchange capacity at pH 7.6** is estimated by the selectivity factor between benzylamine and phenol and is a measure of the total silanol activity on the surface of the silica. At pH > 7 the silanol groups are fully dissociated and combine with the ion exchange sites to influence the retention of benzylamine.	Benzylamine  Phenol 
C	Silica surface metal interactions can cause changes in selectivity and peak shape for analytes which are able to chelate. Changes in the capacity factor and **tailing factor of quinizarin**, which is a chelator, are indicative of secondary metal interactions.	Quinizarin 
BA	The presence of dissociated silanols at pH > 7 can cause poor peak shapes of protonated basic compounds such as amitriptyline. Secondary ion exchange and silanolic interactions can cause shifts in retention and asymmetrical peaks. The capacity factor and **tailing factor of amitriptyline** are indicative of the overall performance of the column.	Amitriptyline 

Table 3: Secondary interactions and ion exchange tests at neutral pH

Characterization tests

Material	Particle Diameter	Pore Diameter	Bonded Phase	Dimensions
Accucore	2.6 µm	80 Å	RP-MS	100 × 2.1 mm
Accucore	2.6 µm	80 Å	C18	100 × 2.1 mm
Kinetex®	2.6 µm	100 Å	C18	100 × 2.1 mm
Poroshell® 120	2.7 µm	120 Å	SB-C18	100 × 2.1 mm
Ascentis® Express	2.7 µm	90 Å	C18	100 × 2.1 mm
Halo®	2.7 µm	90 Å	C18	100 × 2.1 mm
Nucleoshell®	2.7 µm	90 Å	RP C18	100 × 2.0 mm
SunShell®	2.6 µm	90 Å	C18	100 × 2.1 mm

Table 4: Columns tested

Experimental Conditions	Hydrophobic Test	Low pH Test	Neutral pH Test
Mobile phase	65:35 (v/v) methanol /water	45:55 (v/v) methanol/ 10 mM phosphate buffer pH 2.7	80:20 (v/v) methanol/ 10 mM phosphate buffer pH 7.6
Flow rate (mL/min)	0.55	0.55	0.55
Column temperature (°C)	40	40	40
Detection	UV at 254 nm	UV at 254 nm	UV at 254 nm
Injection volume (µL)	1	1	1

Table 5: Experimental conditions

Selectivity comparison

Figures 1 to 3 give examples of the chromatograms from the three tests on the Accucore RP-MS column, demonstrating typical elution order for the test probes in each test. Figure 4 provides an overview of the relative hydrophobic retention (HR) and steric selectivity (SS) of all the phases compared in this document. Accucore RP-MS, Poroshell 120 SB-C18, and Kinetex C18 phases all exhibit similar HR. HR is significantly higher on SunShell C18, Nucleoshell RP-C18, and Halo C18 phases. The latter exhibits the highest hydrophobic retention of all the phases tested. The phase that exhibits the highest steric selectivity is Accucore C18 (SS 1.4). Accucore RP-MS, Poroshell 120 SB-C18, and Kinetex C18 phases all exhibit similar steric selectivity (SS around 1.1); for the other four phases, SS varies between 1.17 and 1.30. Therefore, out of all the phases tested, Accucore C18 provides the best balance between hydrophobic retention, and thus retention time, and steric selectivity, which is a measure of the ability of the phase to separate compounds with similar structures.

From Figure 5, we can observe that the hydrophobic selectivity (HS) is comparable for all the materials. The hydrogen bonding capacity (HBC) is very low for all the materials assessed in this study, providing evidence that all the phases are effectively endcapped.

The activity towards bases (BA, tailing factor of amitriptyline) is comparable for most materials (average of 1.3), but slightly higher for SunShell C18 (1.6), which is evidence of the presence of dissociated silanols, which interact with protonated bases. The activity towards chelators (C) is relatively low, reflected by an average tailing factor for quinizarin of 1.5; however, the quinizarin tailing factor values for Poroshell 120 SB-C18 and SunShell C18 phases are 2.0 and 1.9, respectively, which demonstrate higher metal contents on the silica supports in these phases. The ion exchange capacity at pH 7.6 is very low for Accucore C18 and RP-MS, Halo C18, Ascentis Express C18, and SunShell C18 columns (IEX 7.6 of 1.0) but high for Poroshell 120 SB-C18 (IEX 7.6 of 1.6), highlighting a higher silanolic activity on the surface of the silica. In contrast, there is no evidence of dissociated acidic silanols on any of the phases tested, since the values of IEX 2.7 are very low for all phases. The activity towards acids (AI, tailing factor for chlorocinnamic acid) is similarly low for all the columns (average value 1.2), with the exception of SunShell, which has a value of 1.5.

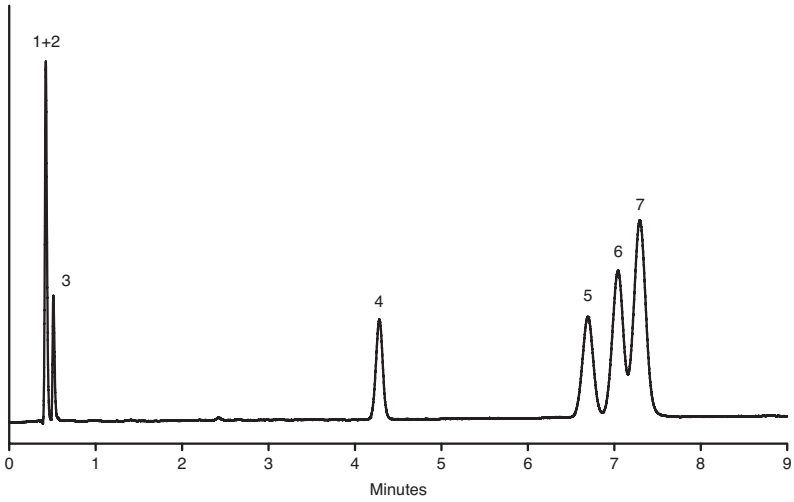


Figure 1: Example chromatogram for the hydrophobic interactions test on the Accucore RP-MS column
1. Theophylline (t_0 marker); 2. Caffeine; 3. Phenol; 4. Butylbenzene; 5. o-Terphenyl; 6. Pentylbenzene; 7. Triphenylene

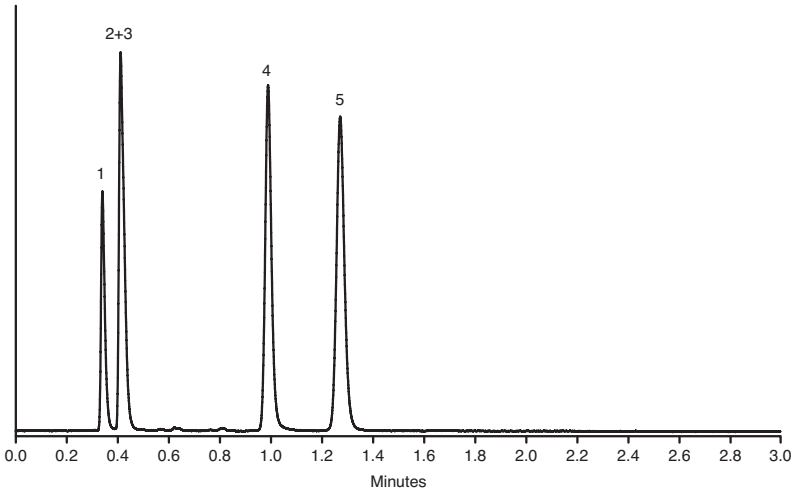


Figure 2: Example chromatogram for the neutral pH test on the Accucore RP-MS column
1. Theophylline (t_0 marker); 2. Phenol; 3. Benzylamine; 4. Quinizarine; 5. Amitriptyline

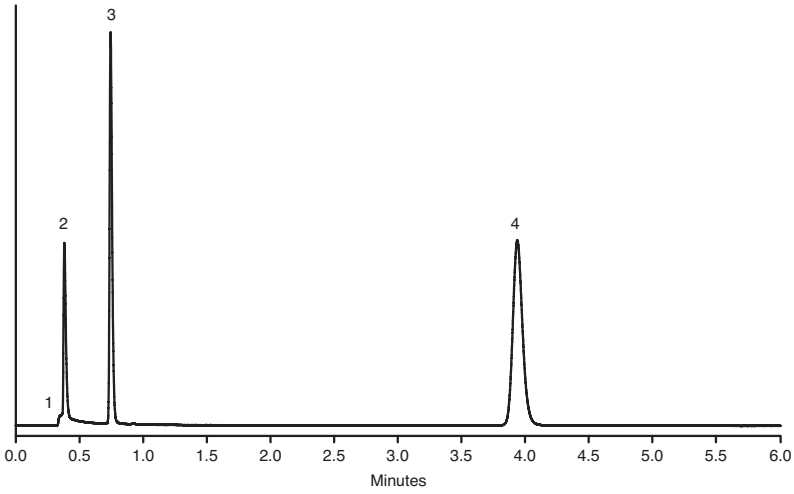


Figure 3: Example chromatogram for the lower pH test on the Accucore RP-MS column
1. Cytidine triphosphate (t_0 marker); 2. Benzylamine; 3. Phenol; 4. Chlorocinnamic acid

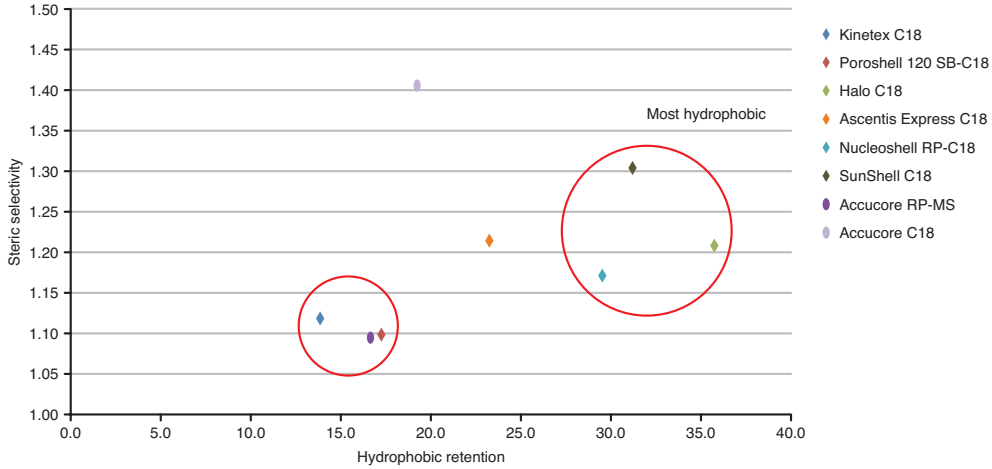


Figure 4: Comparison of the steric selectivity and hydrophobic retention of the tested stationary phases

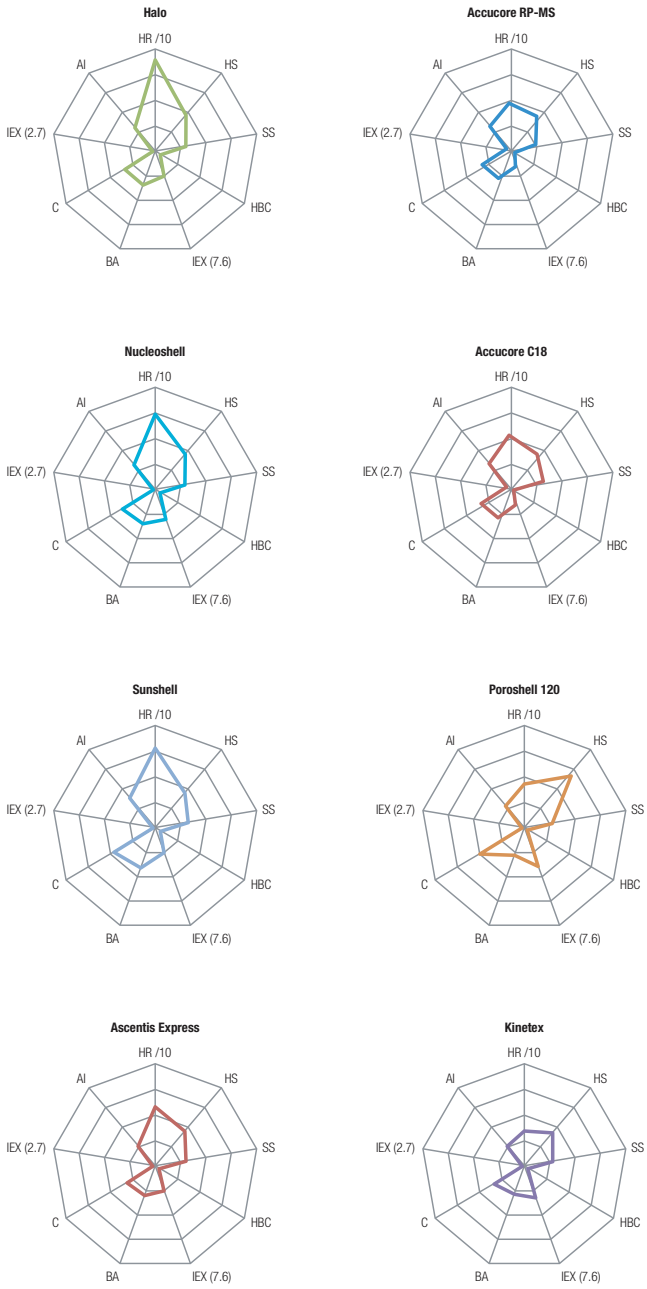


Figure 5: Radar plots for the phases tested, showing the differences in selectivity. HR = hydrophobic retention; HS = hydrophobic selectivity; SS = steric selectivity; HBC = hydrogen bonding capacity; IEX (7.6) = ion exchange capacity at pH 7.6; BA = activity towards bases; C = activity towards chelators; IEX (2.7) = ion exchange capacity at pH 2.7; AI = activity towards acids

Conclusion

The selectivities of the Accucore C18 and RP-MS phases were assessed against six other comparable solid core reversed-phase stationary phases. The retention properties of the stationary phases were categorized by analyzing primary modes of interaction (hydrophobicity, steric selectivity, and hydrogen bonding) and secondary or unwanted modes of interaction (ion exchange and chelation). The results were summarized in radar plots, which identified the following main differences:

1) Primary interactions

- Halo C18, SunShell C18, and Nucleoshell RP-C18 are the most hydrophobic phases and therefore the most retentive towards hydrophobic solutes. A varying degree of hydrophobicity was observed throughout the materials, reflecting different degrees of surface area and coverage of this silica surface.
- Accucore C18 shows the highest steric selectivity, achieved without excessive retention. This attribute of the phase facilitates good separation of solutes that have similar hydrophobicity but dissimilar steric structures.

2) Secondary interactions

- All phases show evidence of efficient endcapping and the absence of dissociated acidic silanols.
- Poroshell 120 SB-C18 and SunShell C18 have the highest levels of chelation, indicating the highest metals content; analysis of solutes which can chelate on these phases may result in asymmetrical peaks.
- Poroshell 120 has the highest silanolic activity at neutral pH; this can cause tailing of solutes capable of ion exchanging with silanols under these pH conditions.
- SunShell C18 has the highest activity towards acids and protonated bases, and therefore asymmetrical peaks can be expected for these types of compounds on this phase.

References

[1] Tanaka, N. et al., *Journal of Chromatographic Science*, **1989**, *27*, 721-728.

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Comparison of Solid Core HPLC Column Performance

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Key Words
Solid core, fused core, superficially porous, pressure, efficiency, impedance

Abstract
In this technical note the performance of Thermo Scientific™ Accucore™ HPLC columns is compared to a number of competitive columns. The experimentally derived parameters that are used for the comparison are column pressure as a function of flow rate, efficiency, and impedance.

Introduction

The use of partially porous particles, with a diameter between 2 and 3 µm, is gaining momentum, as these provide similar efficiency to sub-2 µm particles but with significantly lower column backpressures.

The Accucore HPLC column range uses Core Enhanced Technology™ to produce a 2.6 µm solid-core material with a very tight particle size distribution. The particles in the Accucore columns are not fully porous but instead have a solid silica core surrounded by a porous outer layer. The very tight particle size distribution results in columns with high permeability. Therefore, "bar for bar", Accucore columns produce improved separation efficiency when compared to fully porous materials.

Equation 1, known as the Burke-Plummer equation, shows the dependency of the pressure drop across the column on a variety of experimental parameters. The pressure is directly proportional to the column length, flow rate, and mobile phase viscosity and is inversely proportional to the square of the particle size diameter and the square of the column internal diameter ID. The interstitial porosity (the spaces between the particles that are accessible by the mobile phase) has a more complicated relationship to the pressure. There are other operating parameters that have an impact on the overall system pressure, such as the ID and length of the connecting tubing in the LC system, detector setup parameters, such as flow cell volume in UV or the ID and length of the capillary components in ESI and APCI sources in LC/MS.

Equation 1

$$\Delta P = a \frac{(1 - \varepsilon_i)^2}{\varepsilon_i^3} \frac{F L \eta}{d_c^2 d_p^2}$$

where ΔP = pressure drop across the column
a = constant (dependent on packing, normal values in the range 150 -225)
ε_i = interstitial porosity of the packed bed
F = flow rate through the column
L = length of the column
η = viscosity of the mobile phase
d_p = particle diameter
d_c = column internal diameter

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The conventional approach to compare the chromatographic performance of columns is to plot normalized efficiency (HETP - height equivalent to a theoretical plate) as a function of mobile phase flow rate or linear velocity, often referred to as a van Deemter plot. This approach does have limitations, since it does not account for analysis time or pressure restrictions of the chromatographic system. Kinetic plots [1] are an alternative method of plotting the same experimental data that allow other parameters, such as pressure, to be incorporated. Therefore, we can infer the kinetic performance limits of the tested chromatographic materials. There are a variety of ways in which this data can be presented, and all of these plots are referred to as kinetic plots. In one of the most useful forms of kinetic plots, a term called impedance is used. Impedance (Equation 2) defines the resistance a compound is subjected to as it moves down the column, relative to the performance of that column. This term gives a true measure of the performance of the column as it incorporates efficiency, time, and pressure, which are critical practical considerations of a chromatographic separation.

Equation 2

$$E = \frac{\Delta P\, t}{\eta\, N^2}$$

where E = impedance
ΔP = pressure drop
t = dead time of chromatographic system
η = kinematic viscosity of mobile phase
N = efficiency

In kinetic plots, the linear velocity, conventionally plotted on the x-axis in the van Deemeter plot, is transformed into the pressure drop limited plate number. Using a maximum pressure drop for the system, any experimental set of data of HETP- linear velocity obtained in a column with arbitrary length and pressure drop can be transformed into a projected efficiency (N)-t_0. This represents the plate number and t_0-time, which could be obtained if the same chromatographic support was used in a column that was long enough to provide the maximum allowed inlet pressure for the given linear velocity.

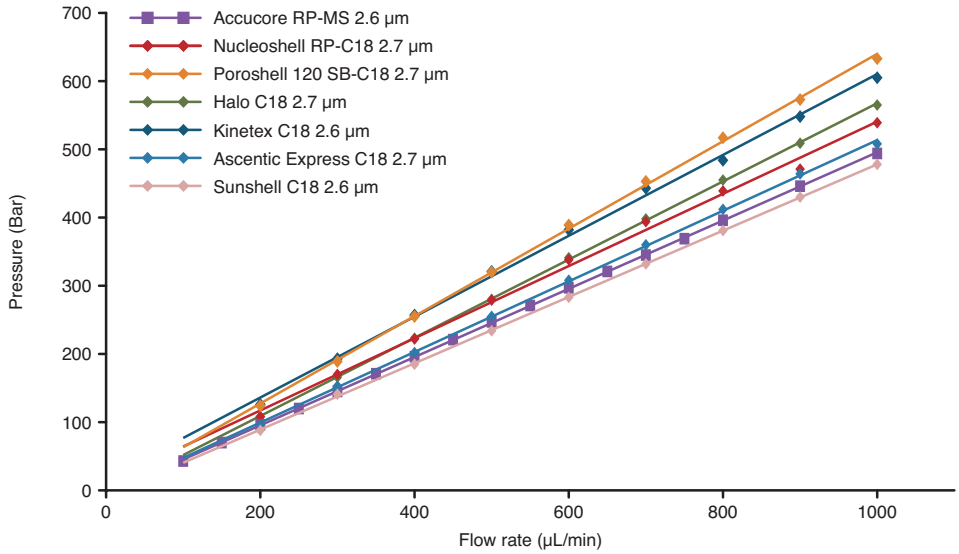

Figure 1: Comparison of column pressure for Accucore and competitor solid core columns.
All columns: 100 x 2.1 mm; test conditions: mobile phase water / acetonitrile (50:50 v/v), column temperature: 30 °C.

Material	Particle Diameter	Pore Diameter	Bonded Phase	Dimensions
Accucore	2.6 µm	80 Å	RP-MS	100 x 2.1 mm
Kinetex®	2.6 µm	100 Å	C18	100 x 2.1 mm
Poroshell® 120	2.7 µm	120 Å	SB-C18	100 x 2.1 mm
Ascentis® Express	2.7 µm	90 Å	C18	100 x 2.1 mm
Halo®	2.7 µm	90 Å	C18	100 x 2.1 mm
Nucleoshell®	2.7 µm	90 Å	RP 18	100 x 2 mm
SunShell®	2.7 µm	90 Å	C18	100 x 2.1 mm

Table 1: Columns used in this study

Column Backpressure Comparison

The solid core particles, tight control of particle diameter, and automated packing processes used in Accucore HPLC columns all contribute to low backpressures. Figure 1 shows how the column backpressure of an Accucore 2.6 µm column compares with the other solid core columns tested (Table 1). With the exception of the SunShell® 2.6 µm column (ChromaNik Technologies, Inc., Osaka, Japan) the Accucore column exhibits the lowest backpressure, across the flow rate range, tested for all of the columns tested. However, the SunShell material exhibits lower efficiencies.

Even when run at a flow rate of 1 mL/min, the backpressure of the 100 x 2.1 mm Accucore column is below 500 bar. This is 22% lower than the backpressure generated by the Poroshell 120 2.7 µm column (Agilent Technologies Inc., Santa Clara, CA, USA) under the same conditions, which is the column with the highest backpressure across the flow rate range.

Efficiency Comparison

In Figure 2, the Accucore column's speed in generating plates is compared to the competitor phases. This kinetic plot is often referred to as a Poppe plot [2]. In this type of plot the plate generation rate is plotted against efficiency. Lower values on the y-axis represent the ability to generate narrow peaks quickly. The Accucore column is the best-performing column when using this comparison, demonstrating that it provides the most efficient peaks per unit time. At the optimum point of the curve, the Accucore 2.6 µm column shows the best combination of plate generation rate / efficiency. On average, the plate generation rate of the Accucore 2.6 µm, Halo 2.7 µm (Advanced Materials Technology, Inc., Wilmington, DE, USA) and Ascentis® Express 2.7 µm (Sigma-Aldrich Co., St. Louis, MO, USA) columns are similar and 28% better than the column with the worst plate generation rate (Sunshell 2.6 µm).

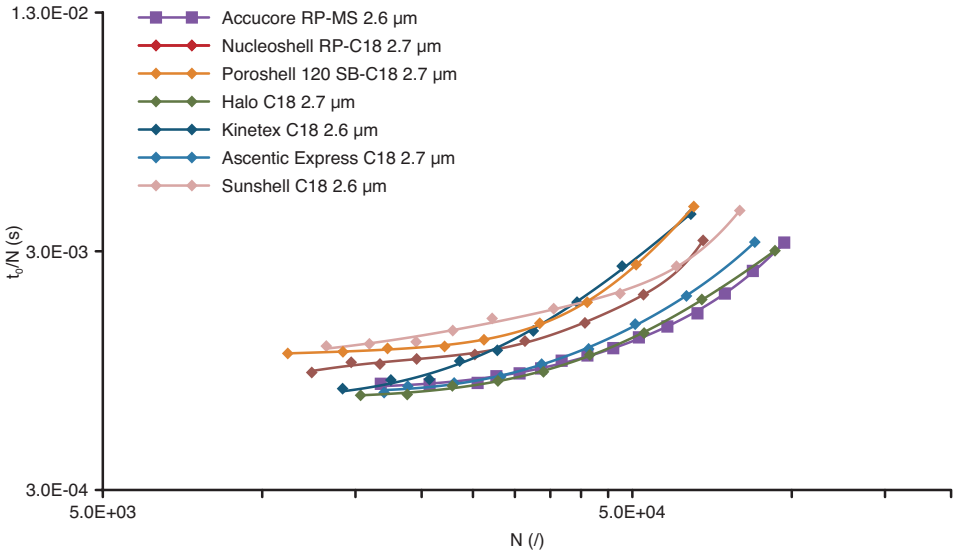

Figure 2: Performance comparison using Poppe plot (plate generation time versus efficiency) for Accucore and competitor solid core columns. All columns: 100 x 2.1 mm; test conditions: mobile phase water / acetonitrile (50:50 v/v), column temperature: 30 °C, test probes: o-xylene and theophylline (t_0 marker).

Impedance Comparison

Impedance is a term that gives a true measure of the performance of the column as it incorporates efficiency, time and pressure, which are critical parameters for chromatographers. Lower impedance values indicate faster chromatography and generation of narrower peaks at lower backpressures. The solid core particles, tight control of particle diameter, and automated packing processes used in Accucore HPLC columns all contribute to low impedance. As demonstrated in Figure 3, the Accucore column exhibits the lowest impedance of all solid core columns tested. The average impedance of the Accucore 2.6 µm column is 7% lower than the material with the second lowest impedance (Halo 2.7 µm) and 51% lower than the material with the highest impedance across the range (Poroshell 120 2.7 µm).

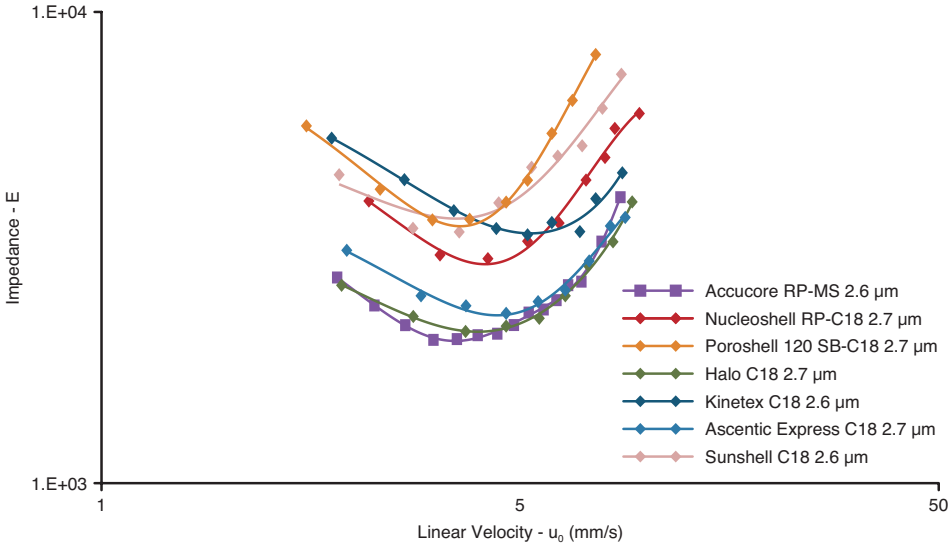

Figure 3: Performance comparison of Accucore and competitor solid core columns using kinetic plots: column impedance (E) relative to linear velocity (u). All columns: 100 x 2.1 mm; test conditions: mobile phase water / acetonitrile (50:50 v/v), column temperature: 30 °C, test probes: o-xylene and theophylline (t_0 marker).

Conclusion

- Accucore HPLC columns generate a lower backpressure than the majority of solid core competitors.
- Accucore HPLC columns generate higher efficiencies than all solid core competitors.
- Accucore HPLC columns generate lower impedances than all solid core competitors.

References

[1] G. Desmet, P. Gzil, D. Clicq, LC GC Europe, 18 (2005) 403
[2] Hans Poppe, J. Chromatogr. A, 778 (1997) 3

The data is a mixture of averages and representative data points, but is always consistent from column to column.
Testing was performed by members of our Applications R&D team.
Comparative performance may not be representative of all applications.
Purchasers must determine the suitability of products for their particular use.

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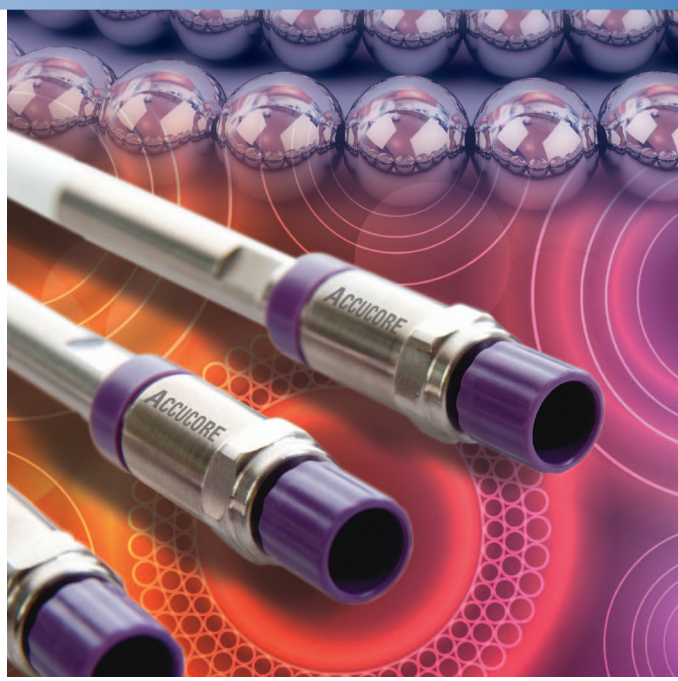
Technical Note 20755

Comparison of Solid Core HPLC Column Performance: Effect of Particle Diameter

Luisa Pereira, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words
Solid core, fused core, superficially porous, pressure, efficiency, impedance

Abstract
In this technical note, the chromatographic performance of solid core 4 µm and 2.6 µm particle-packed columns is compared. Parameters compared are column pressure, efficiency, and impedance.



Introduction
The use of partially porous particles is gaining momentum as they provide higher efficiency than fully porous particles of equivalent particle size. Initially introduced with a particle size in the range 2–3 µm, they are now commercially available in a range of particle sizes, from sub-2 to 5 µm. This gives the chromatographer the flexibility of being able to select the most appropriate particle size for each specific assay; however, it may not be clear what the most suitable particle size is. This technical note partially addresses this gap in information by providing advice on what particle size to select under which experimental conditions. We compare the performance of the Thermo Scientific™ Accucore™ XL 4 µm and Accucore 2.6 µm particle packed columns.

Accucore HPLC columns are based on Core Enhanced Technology™, which features solid core materials with a very tight particle size distribution and advanced bonding technology to functionalize the surface. The particles in the Accucore stationary phases can be described as a solid silica core surrounded by a porous outer layer. The very tight particle size distribution of these materials results in columns with high permeability. Therefore, for the same nominal pressure, Accucore provides better separations than fully porous materials.

Equation 1, known as the Blake-Kozeny equation, shows the dependency of the pressure drop across the column on a variety of experimental parameters under laminar flow conditions. It can be seen that the pressure is directly proportional to the column length, flow rate, and mobile phase viscosity and inversely proportional to the square of the particle size diameter and the square of the column internal diameter. The interstitial porosity (the spaces between the particles that are accessible by the mobile phase) has a more complicated relationship to the pressure. There are other operating parameters that will have an impact on the overall system pressure. Some of these are the inner diameter and length of the connecting tubing in the LC system, the detector set-up parameters, such as flow cell volume in UV, or the inner diameter and length of the capillary components in ESI or APCI sources in LC/MS.

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Equation 1

$$\Delta P = a \frac{(1-\varepsilon_i)^2}{\varepsilon_i^3} \frac{F\,L\,\eta}{d_c^2\,d_p^2}$$

where ΔP – pressure drop across the column
a – constant (dependent on packing, normal values in the range 150 -300 [1,2])
ε_i – interstitial porosity of the packed bed
F – flow rate through the column
L – length of the column
η – kinematic viscosity of the mobile phase
d_p – particle diameter
d_c – column internal diameter

The conventional approach to compare the chromatographic performance of columns is to plot a HETP - height equivalent to a theoretical plate as a function of mobile phase flow rate or linear velocity, often referred to as a van Deemter plot. This approach does have limitations, since it does not account for analysis time or pressure restrictions of the chromatographic system. Kinetic plots [3] are an alternative method of plotting the same experimental data but allowing other parameters such as pressure to be incorporated, and therefore allow us to infer the these performance limits of the tested chromatographic materials. There are a variety of ways in which this data can be presented and all of these plots are referred to as kinetic plots. In one of the most useful forms of these plots a term called impedance is used. Impedance (Equation 2) is a term that defines the resistance a compound is subjected to as it moves down the column relative to the performance of that column. This term gives a true measure of the performance of the column as it incorporates efficiency, time, and pressure, which are critical practical considerations of a chromatographic separation.

Equation 2

$$E = \frac{\Delta P\,t_0}{\eta\,N^2}$$

where E – impedance
ΔP – pressure drop across the column
η – kinematic viscosity of mobile phase
N – efficiency
t_0 – column dead time

Pressure comparison

Figure 1 shows how the column backpressure of the Accucore XL 4 µm column compares with that of the Accucore 2.6 µm column. On average, across the flow rate range tested, the pressure measured on the Accucore 2.6 µm column is 2.2 times higher. At 1 mL/min flow rate the pressures measured are 94 and 202 bar for the 4 and 2.6 µm columns, respectively.

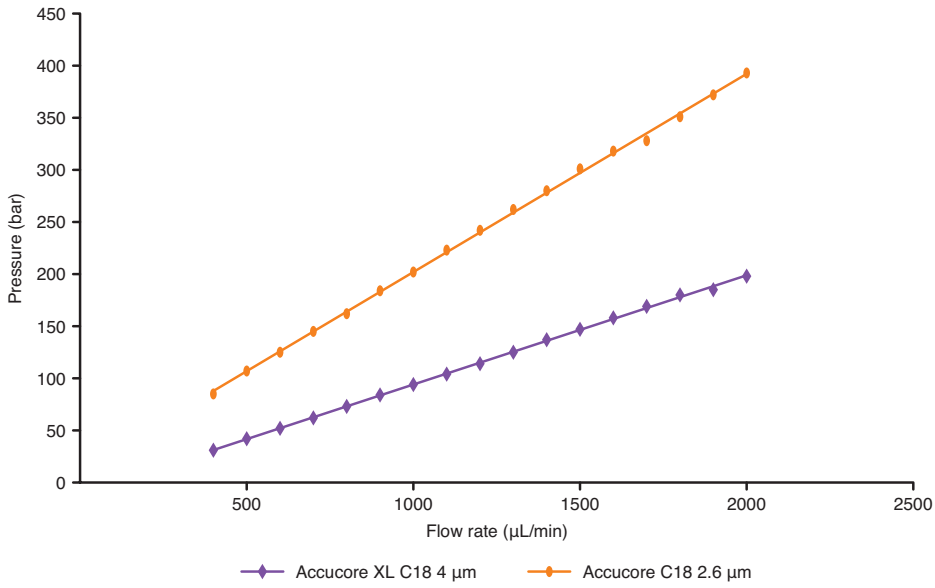

Figure 1: Comparison of column pressure for Accucore XL 4 µm and Accucore 2.6 µm columns

All columns 150 × 4.6 mm; test conditions: water / acetonitrile (50:50 v/v) mobile phase, 30° C column temperature

Efficiency comparison

Figure 2 compares the efficiency of the Accucore XL 4 µm column with that of the Accucore 2.6 µm column using a van Deemter plot. On average (across the flow rate range tested) Accucore 2.6 µm gives 27% higher efficiency than the Accucore XL 4 µm column, and the improvement in efficiency increases as the linear velocity increases.

The curves for both columns are very flat, and therefore a wide range of linear velocities (or mobile phase flow rates) can be used without losing chromatographic performance. The flattest regions of the van Deemter curve correspond to a mobile phase flow rate range of 0.9 to 1.4 mL/min for the Accucore XL 4 µm column and 1.2 to 1.8 mL/min for the Accucore 2.6 µm column.

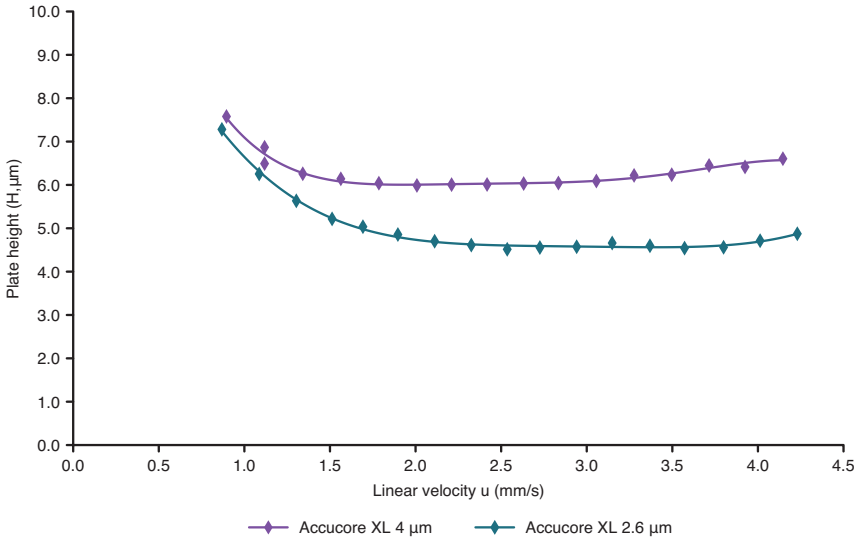

Figure 2: Efficiency comparison using van Deemter plots for Accucore XL 4 µm and Accucore 2.6 µm columns

All columns 150 × 4.6 mm; test conditions: water / acetonitrile (50:50 v/v) mobile phase, 30 °C column temperature, test probes: phenetole and theophylline (t_0 marker)

Impedance comparison

Impedance is a term that gives a true measure of the performance of the column as it incorporates efficiency, time, and pressure, which are critical parameters for chromatographers. Lower impedance values indicate faster chromatography and generation of narrower peaks at lower backpressures. The solid core particles, tight control of particle diameter, and automated packing processes used in Accucore HPLC columns contribute to low impedances.

On average (across the flow rate range tested) the Accucore 2.6 µm column provides 20% more efficiency per unit time than the Accucore XL 4 µm column (Figure 3). In terms of overall performance of both 4 and 2.6 µm materials, the Accucore 2.6 µm column demonstrates 37% lower impedance (Figure 4).

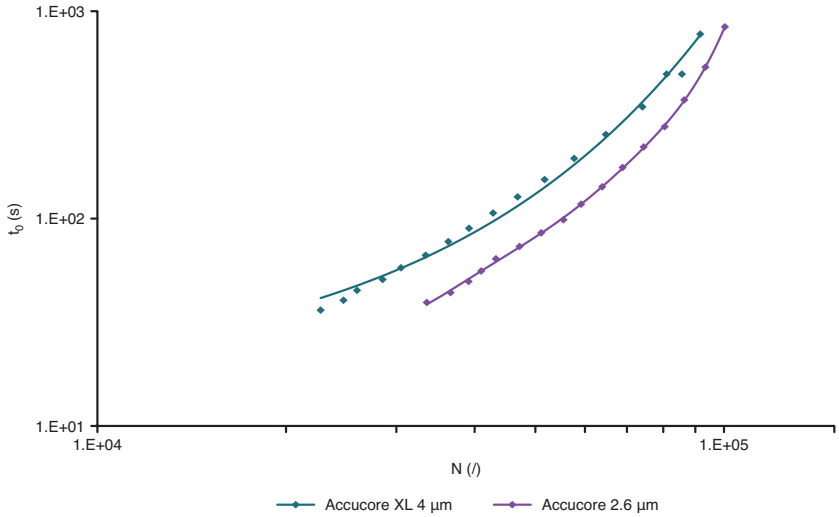

Figure 3: Performance comparison of Accucore XL 4 µm and Accucore 2.6 µm columns using kinetic plots: efficiency per unit time

All columns 150 × 4.6 mm; test conditions: water / acetonitrile (50:50 v/v) mobile phase, 30 °C column temperature, test probes: phenetole and theophylline (t_0 marker)

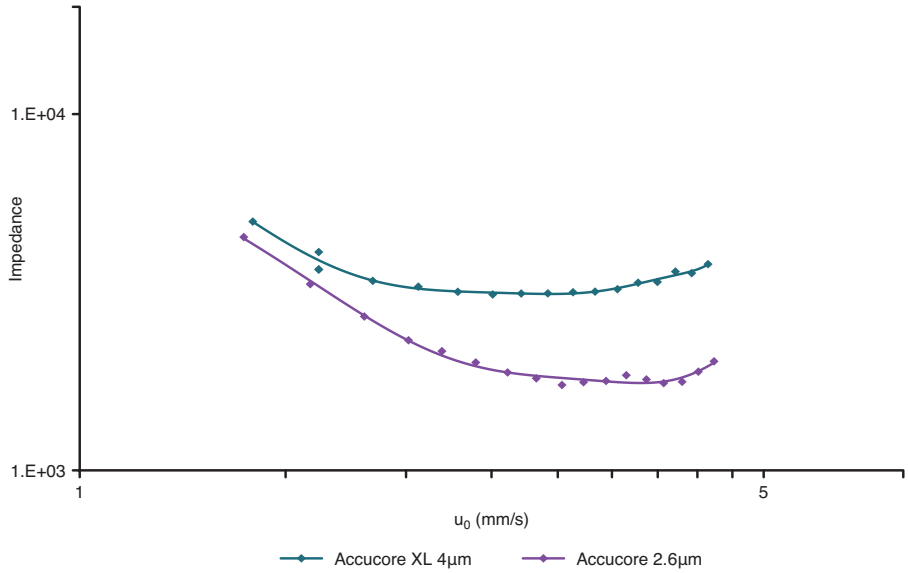

Figure 4: Performance comparison of Accucore XL 4 µm and Accucore 2.6 µm columns using kinetic plots: column impedance (E) relative to linear velocity (u)

All columns 150 × 4.6 mm; test conditions: water / acetonitrile (50:50 v/v) mobile phase, 30 °C column temperature, test probes: phenetole and theophylline (t_0 marker)

Conclusion

Comparison of the Accucore 2.6 µm and Accucore XL 4 µm solid core columns shows that:
- The backpressure of the Accucore 2.6 µm column is 2.2 times higher.
- The Accucore 2.6 µm column is 20% more efficient per unit time.
- The Accucore 2.6 µm column has 37% lower impedance.

The choice between these two solid core materials should be based on the assay goals and the equipment available. The Accucore XL 4 µm columns dramatically improve separation efficiency, and therefore resolution and sensitivity over those obtained with conventional fully porous 5 and 3 µm particle packed columns, without the need to make changes to the operating parameters or system configuration [4]. As demonstrated above, the Accucore 2.6 µm columns provide even higher efficiency and lower impedance, but often system dead volume and operating parameters have to be optimized to get the best possible performance out of these columns [5]. Additionally, when operating at the higher linear velocities, a 600 bar pressure limit LC system may be required.

Therefore, Accucore XL 4 µm columns should be used when:
- There is large dead volume in the system.
- The maximum operating pressure of the pumps is 400 bar.
- The same method as used with a fully porous particle packed column must be maintained.

In contrast, Accucore 2.6 µm columns should be used when even higher efficiency is required and:
- The dead volume of the system is minimal (<100 µL).
- The maximum operating pressure of the pumps is greater than 400 bar.
- The method can be optimized.

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[4] Thermo Fisher Scientific, Technical Note 2064, 2012.
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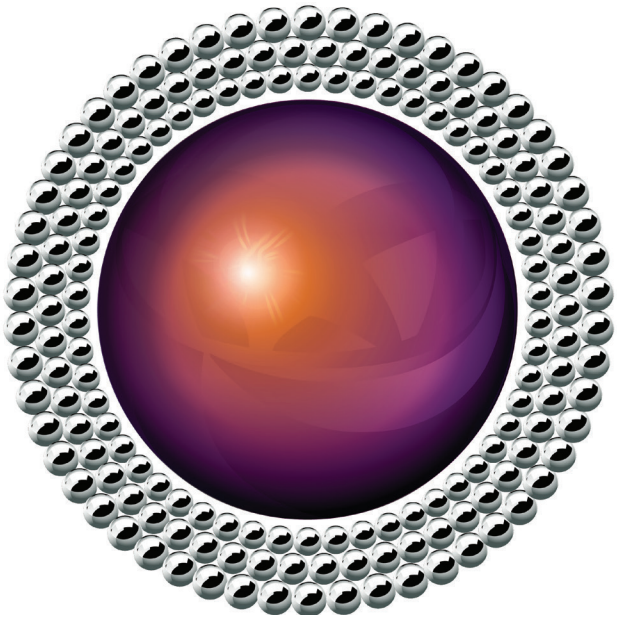

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Solid Core 4 µm HPLC Column Comparison to Fully Porous 3 µm and 5 µm Columns: Efficiency and Pressure

Luisa Pereira, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words
Solid core, fused core, superficially porous, pressure, efficiency, impedance

Abstract
In this technical note, the chromatographic performance of solid core 4 µm particle packed HPLC columns is compared with that of fully porous 5 µm and 3 µm particle packed columns. Parameters compared are column pressure, efficiency, and impedance.

Introduction
The use of partially porous particles, with a diameter between 2 and 3 µm, is gaining momentum, as these provide similar efficiency to sub-2 µm particles but with significantly lower column backpressures.

Often, however, solid core particles are packed into short, narrow-bore columns, which has implications in terms of the system set up. System dead volume and operating parameters have to be optimized to get the best possible performance out of the column.

It is demonstrated herein that by 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 set up. With Thermo Scientific™ Accucore™ XL 4 µm HPLC columns, it is possible to dramatically improve separation efficiency, and therefore resolution and sensitivity over those obtained with conventional fully porous 5 µm and 3 µm particle packed columns. These improvements are obtained with only a 40% increase in backpressure over the 5 µm and a reduction in backpressure compared to the 3 µm material. The Accucore XL 4 µm solid core HPLC columns exhibit significantly lower impedance than fully porous materials.

Equation 1, known as the Burke-Plummer equation, shows the dependency of the pressure drop across the column on a variety of experimental parameters. The pressure is directly proportional to the column length, flow rate, and mobile phase viscosity and is inversely proportional to the square of the particle size diameter and the square of the column internal diameter ID. The interstitial porosity (the spaces between the particles that are accessible by the mobile phase) has a more complicated relationship to the pressure. There are other operating parameters that have an impact on the overall system pressure, such as the ID and length of the connecting tubing in the LC system, detector setup parameters such as flow cell volume in UV or the ID and length of the capillary components in ESI or APCI sources in LC/MS.

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Equation 1

$$\Delta P = a \frac{(1 - \varepsilon_i)^2}{\varepsilon_i^3} \frac{F L \eta}{d_c^2 d_p^2}$$

where

ΔP = pressure drop across the column
a = constant (dependent on packing, normal values in the range 150-225)
ε_i = interstitial porosity of the packed bed
F = flow rate through the column
L = length of the column
η = viscosity of the mobile phase
d_p = particle diameter
d_c = column internal diameter

The conventional approach to compare the chromatographic performance of columns is to plot a normalized efficiency (HETP - height equivalent to a theoretical plate) as a function of mobile phase flow rate or linear velocity, often referred to as a van Deemter plot. This approach does have limitations, since it does not account for analysis time or pressure restrictions of the chromatographic system. Kinetic plots [1] are an alternative method of plotting the same experimental data but allow other parameters, such as pressure, to be incorporated. Therefore, we can infer the kinetic performance limits of the tested chromatographic materials. There are a variety of ways in which this data can be presented, and all of these plots are referred to as kinetic plots. In one of the most useful forms of kinetic plots, a term called impedance is used. Impedance (Equation 2) defines the resistance a compound is subjected to as it moves down the column, relative to the performance of that column. This term gives a true measure of the performance of the column as it incorporates efficiency, time, and pressure, which are critical practical considerations of a chromatographic separation.

Equation 2

$$E = \frac{\Delta P\, t}{\eta\, N^2}$$

where E = impedance
t = dead time of chromatographic system
ΔP = pressure drop
η = kinematic viscosity of mobile phase
N = efficiency

In kinetic plots, the linear velocity, conventionally plotted on the x-axis in the van Deemeter plot, is transformed into the pressure drop limited plate number. Using a maximum pressure drop for the system, any experimental set of data of HETP- linear velocity obtained in a column with arbitrary length and pressure drop can be transformed into a projected efficiency (N)-t_0. This represents the plate number and t_0-time, which could be obtained if the same chromatographic support was used in a column that was long enough to provide the maximum allowed inlet pressure for the given linear velocity.

Pressure Comparison

Figure 1 shows how the column backpressure of the Accucore XL 4 µm HPLC column compares with that of the fully porous 5 µm and 3 µm columns tested. On average (across the flow rate range tested), the Accucore XL 4 µm HPLC column gives 42% higher pressure than fully porous 5 µm and 13% lower pressure than fully porous 3 µm HPLC columns. Even when running the 150 x 4.6 mm Accucore XL 4 µm HPLC columns at a flow rate of 2 mL/min, the backpressure is only 200 bar.

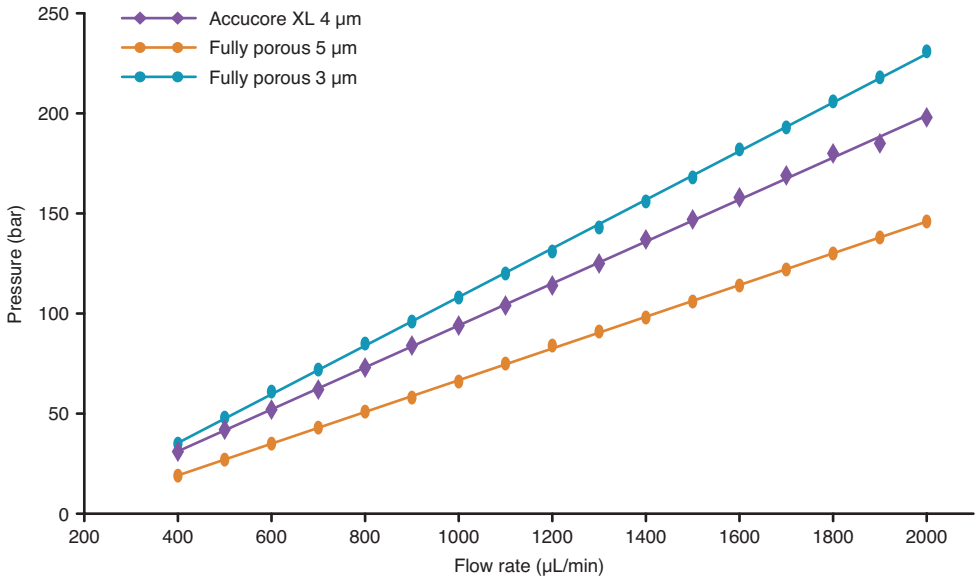

Figure 1: Comparison of column pressure for Accucore XL 4 µm HPLC column and fully porous 5 µm and 3 µm columns. All columns: 150 x 4.6 mm; test conditions: mobile water / acetonitrile (50:50 v/v); column temperature: 30 °C.

Efficiency Comparison

Figure 2 compares the efficiency of the Accucore XL 4 µm material with that of the fully porous 5 and 3 µm materials tested using a van Deemter plot. On average (across the flow rate range tested), the Accucore XL 4 µm material gives 75% more efficiency than fully porous 5 µm and 50% more efficiency than fully porous 3 µm. The curve for the Accucore XL 4 µm HPLC column is very flat; therefore, a wide range of linear velocities (or flow rates) can be used without losing chromatographic performance.

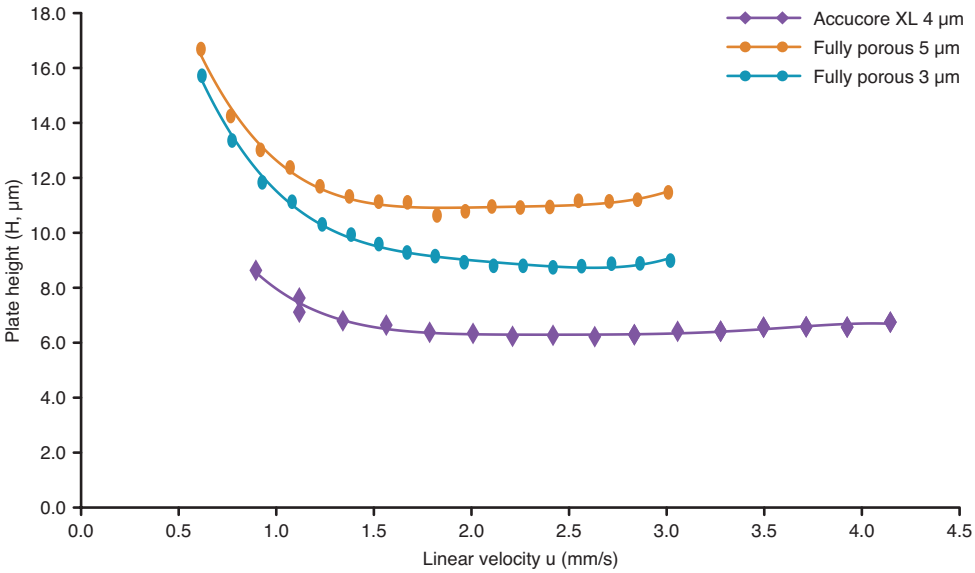

Figure 2: Efficiency comparison using van Deemter plots for Accucore XL 4 µm HPLC column and fully porous 5 µm and 3 µm columns. All columns: 150 x 4.6 mm; test conditions: water / acetonitrile (50:50 v/v); column temperature: 30 °C; test probes: o-xylene and theophylline (t_0 marker).

Impedance Comparison

Impedance is a term that gives a true measure of the performance of the column, as it incorporates efficiency, time, and pressure, which are critical parameters for chromatographers. Lower impedance values indicate faster chromatography and generation of narrower peaks at lower backpressures. The solid core particles, tight control of particle diameter, and automated packing processes used in Accucore HPLC columns all contribute to low impedances. On average (across the flow rate range tested), the Accucore XL 4 µm HPLC column provides the following:

- 59% more efficiency per unit time than fully porous 5 µm and 53% more efficiency per unit time than fully porous 3 µm (Figure 3).
- 79% lower impedance than fully porous 5 µm and 72% lower impedance than fully porous 3 µm (Figure 4).

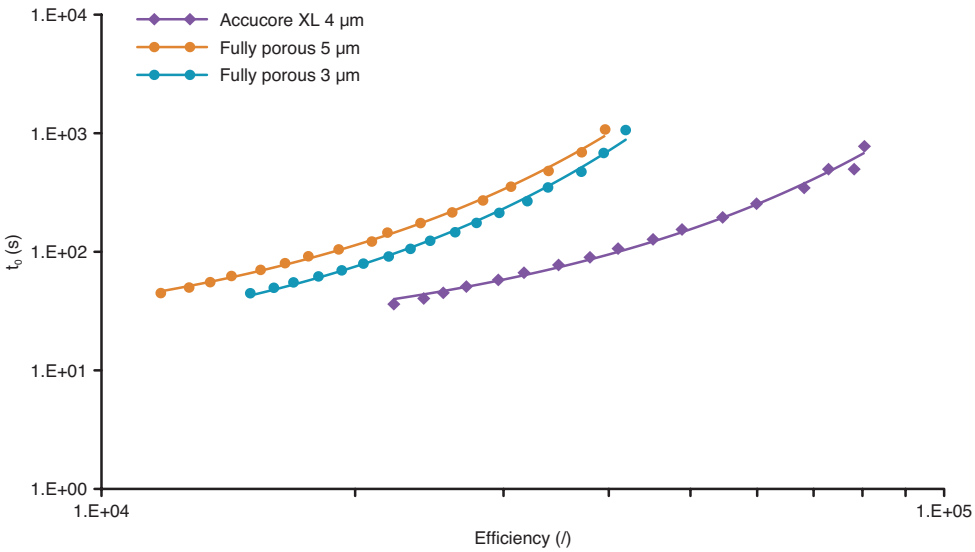

Figure 3: Performance comparison of Accucore XL 4 µm HPLC column and fully porous 5 µm and 3 µm columns using kinetic plots: efficiency per unit time. All columns: 150 x 4.6 mm; test conditions: water / acetonitrile (50:50 v/v); column temperature: 30 °C; test probes: o-xylene and theophylline (t_0 marker).

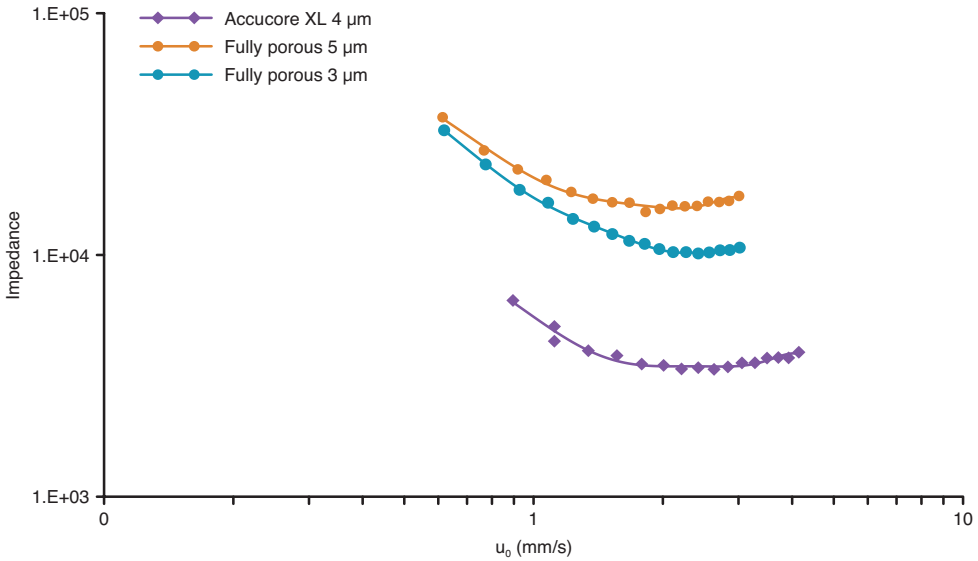

Figure 4: Performance comparison of Accucore XL 4 µm HPLC column and fully porous 5 µm and 3 µm columns using kinetic plots: column impedance (E) relative to linear velocity (u). All columns: 150 x 4.6 mm; test conditions: water / acetonitrile (50:50 v/v); column temperature: 30 °C; test probes: o-xylene and theophylline (t_0 marker)

Conclusion

- The Accucore XL 4 µm solid core HPLC columns provide improvements in efficiency in excess of 50% over fully porous 5 µm and 3 µm columns.
- The backpressure of the Accucore XL 4 µm solid core HPLC column is 42% higher than fully porous 5 µm, but 13% lower than fully porous 3 µm.
- The Accucore XL 4 µm solid core HPLC columns exhibit significantly lower impedance than fully porous materials.

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

Luisa Pereira, Ken Meadows, Anila Khan, Thermo Fisher Scientific, Runcorn, Cheshire, UK

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

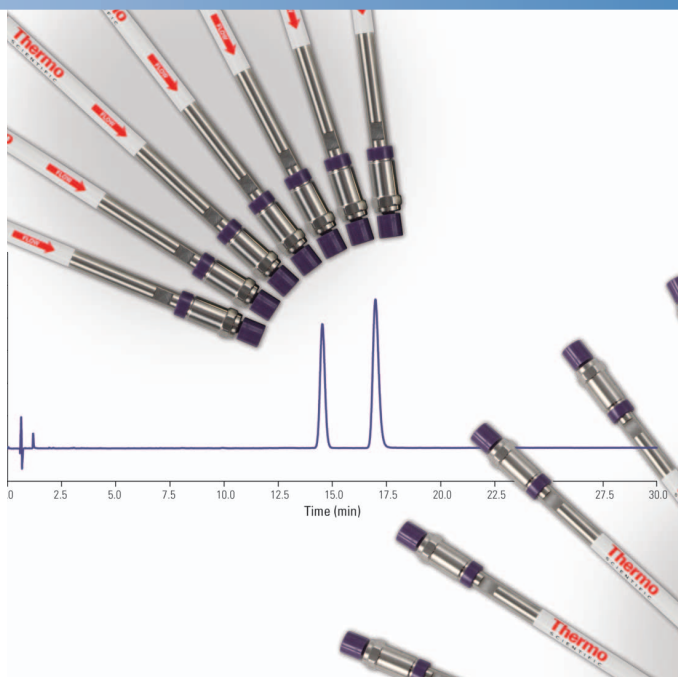
The primary goal of developing a chromatographic separation is to resolve a mixture of analytes. From the general resolution equation (Equation 1), it is evident there are three parameters that control resolution, namely efficiency (N), selectivity (α), and retention (k') factors. Selectivity and retention 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).

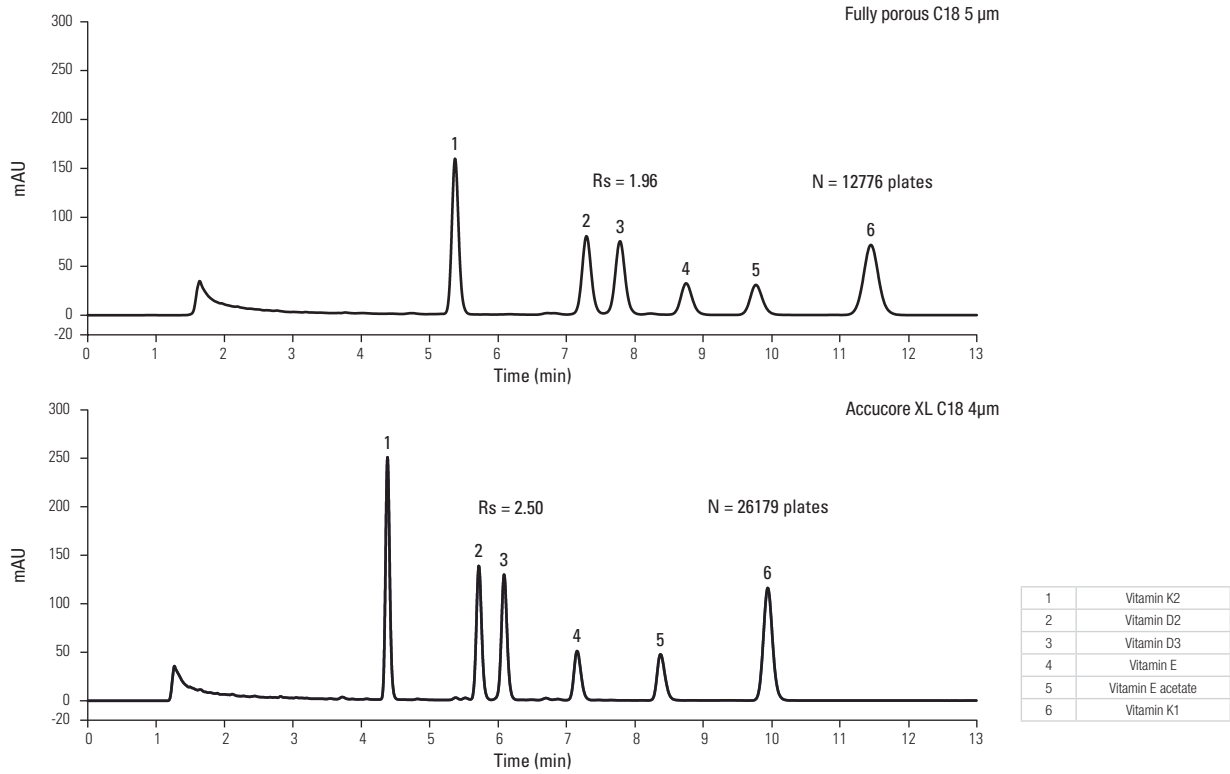


Figure 1: Comparison of the resolution of a critical pair (peaks 2 and 3) on 5 µm fully porous C18 and 4 µm Accucore XL C18 HPLC columns. Experimental conditions: columns – 150 x 4.6 mm; mobile phase – acetonitrile / methanol (80:20 v/v); flow rate – 1 mL/min; column temperature – 30 °C; UV detection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

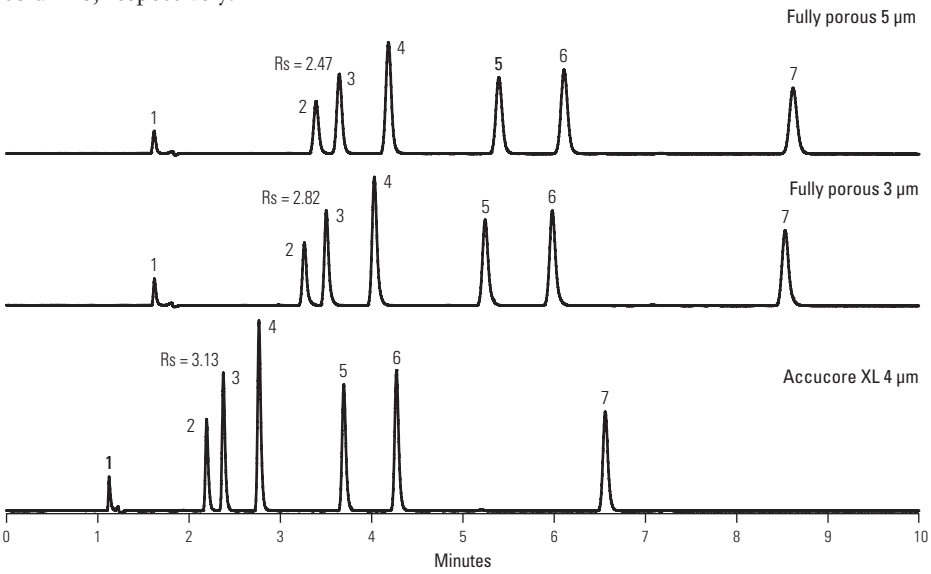
	Efficiency (USP)	
	Accucore XL 4 µm	Fully porous 5 µm
Vitamin K2	23826	13599
Vitamin D2	25566	13963
Vitamin D3	25710	13985
Vitamin E	22788	13288
Vitamin E acetate	24568	13880
Vitamin K1	26179	12776

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

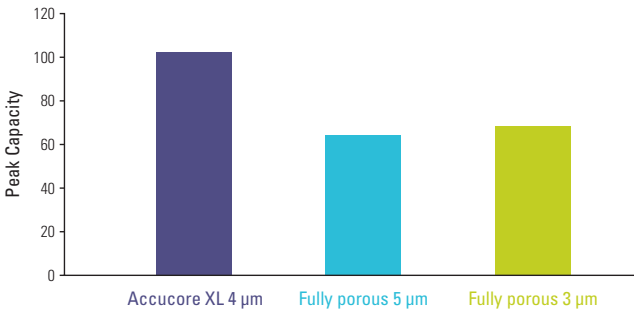


Figure 3: Peak capacity comparison for the herbicides method

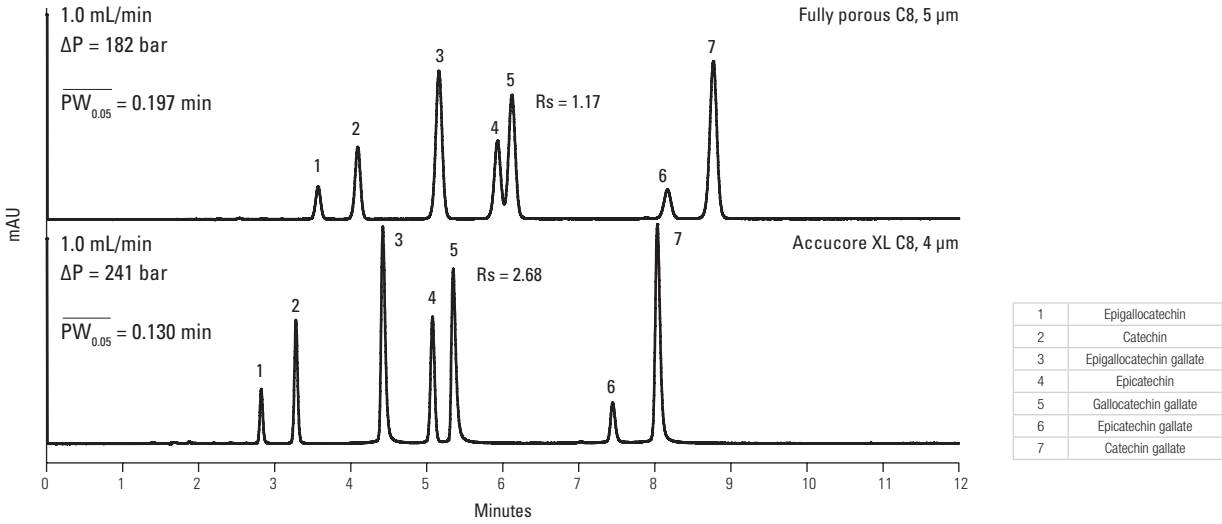


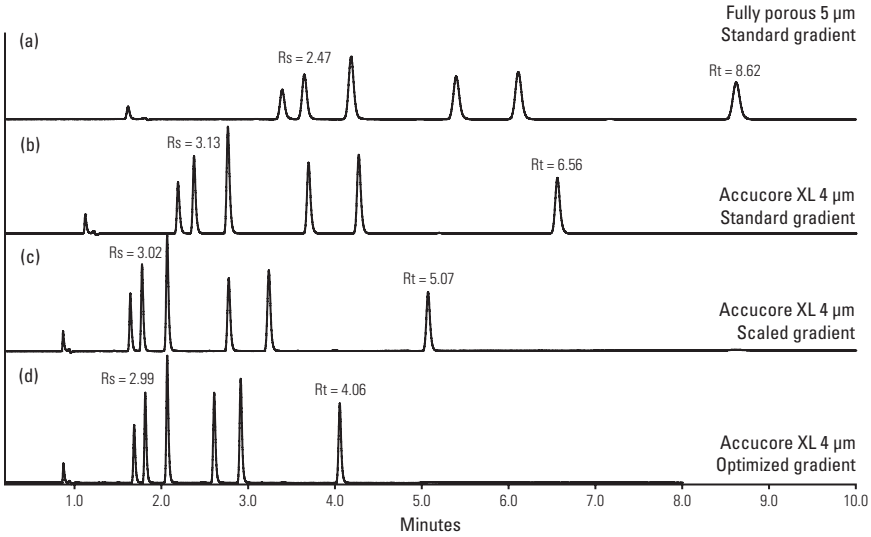
Figure 4. Comparison of the resolution of a critical pair (peaks 4 and 5) on 5 µm fully porous C8 and 4 µm Accucore XL C8 HPLC columns. Experimental conditions: columns – 150 x 4.6 mm; mobile phase A – water + 0.1% formic acid; mobile phase B – methanol + 0.1% formic acid; gradient: 20% to 50% in 15 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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Adjusting Conventional HPLC Methods

For users of conventional HPLC methods working in regulated environments there may be regulatory issues to consider when changing columns in order to realise the improvements offered by newer technologies. For example USP (United States Pharmacopeia) General Chapter <621> Chromatography-System Suitability describes the maximum adjustments that can be made to an analysis so that a method still fulfils the requirements of the system suitability test.

Column Parameter	Allowed Change
Column length	± 70%
Column internal diameter	± 25%
Particle size	Reduction of up to 50%; no increase

Method Parameter	Allowed Change
Flow rate	± 50%
Injection volume	System suitability testing (SST) criteria must be met
Column temperature	± 10%
Mobile phase pH	± 0.2
UV wavelength	No changes outside manufacturer specifications
Concentration of salts in buffer	± 10%
Composition of mobile phase	Minor component adjustment ± 30% or ± 10% absolute, whichever is smaller

Transferring a method from a column packed with a 5 µm fully porous material to an Accucore XL 4 µm HPLC column requires no changes to method parameters and involves only a 20% reduction in particle size–thus meeting the above requirements.

Solid Core 4 µm Particles – High Peak Capacity for Complex Samples

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Key Words
Accucore XL, solid core, superficially porous, peak capacity

Abstract
Using green tea extract as a complex sample, separation of multiple compounds was performed to demonstrate peak capacity on the Thermo Scientific™ Accucore™ XL HPLC column. The benefits of shortening the Accucore XL HPLC column length to maximize the number of peaks per minute through peak capacity to analysis time ratio was shown.

Introduction

The ultimate goal in chromatography is to fully resolve all the compounds within a sample in the shortest possible time with the instrument at hand. Therefore it is important to maximize the efficiency of a separation. The separation can be measured through peak capacity or the peak capacity to analysis time ratio. Maximizing the efficiency can be achieved by improving separation properties of a packed column through the use of superficially porous or solid core particles. Also, as demonstrated in this technical note, peak capacity can be maximized through the use of longer columns or column chains while staying within the pressure restrictions of an instrument.

Based on Core Enhanced Technology™ using 4 µm solid core particles, Accucore XL HPLC columns allow users of conventional HPLC methods to enjoy performance far beyond that of columns packed with 5 µm or even 3 µm fully porous particles. Very high separation efficiencies using standard HPLC instruments and conditions provide increased peak resolution and lower limits of detection. An ultra-stable packed bed results in exceptionally robust columns that demonstrate excellent retention and response reproducibility. In addition, higher flow rates can be achieved without significantly affecting the separation efficiency, which means that faster separations can be performed without compromising performance.

Assessment of Peak Capacity for Gradient Separations

Peak capacity is a broad measure of the separation performance of a column. For gradient separations, peak capacity is calculated using equation 1 [1].

Equation 1

$$n_c = 1 + \left(\frac{t_g}{w}\right)$$

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When total peak capacity (n_c) is calculated based on a gradient elution, t_g is total gradient time and $\overline{w}$ is the average peak width. In this case peak width is measured at the baseline of the peak.

As shown in Equation 1, peak capacity is influenced by peak width, which is directly related to efficiency. Solid core particles maximize efficiency and therefore, under the conditions used for this application, peak capacity by reducing the degree of eddy and longitudinal diffusion through the column [2]. This means that Accucore XL HPLC columns exhibit greater peak capacities compared to columns packed with fully porous particles of a similar size.

The separation of highly complex samples is a major challenge in chromatography and the properties of the Accucore XL HPLC columns provide a means of providing highly efficient separations in a short analysis time. The separation properties, including assessment of efficiency through peak capacity of an Accucore XL HPLC column were demonstrated through the analysis of a complex mixture of a green tea extract. In addition, the peak capacity to analysis time ratio was calculated to show the benefits of shortening the column length on an Accucore XL HPLC column. This provides improvements in analysis times with some sacrifice in resolution and peak capacity and presents the user with a choice between maximizing peak capacity or reducing their analysis times for their separation.

Experimental Conditions

Sample Preparation

Green tea extract was removed from a 315 mg capsule and dissolved in 5 mL methanol / water (50:50 v/v). The sample was vortexed and centrifuged at 14,000 rpm for 10 minutes to remove insoluble particulates and also maximize the number of analytes in the sample for analysis. The supernatant was transferred and diluted 1:5 in mobile phase A for injection onto the HPLC.

Columns	Part Number
Accucore XL C18 4 µm, 150 × 2.1 mm	74104-152130
Accucore XL C18 4 µm, 300 × 2.1 mm	
Accucore XL C18 4 µm, 450 × 2.1 mm	

Separation Conditions	
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC system
Column temperature:	30 °C
Injection volume:	2 µL (partial loop)
Flow rate:	0.3 mL/min
UV detection:	254 nm (data rate 20 Hz)
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient conditions:	Dependent on column length (Table 1a-c)

Results and Discussion

Using a gradient composed of 0.1% formic acid in methanol and water in conjunction with the Accucore XL HPLC column, separation of a complex green tea extract was performed. Column lengths of 450 mm, 300 mm, and 150 mm were examined to investigate the effect of column length on peak capacity, as well as peak capacity to analysis time ratio. The gradient timetables were adjusted to keep the % B change per unit column length constant. Therefore, a similar separation of the compounds on the different column lengths based on the critical pair was achieved. The gradient timetables for three column lengths are shown in Tables 1a-c.

(a) Accucore XL 4 µm, 150 × 2.1 mm

Gradient Timetable	
Time	% B
0.0	5
0.5	5
13.0	75
15.0	75
15.1	5
20.0	5

(b) Accucore XL 4 µm, 300 × 2.1 mm

Gradient Timetable	
Time	% B
0.0	5
1.0	5
26.0	75
30.0	75
30.2	5
40.0	5

(c) Accucore XL 4 µm, 450 × 2.1 mm

Gradient Timetable	
Time	% B
0.0	5
1.5	5
39.0	75
45.0	75
45.3	5
60.0	5

Tables 1a-c: Gradient timetables for Accucore XL HPLC column lengths of 150 mm (a), 300 mm (b), and 450 mm (c)

The lower backpressures generated on the Accucore XL HPLC column allowed column lengths of up to 450 mm to be used to maximize peak capacity with backpressures ≤600 bar. This means, if necessary, for complex samples highly efficient separations can be performed within the pressure limits of conventional HPLC instrumentation.

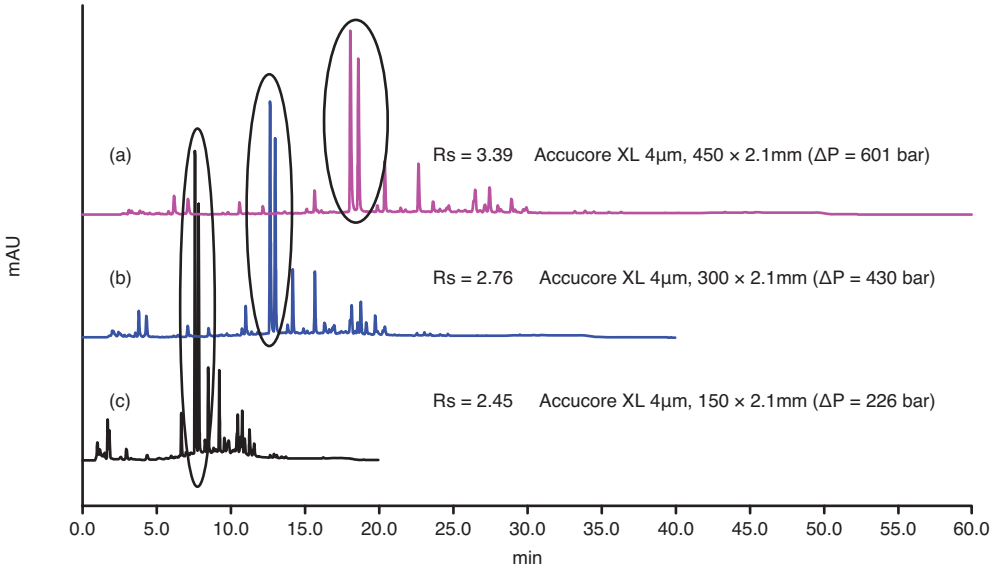

Figure 1: Overlaid chromatograms for the separation of green tea extract on Accucore XL HPLC column lengths 150 mm (a), 300 mm (b), and 450 mm (c). The critical pair is circled on each chromatogram and the resolution for each critical pair is indicated. There is low resolution for the early eluters on the 150 mm column (c), as the focus of the gradient is based on the critical pair.

The Accucore XL HPLC column is shown to offer the analyst a choice of either maximizing peak capacity or sample throughput. Resolution and peak capacity can be maximized using the longer 450 mm column, as seen in Figure 1 and a summary of the peak capacities achieved on each column is seen in Table 2. However, by shortening the column length of the Accucore XL HPLC column, the number of detectable peaks per minute (peak capacity to analysis time ratio) increased by up to 50%, as seen in Figure 2, with only a 28% loss in resolution of the critical pair. This means that sample throughput can be increased through a reduced analysis time without significantly affecting the quality of the data produced based on the critical pair.

Column Length (mm)	Run Time (mins)	Peak Capacity
150	20	167
300	40	280
450	60	336

Table 2: Summary of the peak capacities achieved on the different Accucore XL HPLC column lengths

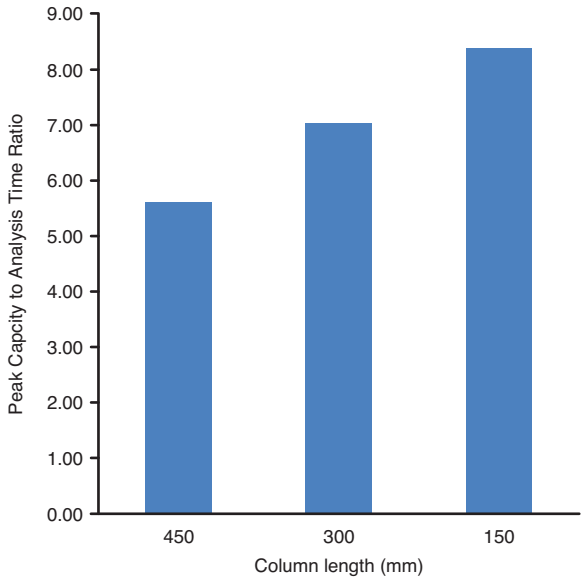

Figure 2: The number of peaks per minute increases by up to 50% as column length decreases by 67%. Therefore, shorter analysis times with good resolution can be achieved using the shorter column.

Statistical examination of the critical pair on the different column lengths, seen in Tables 3a-c, show that the data is matched with excellent precision. Run-to-run reproducibility is of particular importance when analyzing complex mixtures, as it enables the examination of batch-to-batch differences between complex samples. In addition, the selectivity between the critical pair does not change with decreasing column length, which indicates that the separation remains reproducible for the analysis of this complex sample on the different Accucore XL HPLC column lengths.

(a) 450 mm

	Peak 1 RT (min)	Peak 2 RT (min)	Resolution	Selectivity
Rep 1	18.06	18.59	3.35	1.03
Rep 2	18.05	18.58	3.40	1.03
Rep 3	18.07	18.61	3.42	1.03
Average	18.06	18.59	3.39	1.03
%RSD	0.05	0.06	1.06	0.02

(b) 300 mm

	Peak 1 RT (min)	Peak 2 RT (min)	Resolution	Selectivity
Rep 1	12.62	12.97	2.83	1.03
Rep 2	12.65	13.00	2.72	1.03
Rep 3	12.64	12.99	2.73	1.03
Average	12.64	12.99	2.76	1.03
%RSD	0.16	0.09	2.20	0.06

(c) 150 mm

	Peak 1 RT (min)	Peak 2 RT (min)	Resolution	Selectivity
Rep 1	7.58	7.82	2.45	1.03
Rep 2	7.58	7.83	2.45	1.03
Rep 3	7.58	7.82	2.45	1.03
Average	7.58	7.83	2.45	1.03
%RSD	0.03	0.04	0.00	0.01

Tables 3a-c: Statistical examination of the critical pair detected in the complex mixture for Accucore XL HPLC column lengths of 450 mm (a), 300 mm (b) and 150 mm (c)

Conclusion

The high efficiency of the Accucore XL HPLC column has been demonstrated. It has been shown longer Accucore XL HPLC columns provide the greatest separation and peak capacity. However, separation of a complex mixture can be maintained when decreasing the column length to improve analysis times. The shortest Accucore XL HPLC column was shown to provide the greatest number of peaks per minute without compromising the separation.

Therefore, the high resolution offered by the Accucore XL HPLC column can be used to improve complex separations through an increase in peak capacity to analysis time ratio, which makes it an ideal candidate for improving the overall performance of a separation.

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Technical Note 20742

Improving Analysis Sensitivity with Solid Core 4 µm Columns

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Key Words
Solid core, fused core, superficially porous, sensitivity, signal-to-noise, efficiency

Abstract
In this technical note the sensitivity, measured as signal-to-noise ratio, achieved with solid core 4 µm particle packed columns is compared to that of fully porous 5 and 3 µm particle packed columns.

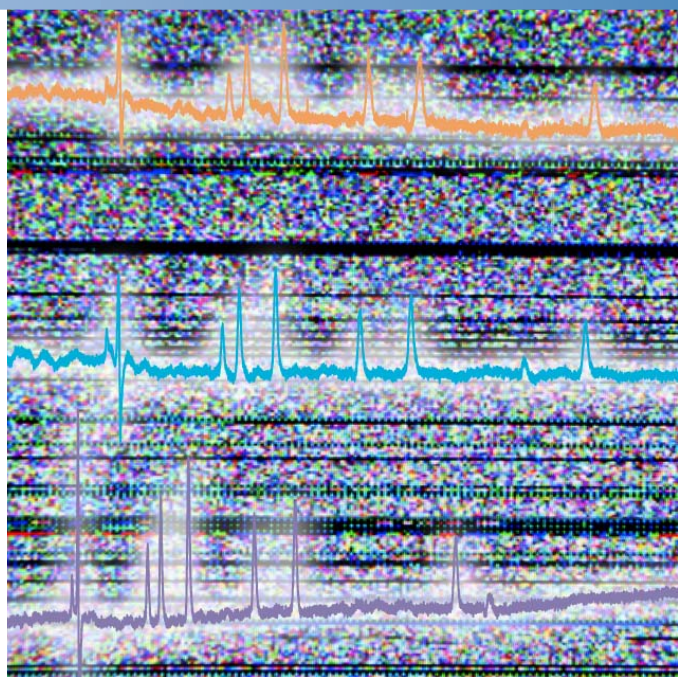
Introduction

Sensitivity is often an important characteristic of a method that needs to be considered in the method optimization strategy. The chromatographic parameters that affect sensitivity are column length and diameter, column performance (peak shape and efficiency), thermodynamic parameters (retention time and temperature), and injection conditions. Sensitivity is related to the concentration at the peak apex C_{max}, which depends on the chromatographic parameters as described by Equation 1 [1].

$$C_{max} = \frac{4}{\varepsilon_t \pi \sqrt{2\pi}} \frac{\sqrt{N}}{L\,(1+k)\,d_c^2} \frac{c_0 V_i}{(T_f - 1)\kappa}$$

Where ε_t – the total column porosity
L – column length
d_c – column diameter
N – peak efficiency
k – peak retention factor
c_0 – sample concentration
V_i – injection volume
T_f – peak tailing factor (measured at a given peak height fraction h)
κ – constant dependent on h

From Equation 1 it is clear that high efficiency and symmetrical peaks produce higher response peaks (higher C_{max}) and therefore higher sensitivity. C_{max} is also inversely proportional to column porosity; thus, lower porosity columns such as those packed with partially porous particles should also produce higher C_{max}, assuming all other conditions remain unchanged.



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. Using solid core 4 µm particles 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. Very high peak efficiencies using standard HPLC instrumentation and conditions allow for increased peak resolution and limits of detection.

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Peak efficiency comparison

Figure 1 illustrates the separation of ibuprofen and valerophenone on a Accucore XL C18 4 µm HPLC column and a fully porous C18 5 µm column using the same isocratic method based on the USP monograph [2]. Efficiency for both compounds improved by more than 70% when using the Accucore XL HPLC column compared to the fully porous column (Table 1). This improvement in peak efficiency results in increased signal to noise ratio (112% on average) for exactly the same amount injected on column, representing a dramatic improvement in analysis sensitivity.

The backpressure for the Accucore XL C18 4 µm HPLC column was measured at 312 bar, and the 5 µm fully porous column backpressure was measured at 239 bar. Therefore, the improvement in analysis sensitivity is gained with a small increase in backpressure, which is still within the operating limits of a conventional HPLC system.

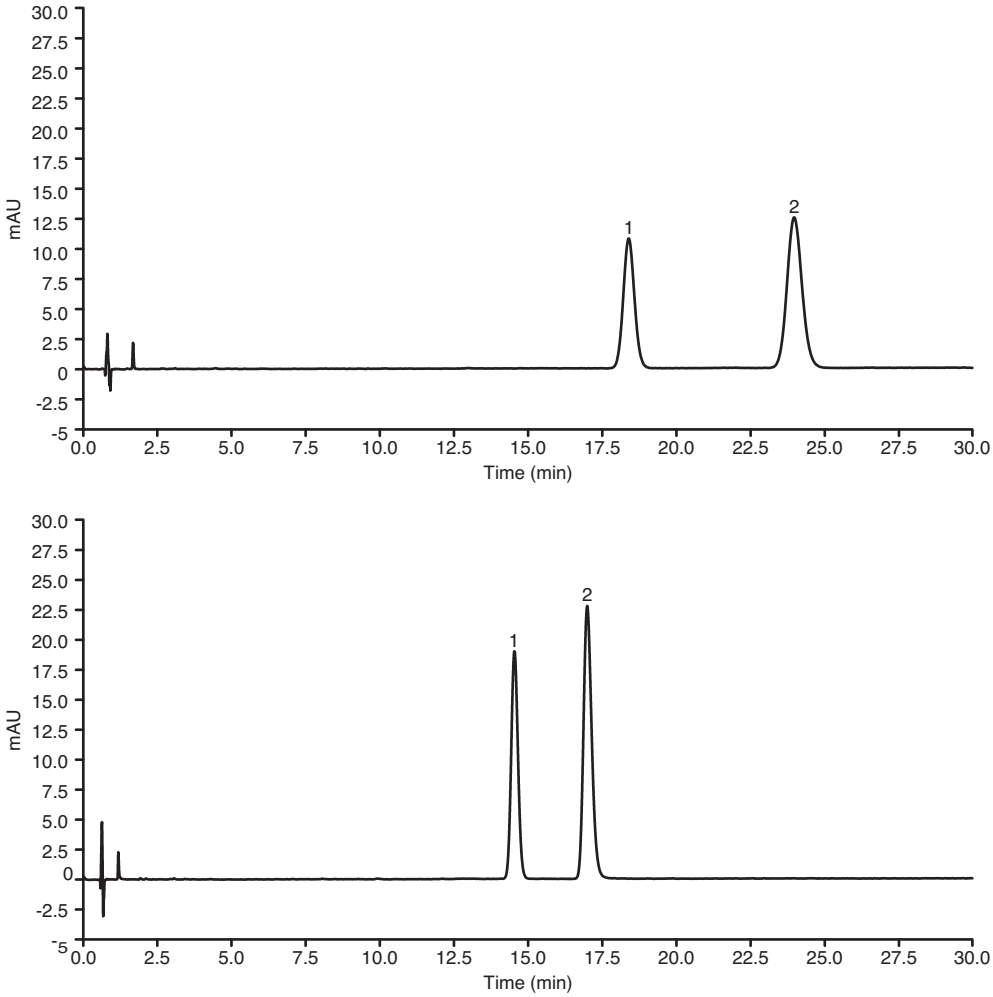

Figure 1: Chromatogram of valerophenone (1) and ibuprofen (2) analyzed using an Accucore XL C18 4 µm HPLC column (bottom trace) compared to a fully porous C18 5 µm column (top trace)

Experimental conditions: columns – 150 × 4.6 mm; mobile phase – water with phosphoric acid, pH 2.5 / acetonitrile (66.3:33.7 v/v); flow rate – 2 mL/min; column temperature – 30 °C; UV detection – 214 nm; injection volume – 5 µL

Compound	Plates (USP)			Signal-to-Noise Ratio		
	Accucore XL	Fully Porous	% improvement	Accucore XL	Fully Porous	% improvement
Valerophenone	19532	11218	74	908	462	96
Ibuprofen	18274	10538	73	1202	534	125

Table 1: Efficiency and signal-to-noise ratio data for valerophenone and ibuprofen

Sensitivity comparison with gradient mobile phase conditions

In Figure 2 the analysis performance of an Accucore XL C8 4 µm HPLC column is compared to that of a fully porous C8 5 µm using gradient mobile phase conditions, and maintaining all other experimental conditions for the two columns. The peak widths for the seven triazines narrowed significantly (on average by 29%) when using the Accucore XL HPLC column compared to the fully porous column (Table 2). As a result, the signal-to-noise ratio increased by 140% on average for exactly the same amount injected on column, significantly improving analysis sensitivity (Figure 3). Additionally, the resolution between the critical pair (peaks 5 and 6) on the fully porous column was 1.92, which improved by 54% to 2.95 with the Accucore XL C8 HPLC column.

The backpressure for the Accucore XL C8 4 µm HPLC column was measured at 215 bar, and the 5 µm fully porous column backpressure was measured at 165 bar. The improvement in performance was gained with a small increase in backpressure, which was still within the operating limits of a conventional HPLC system.

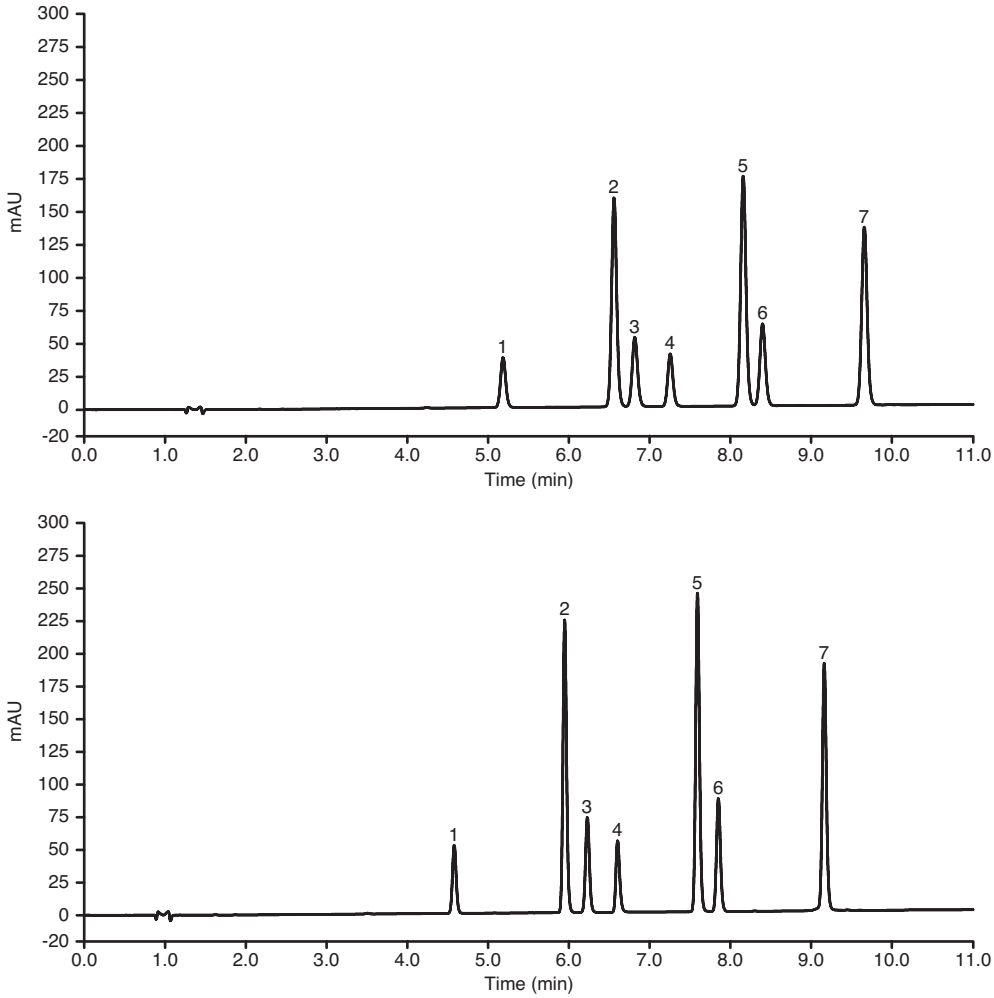

Figure 2: Chromatogram of simazine (1), simetryn (2), atrazine (3), prometon (4), ametryn (5), propazine (6), and prometryn (7) analyzed using an Accucore XL C18 4 µm HPLC column (bottom trace) compared to a fully porous C18 5 µm column (top trace)

Experimental conditions: columns – C18, 150 × 4.6 mm; mobile phase – water and acetonitrile; gradient – 20% to 60% acetonitrile in 10 min; flow rate – 1.5 mL/min; column temperature – 25 °C; UV detection – 220 nm; injection volume – 5 µL

Compound	Peak Width		Resolution		Signal-to-Noise Ratio	
	Accucore XL	Fully Porous	Accucore XL	Fully Porous	Accucore XL	Fully Porous
Simazine	0.108	0.151	N/A	N/A	3453	1468
Simetryn	0.106	0.151	16.42	11.75	14790	6109
Atrazine	0.112	0.160	3.31	2.13	4828	2027
Prometon	0.112	0.153	4.30	3.61	3603	1534
Ametryn	0.111	0.158	11.56	7.49	16170	6718
Propazine	0.114	0.163	2.95	1.92	5745	2390
Prometryn	0.115	0.162	14.64	9.98	12363	5200

Table 2: Peak width, resolution, and signal-to-noise ratio data for seven triazines

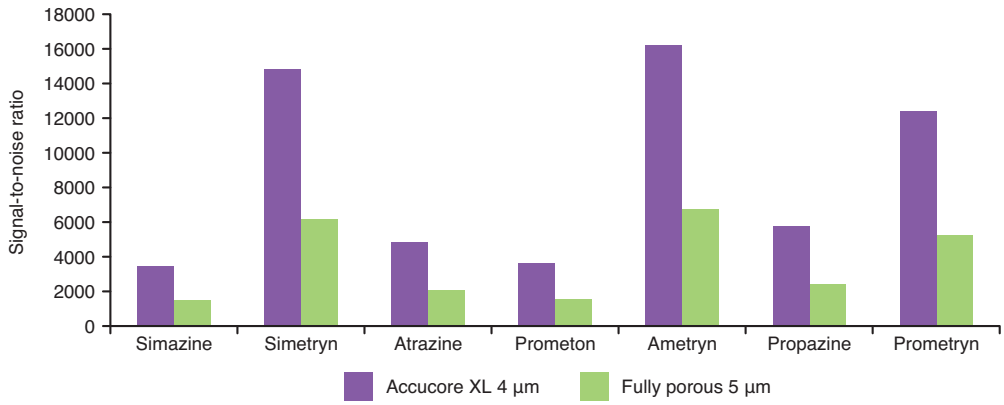

Figure 3: Signal-to-noise ratio comparison for the triazines gradient method in Figure 2, showing improvements between 135% and 142% for the 7 triazines

Sensitivity comparison in trace analysis

In trace analysis, the analyst is challenged to achieve the lowest possible limit of detection (LOD), the lowest concentration that can be detected, and the limit of quantification (LOQ), the lowest concentration that can be reliably quantified at a given signal-to-noise. For example, LOD is typically defined as the concentration on column that gives S/N = 3 and LOQ where S/N = 10. To achieve this goal, it is important to select the chromatographic parameters that will maximize C_{max}, namely high efficiency columns that produce symmetrical peaks, and which are not excessively retained.

In Figure 4, a comparison is made of the signal-to-noise ratios obtained for a series of triazines at trace level (1 ng injected on column) separated on Accucore XL 4 µm and fully porous 3 and 5 µm columns. It can be seen that the higher efficiency of the chromatographic peaks on the Accucore XL HPLC column enables greater signal-to-noise ratios, which are on average 116% and 100% higher than those obtained on fully porous 5 and 3 µm columns, respectively. The derived LODs and LOQs (based on this data) are listed on Table 3.

Column	LOD (ng) S/N = 3	LOQ (ng) S/N = 10
Fully porous 5 µm	0.6	2.0
Fully porous 3 µm	0.6	1.9
Accucore XL 4µm	0.3	0.9

Table 3: Derived LODs and LOQs

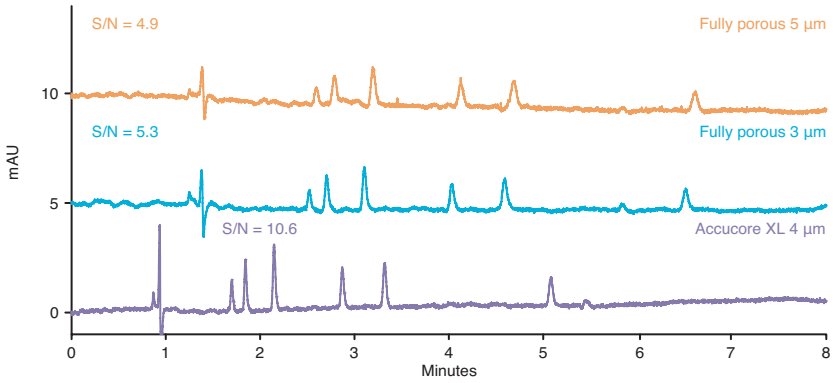

Figure 4: Comparison of average signal-to-noise ratios (S/N) on fully porous 5 and 3 µm and Accucore XL 4 µm columns for 1 ng of each solute loaded on column

Experimental conditions: columns – 150 × 4.6 mm; mobile phase – water and acetonitrile; gradient – 35% to 60% acetonitrile in 7.5 min; flow rate – 1.3 mL/min; column temperature – 30 °C; UV detection – 247 nm; injection volume – 5 µL; solutes – 1. Uracil, 2. Tebuthiuron; 3. Metoxuron; 4. Monuron; 5. Chlorotoluron; 6. Diuron; 7. Linuron

Conclusion

- 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 sensitivity of the analysis.
- The Accucore XL 4 µm columns significantly improve sensitivity over fully porous 5 µm and 3 µm columns, with no changes to methodology or HPLC system configuration.

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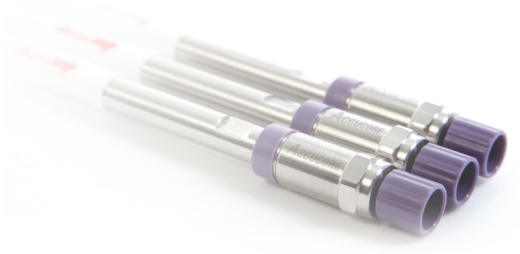
Column Formats

Accucore XL HPLC columns are offered in analytical and micro formats. Optimum conditions and ratings are shown in the table below.

Column ID	Optimum Flow Rate	Optimum Injection Volume	Backpressure Rating	Temperature Rating
2.1 mm	0.3 mL/min	2 µL	600 bar	70 °C
3.0 mm	0.6 mL/min	5 µL	600 bar	70 °C
4.6 mm	1.3 mL/min	10 µL	600 bar	70 °C

Analytical and Narrowbore Columns

Accucore HPLC columns are packed into our high pressure hardware. These stainless steel columns are engineered to the highest quality and have a pressure rating of 600 bar.



Guard Cartridges

Guard cartridges are designed to protect your column from particulates introduced from the matrix or instrument and from any strongly retained components in the injected sample.

Technical Note 20668

Assessment of the Stability of 4 µm Solid Core Particles for the Analysis of Non-Steroidal Anti-Inflammatory Drugs

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Key Words

Accucore XL, column stability, fused core, superficially porous, solid core, ibuprofen

Abstract

The analysis of non-steroidal, anti-inflammatory drugs on a 4 µm solid core C8 HPLC column is described. A method was created using isocratic conditions for separation to demonstrate column stability. The column was proven to remain stable following 2,200 injections and 45,000 column volumes of a test mixture containing naproxen, fenoprofen, and ibuprofen.

Introduction

Thermo Scientific™ Accucore™ XL HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 4 µm diameter particles are not totally porous, but instead have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The tightly controlled 4 µm diameter of Accucore particles results in lower backpressures than typically seen with fully porous particles of the same diameter. The nature of the Accucore XL particles provide an improvement in performance of an analysis compared to that seen on columns using fully porous 3 µm and 5 µm particles.

Stability of a column is of critical importance in obtaining high quality data. Deterioration in column performance can result in batch and validation failures that can effect timelines and result in loss of both time and money. Therefore, a great deal of value is placed on showing that columns remain reproducible when being used for extended periods of time. An analytical method was developed to enable the assessment of Accucore XL HPLC column stability.

Experimental Details

Sample Preparation

Primary standards of theophylline, naproxen, fenoprofen, and ibuprofen were prepared separately in methanol at a concentration of 1000 µg/mL. A working standard was prepared by preparing a 10 mL solution of Ibuprofen, fenoprofen, theophylline, and naproxen by adding 1 mL ibuprofen, 0.5 mL fenoprofen, 0.5 mL theophylline, and 0.025 mL naproxen primary standards to 1.975 mL acetonitrile and 6 mL 20 mM ammonium formate, pH 3.

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Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Dionex™ UltiMate™ 3000 HPLC system	
Column:	Accucore XL C8 4 µm, 50 x 2.1 mm	74204-052130
Mobile phase:	Acetonitrile / 20 mM ammonium formate, pH 3 (40:60 v/v)	
Backpressure:	50 bar	
Column temperature:	30 °C	
Injection volume:	2 µL (partial loop)	
Flow rate:	0.3 mL/min	
UV detection:	233 nm (data rate 20 Hz)	

Results

A stable analytical HPLC method was developed to assess the robustness of the Accucore XL HPLC column. Using isocratic conditions, full separation of the non-steroidal, anti-inflammatory drugs (NSAIDs) naproxen, fenoprofen, and ibuprofen was performed. The Accucore XL C8 HPLC column was shown to be stable for the entire experiment with no indication of deterioration in chromatography even after 2,200 injections, which equates to 45,000 column volumes.

The reproducibility of the Accucore XL HPLC column for the chromatographic separation of the NSAIDs is summarized in Table 1. It is evident that the data for all analytes is matched with excellent precision with the % RSD retention factor of ≤0.80% and % RSD normalized efficiency ≤1.52%. A typical chromatogram of the NSAIDs is seen in Figure 1.

	Naproxen		Fenoprofen		Ibuprofen	
	k'	$N/N_{(n1-5)}$	k'	$N/N_{(n1-5)}$	k'	$N/N_{(n1-5)}$
Mean	2.26	0.986	4.80	0.996	7.99	1.002
% RSD	0.80	1.52	0.77	0.66	0.77	0.36

Table 1: Assessment of the stability on the Accucore XL C8 HPLC column assessed for 2,200 injections. Efficiency was assessed by normalizing efficiency values to the mean of the efficiencies for the first five samples injected.

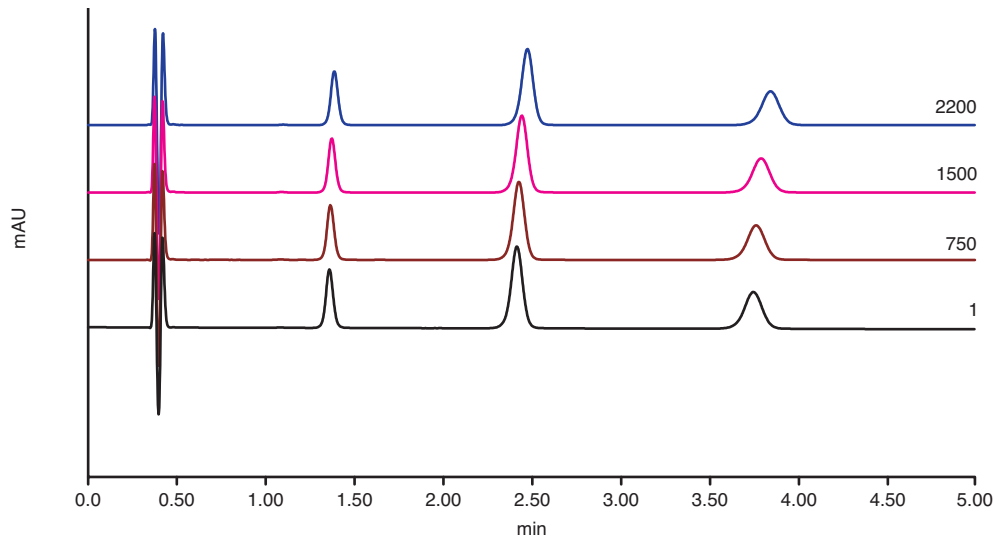

Figure 1: Separation of naproxen, fenoprofen, and ibuprofen, with theophylline as a t0 marker. Injections 1, 750, 1500, and 2,200 are shown.

Figures 2 and 3 are illustrations of the stability data. It is apparent there was a degree of variability within the middle of the analysis, brought about by instrument related issues. However, there is no indication of instability of the Accucore XL HPLC column, even after 2,200 injections, as both the retention and efficiency remained consistent to the end of the analysis.

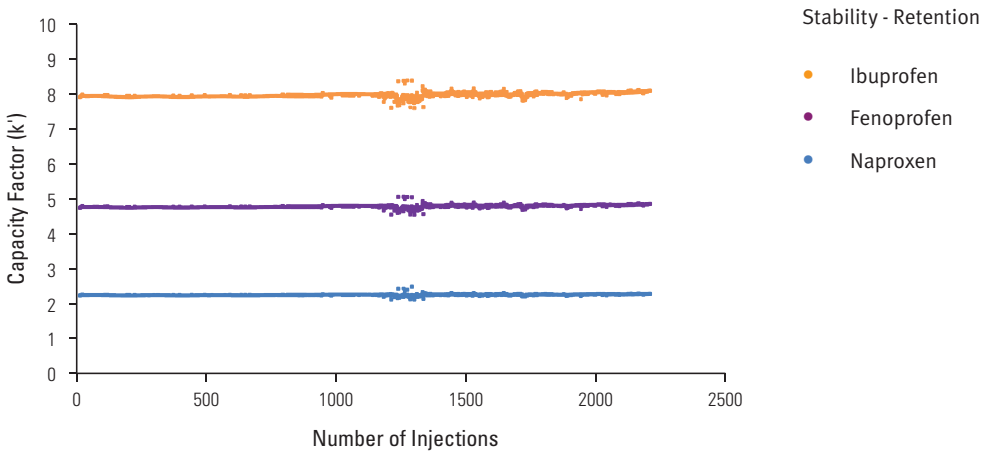

Figure 2: Assessment of the reproducibility of retention for naproxen, fenoprofen, and ibuprofen over 2,200 injections on the Accucore XL C8 HPLC column

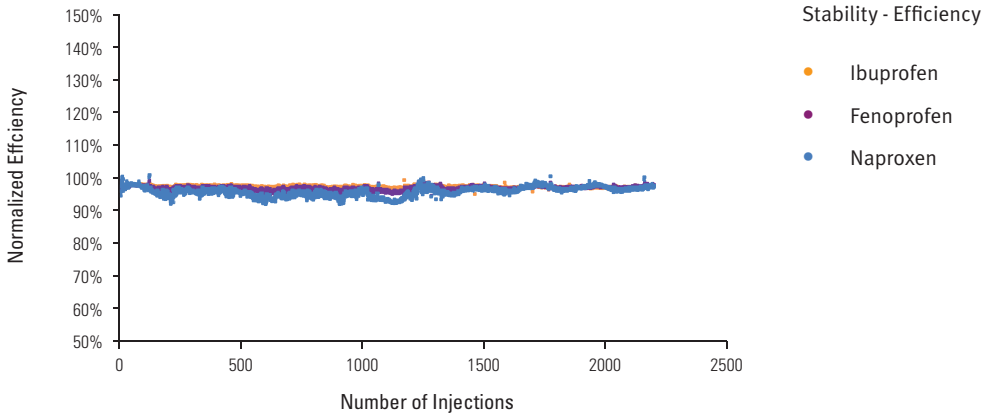

Figure 3: Assessment of the reproducibility of efficiency for naproxen, fenoprofen, and ibuprofen over 2,200 injections on the Accucore XL C8 HPLC column

Conclusion

It has been demonstrated that the Accucore XL C8 HPLC column is stable following 2,200 injections of a solution containing the NSAIDS naproxen, fenoprofen, and ibuprofen. Using isocratic conditions, full separation of the NSAIDs was achieved. The precision of capacity factors and efficiency for the NSAIDs over the course of the investigation is excellent, and the Accucore XL C8 HPLC column was shown to provide excellent peak shape for these compounds.

The Accucore XL HPLC columns have been proven to remain stable over the course of the analysis, which makes them ideal columns to choose to obtain high-performing and robust HPLC separations.

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