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.

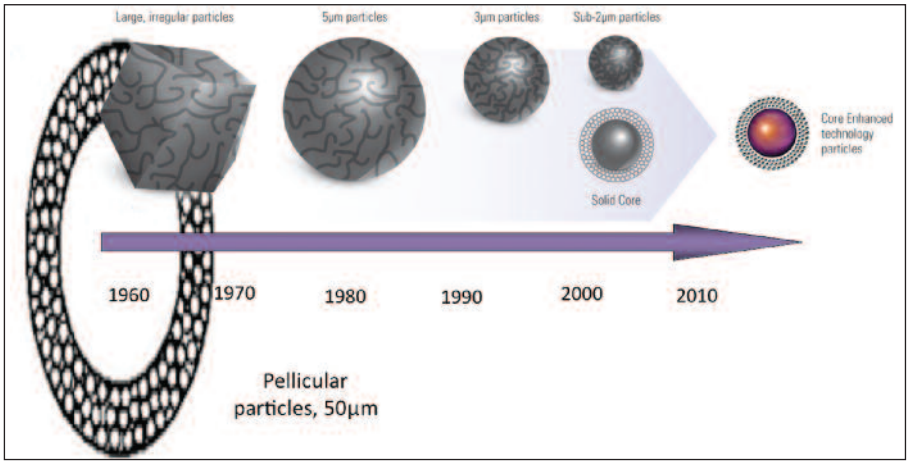


Figure 1: Particle evolution: packing materials have changed from large pellicular particles via smaller totally porous particles to spherical particles with diameters of less than 2µm, to 2.6µm solid-core particles

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

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

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;

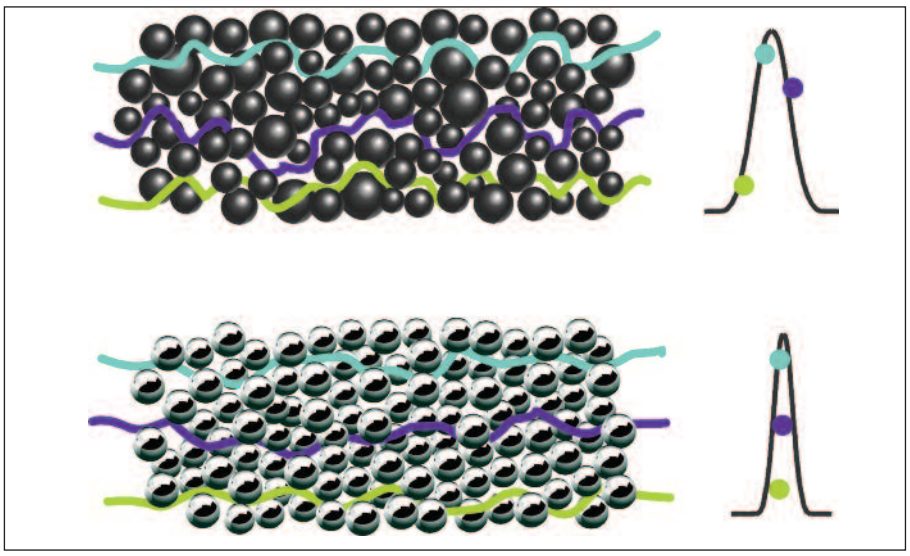


Figure 2: Representation of the effect of average particle size distribution (D90/10) on the packed bed homogeneity and band broadening through Eddy diffusion. Top - D90/10 ~ 1.5; Bottom - D90/10 ~ 1.1.

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

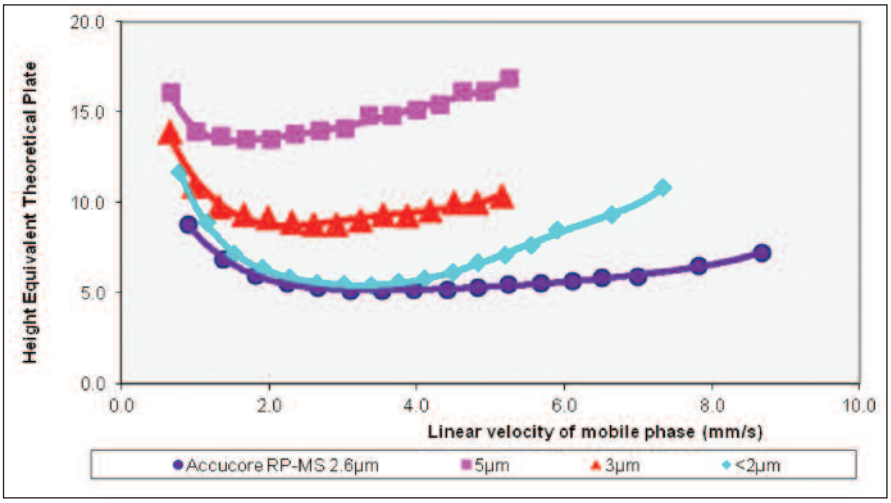


Figure 3: Efficiency comparison using Van Deemter plots for Accucore 2.6µm and fully porous 5, 3 and sub-2µm.

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.

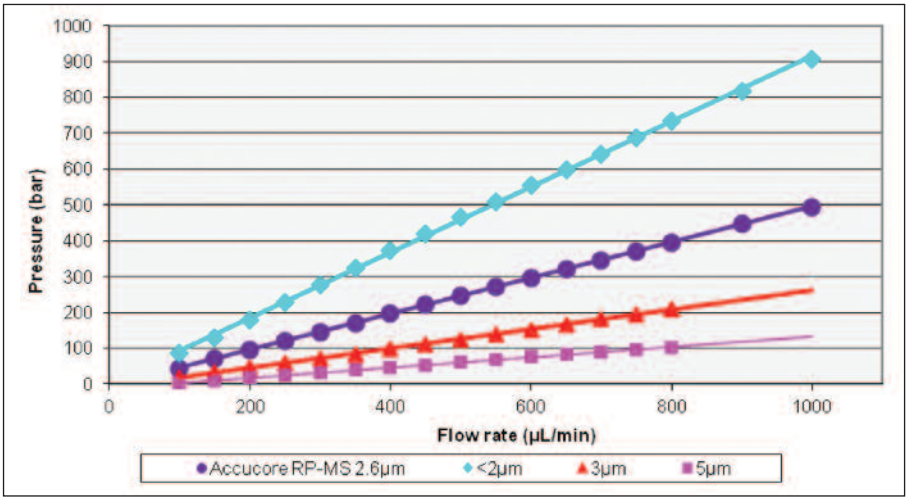


Figure 4: Comparison of column pressure for Accucore 2.6 µm and fully porous 5 and 3 and sub-2µm (100x2.1 mm columns, mobile phase: water/acetonitrile (1:1), temperature 30°C).

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].

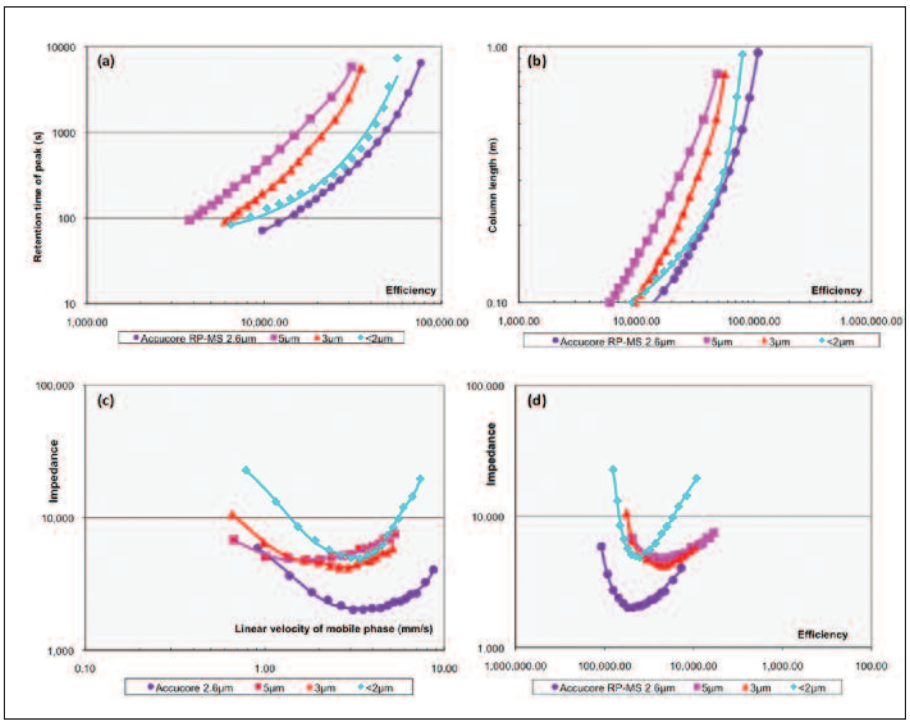


Figure 5: Performance comparison of Accucore 2.6µm and fully porous 5, 3 and sub-2µm using kinetic plots: (a) efficiency per unit time, (b) efficiency per column unit length, (c) impedance relative to mobile phase linear velocity, (d) impedance relative to efficiency.

Equation 6

E – impedance

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

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

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

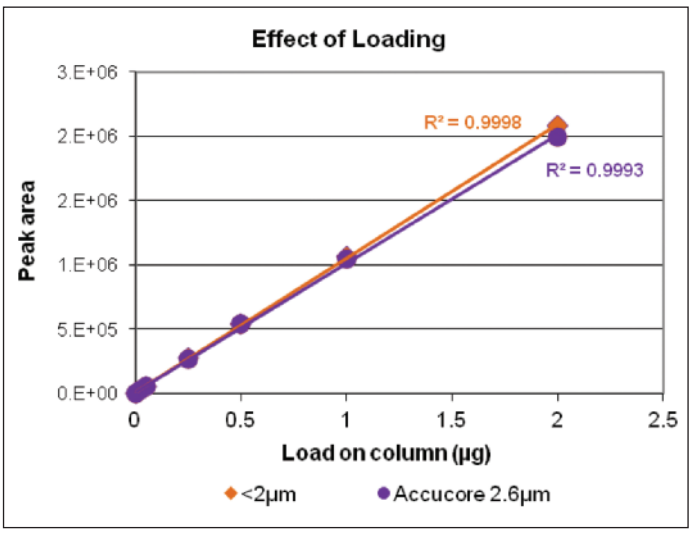
Δt – analysis time for which the peak capacity is calculated

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



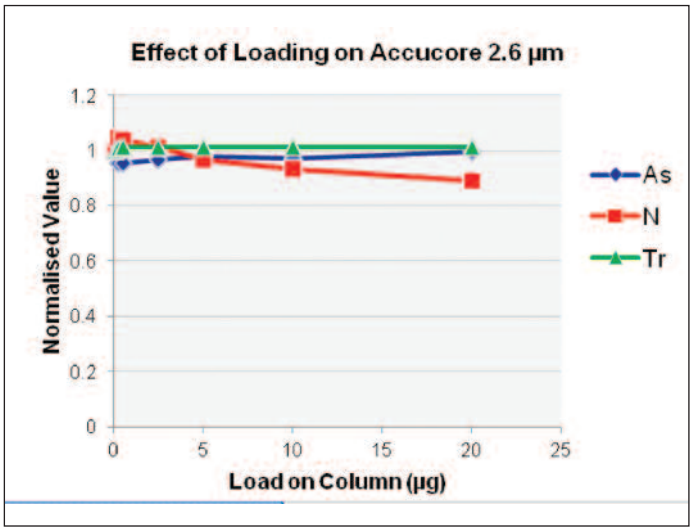


Figure 6: Effect of column loading on chromatographic parameters. (a) Comparison of loading 2µg on solid-core 2.6µm and fully porous sub-2µm; (b) Effect of loading on peak asymmetry (As), retention time (Tr) and efficiency (N) on the solid-core column.

	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

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

R_S – Resolution between a pair of peaks

t_x – retention time of peak x
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.

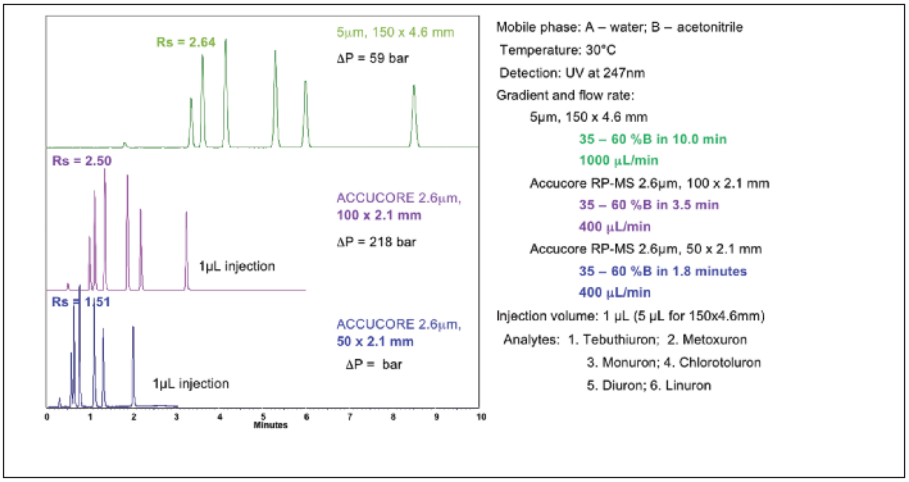


Figure 7. Example of method transfer from fully porous 5µm 150x4.6mm column to Accucore 2.6µm, 100x2.1 ID mm column (similar column chemistry)

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

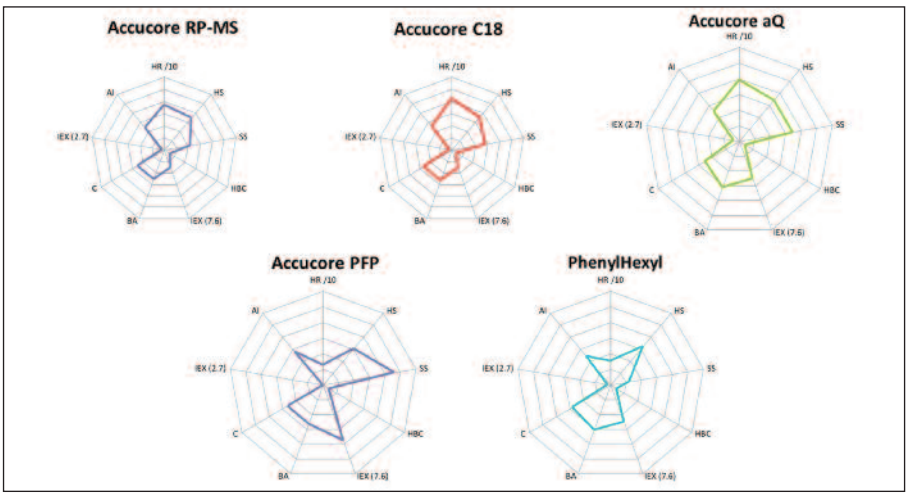


Figure 8: Radar plots for Accucore stationary phases: comparison of the phase selectivities. Tables 2, 3 and 4 for axis labels.

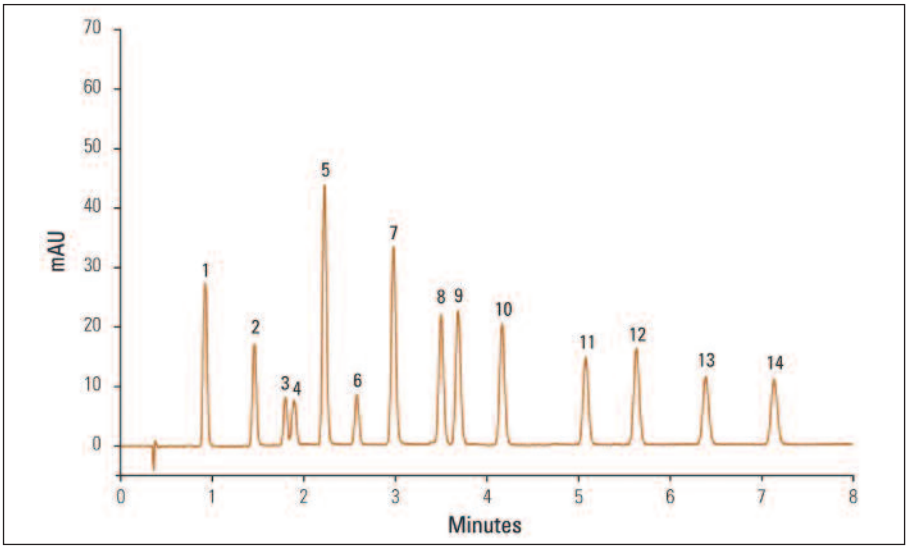


Figure 9: Separation of 14 positional isomers on Accucore PFP. Experimental conditions: Column - Accucore PFP 2.6µm, 50mm x 2.1mm; Mobile phase: A – Water + 0.1% Formic Acid, B – Acetonitrile + 0.1% Formic Acid; Gradient: 15 – 30% B in 7 minutes; Flow rate: 600µL/min; Temperature: 50°C; Detection: UV 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

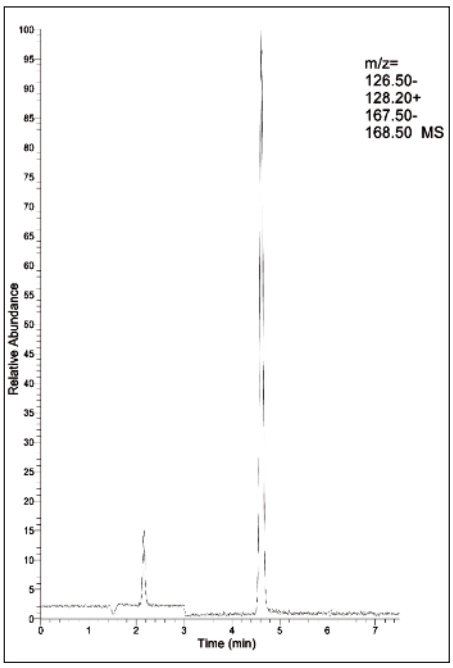


Figure 10. Separation of melamine and cyanuric acid on Accucore HILIC. Experimental conditions: Column Accucore HILIC 2.6µm, 150mm x 4.6mm; Mobile phase: 90:10 (v/v) Acetonitrile:50mM Ammonium Acetate, pH 5; Flow rate: 1mL/min; Temperature: 40°C; Detection: MS at m/z 127, 128, 168 (negative mode 0-3 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

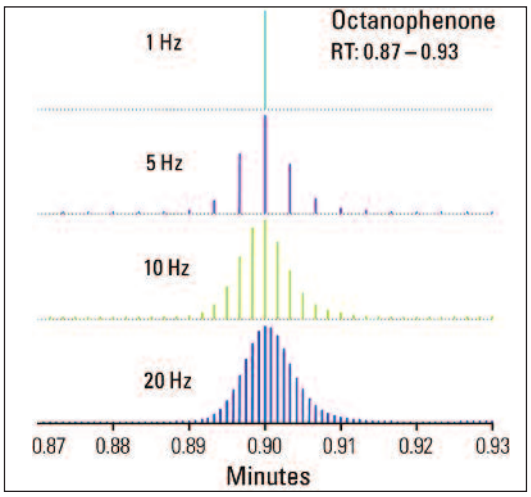


Figure 11: Effect of detector sampling rate on the peak height and peak area.

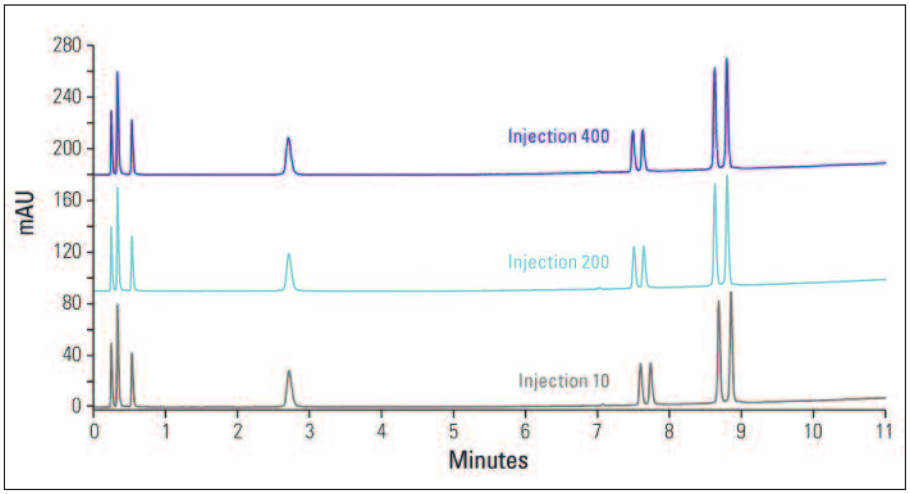


Figure 12: Accucore column stability at pH 1.8. Experimental conditions: Column - Accucore C18 2.6µm, 100 x 2.1mm; Mobile phase: A – Water + 0.1% Trifluoroacetic Acid, B – Methanol + 0.1% Trifluoroacetic Acid; Gradient: 25%B for 0.75 min, then to 100%B by 10 min, hold at 100%B for 2 min, return to 25%B 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.

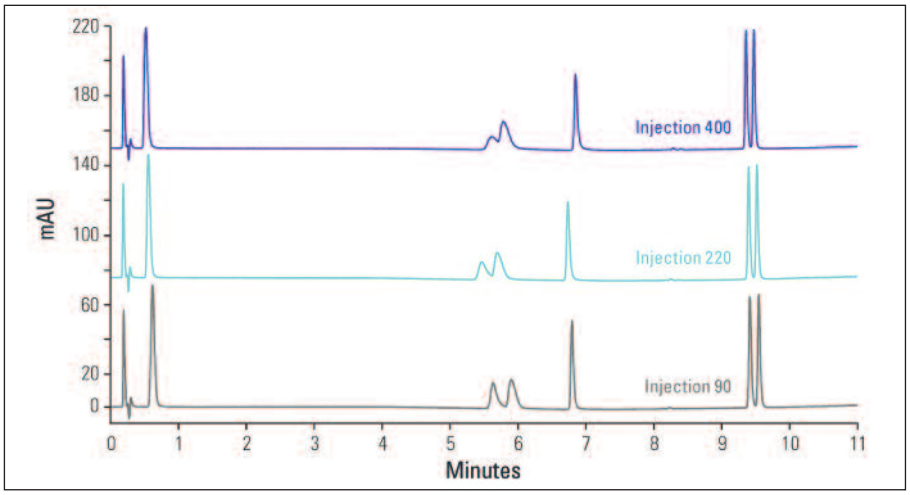


Figure 13: Accucore column stability at pH 10.5. Experimental conditions: Column - Accucore C18 2.6µm, 100 x 2.1m; Mobile phase: A – Water + 0.1% Ammonia, B – Methanol + 0.1% Ammonia; Gradient: 15%B for 1 min, then to 100%B by 8 min, hold at 100%B for 3 min, return to 15%B and hold for 5 min for re-equilibration; 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.

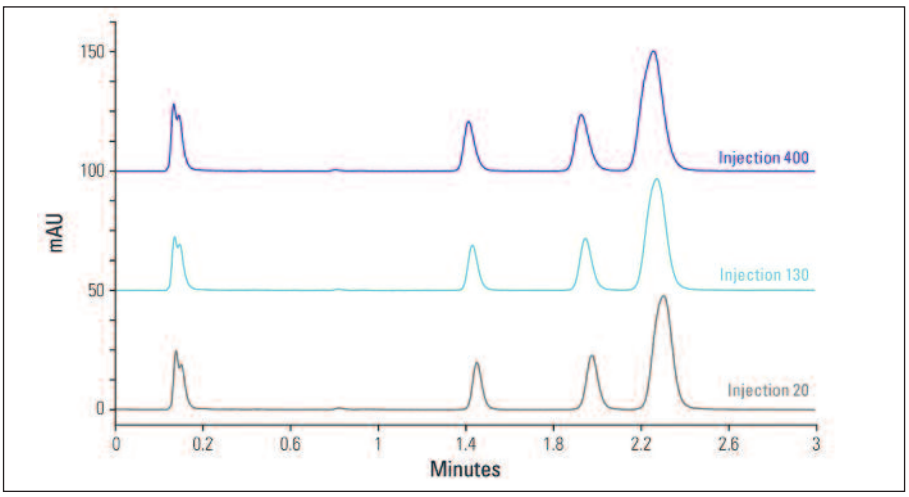


Figure 14: Accucore column stability at 70° C. Experimental Conditions: Column - Accucore C18 2.6µm, 100 x 2.1mm; Mobile phase: 35:65 (v/v) Water/Methanol; Flow rate: 400µL/min; Injection volume: 1.5µL; Temperature: 70°C; Detection: UV at 254nm (0.1s rise time, 20Hz); Order of elution: 1. Theophylline/Caffeine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