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Chromatography Solutions

Chromatography white paper

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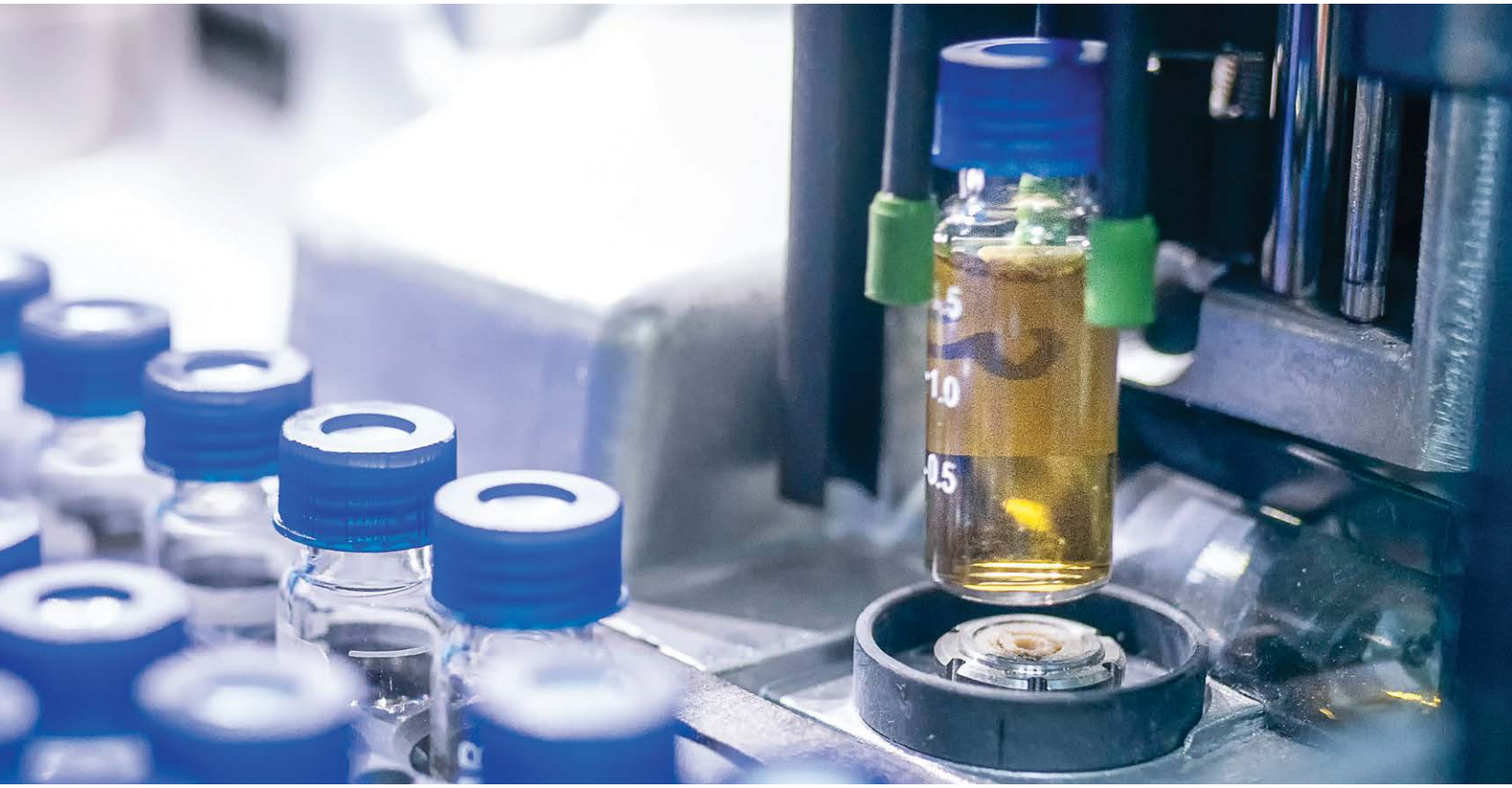
Solid core particles: Application to small molecule LC analysis



ABSTRACT

The current generation of solid core particles for LC analysis were introduced in 2006 and provide higher efficiency separations than equivalently sized fully porous particles. Since then, their use for the analysis of both small and large molecules has increased steadily, in a wide variety of analytical fields, to drive high performance separations and increases in laboratory throughput. This article focusses on the application of solid core particles to small molecule analysis. It begins by introducing the concept of solid core particles, their morphology, history of development and current methods of synthesis, before moving on to discuss the theory behind the performance advantages they offer. The Avantor® ACE® UltraCore range is then introduced, which combines solid core silica particles and a range of novel selectivity stationary phase chemistries. Finally, example applications that demonstrate how these particles can be used to provide solutions for some of the analytical challenges faced by the modern chromatography laboratory are discussed.

INTRODUCTION

Chromatography is a separation technique based on adsorption and/or interaction of a mixture of different molecules with stationary phases. Among different types of chromatography, high performance liquid chromatography (HPLC) has been most widely used as an essential analysis tool for research, manufacturing, environmental monitoring and drug development.[1] This is due to its universal applicability and remarkable assay precision.[2] The silica microsphere is the dominant base architecture for stationary phases found in HPLC, although other materials based on organic polymers and other structures such as monolithic and irregular shaped beads are also available. The challenges that are faced in performing HPLC are best suited by this particle, which is stable in a wide range of aqueous and organic mobile phases, relatively cheap, amenable to a wide range of chemistry modifications, as well as being physically stable to the pressures that are seen within a typical separation process. The spherical nature of the bead has advantages in both the manufacturing process, in ensuring the generation of homogeneous particles, and in ensuring optimal chromatographic performance by minimising the dispersion processes occurring in the column.

For small nonporous particles, the separation is performed on the particle surface and band-broadening is alleviated because of the short diffusion path, thus allowing faster mass transfer.[3] However, due to the low surface area, separation resolution and selectivity are limited. The surface area can be dramatically increased, up to 100-fold, by the introduction of pores into the main bead structure. For liquid phase separation, the pore sizes are required to be greater than ~ 7 nm to allow sufficient mass transport. For separation of large biomolecules, large pores up to 1000 Å may be required for efficient separation.[4]

UHPLC

The size of silica particles and the packing quality can significantly affect the performance of the packed columns. HPLC separation performance (in terms of theoretical plate numbers) can be doubled by reducing the particle diameter by half, however this will quadruple the back pressure at the same time, equation 1.[4]

$$P = \frac{\eta \upsilon L}{d_p^2}$$

EQUATION 1

Where η is mobile phase viscosity, υ is mobile phase velocity, L is column length and d_p is particle size.

Manufacturers have utilised the simplistic relationship between column performance and particle size to allow for the development of ultra-high pressure liquid chromatography (UHPLC), which is based on the utilisation of very small,

sub-2 µm, silica particles. However, the considerable increase in back pressure[5], is often seen as a detrimental feature, due to the technical challenges faced with operating at these elevated pressures. The ability to operate with high efficiencies without the need for higher pressures was ultimately the driver for the development and success of the current generation of solid core particles.

INTRODUCTION TO SOLID CORE

In recent years, solid core silica particles (core shell and porous shell) have been increasingly used for highly efficient separations with fast flow rate and relatively low back pressure[6], thus addressing the pressure issues associated with UHPLC. The reasons for the benefits will be discussed in detail later in this article, however, in summary there are three dominant factors:

- The smaller porous volume which reduces the volume present for broadening from longitudinal diffusion (B term in van Deemter equation)[7]
- The shorter diffusion path length also improves the C term due to faster mass transfer[5, 8]
- The more homogeneous particle size of the packing material allowing for better packing[9]

As the thickness of the porous shell decreases, the faster mass transfer can lead to improved column efficiency and faster elution times [10, 11], however, this will also result in a reduction of the available surface area and consequently affect the loading capacity of the material.

HISTORY

The notion of solid core particles was first proposed by Horváth[12], who suggested that a substantial improvement in chromatographic performance could be obtained by reducing the available dispersion within the column. This initially led to the concept and development of pellicular particles. This term is a Latin-based term derived from pellis which means pelt, or outer skin. Early generations of pellicular particles were covered in the review by Guiochon et al.[6] Unfortunately, the commercialisation of these products struggled due to the large size of the core, typically in the order of 50 µm, compared with contemporary irregular and spherical fully porous materials that had much smaller particle sizes of the order of 10 µm. Particle size contributes significantly to the peak width, which meant at that time, that the advantages of the pellicular material were far outweighed by the difference in particle size.[8]

It was Kirkland who developed the modern format of the superficially porous material, which has seen the generation of spherical particles with diameters comparable to HPLC and UHPLC particles, and with a range of thicknesses of the outer porous layer. With the issues addressed regarding the particle size, there was a rapid growth in the uptake of this technology as it was seen as UHPLC without the need for excessive pressures. It should be stated though that in developing the technology there were several different strategies that were unsuccessfully employed.[13, 14, 15, 16, 17, 18]

CURRENT SYNTHESIS

The approach that Kirkland developed, and many manufacturers employ today, is to attach nanospheres onto a solid core. This approach is referred to as the layer-by-layer (LbL) approach.[6] With this approach the core silica particles are firstly bound with a polyelectrolyte (e.g., negatively charged silica particles bound with a cationic polymer). Any excess polyelectrolyte is removed by rinsing. The coated core particles are then immersed in a dispersion of nanoparticles with charges opposite from those of the organic polyelectrolyte. This process is repeated by alternating immersions between the polyelectrolyte solution and

the nanoparticle suspension until the desired shell thickness is achieved.[19] The resulting particles can then be treated thermally to remove the organic polyelectrolyte and produce solid core porous-shell particles. An example of the resulting structure is shown in Figure 1.

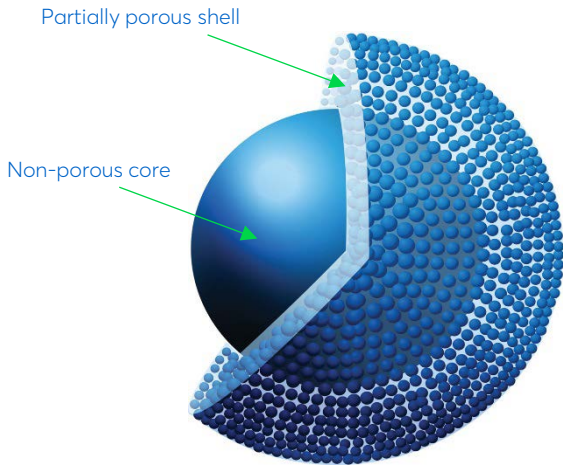


FIGURE 1: Schematic representation of solid core particle morphology.

The resulting structure of the particle is then defined by the size of the silica solid core and the number of layers that are added. This will result in different chromatographic properties, such as loading capacity, column backpressure and chromatographic efficiency. The productivity of manufacturing solid core silica particles using the LbL approach is inherently low, due to the numerous reaction and wash steps that are needed. The manufacturing process can take many weeks to generate a final batch of material. Manufacturers have looked at different approaches to address this issue, and also to circumvent the patents associated with this technology. The different approaches include:

- A multilayer process where multiple layers are added in one stage.[20, 21] These particles had a higher level of porosity than those obtained by the traditional LbL process. The multilayer adsorption phenomenon was attributed to the formation of nanoparticle aggregates, reduced repulsive force between nanoparticles and increased non-electrostatic attraction between nanoparticles and polyelectrolytes[21]
- Silica-polymer solid core particles by silica-supported polymerization[22]
- Solid core hybrid particles and hollow structures by precipitation polymerization[23]
- Silica-metal-organic frameworks (MOFs) solid core microspheres[24, 25]
- Magnetic silica solid core particles.[26, 27] This has the added advantage that the particles are easier to handle during the manufacturing process.

A much quicker process was reported by Ahmed et al.[28] who demonstrated that it was feasible to produce a one-pot synthesis of solid core material. The resulting beads were referred to as having a spheres-on-sphere (SOS) morphology. It was noted that it was feasible to generate beads within a day using this approach, although it should be stated that the resulting morphology was sensitive to a wide range of parameters including stirrer speed and temperature.

The following sections of this white paper begin by briefly discussing dispersion theory and how the architecture of solid core particles leads to improved performance compared to fully porous particles. The Avantor® ACE® UltraCore range of columns is then introduced, including the range of stationary phase chemistries that are available and their applicability to method development. Finally, the application of solid core particles to a variety of chromatographic separations of small molecules in different analytical fields is demonstrated.

ADVANTAGES & THEORY OF SOLID CORE PARTICLES

Solid core particles offer distinct advantages compared to fully porous particles (FPP). In particular, they provide greater column efficiency than equivalently sized fully porous particles. For example, columns packed with 2.5-2.7 µm solid core particles can provide theoretical plate values comparable to 1.7 µm

fully porous particles. Critically, this elevated performance is achieved at substantially reduced backpressure compared to the smaller diameter particles (due to the inverse squared relationship between pressure and particle size). This makes their use highly attractive; columns packed with solid core particles are compatible with standard 400 bar HPLC systems and can be used to increase separation efficiency and drive improvements in sample throughput. This makes solid core particles an ideal option for increasing separation efficiency, without the need for Ultra High Performance LC (UHPLC) equipment.

WHY ARE SOLID CORE PARTICLES MORE EFFICIENT?

To fully understand the benefits offered by solid core particles, it is necessary to consider band broadening, or dispersion theory. In liquid chromatography, the term band broadening (i.e. an increase in peak width) refers to processes that cause spreading of an analyte band as it migrates through the LC system and column. In practical terms, band broadening results in loss of efficiency, loss of resolution and deterioration of the chromatographic performance of a method.

The van Deemter equation describes the various physical processes that contribute to band broadening and relates the column efficiency obtained (expressed as Height Equivalent to a Theoretical Plate, *HETP*) to the linear velocity of the mobile phase as it flows through the column. In its simplified form (equation 2), the van Deemter equation describes three terms which contribute to band broadening inside an LC column (*A*, *B* and *C*) and relates them to the mobile phase linear velocity (*u*).[8, 29, 30]

$$HETP = A + B/u + C.u$$

EQUATION 2

A = Eddy diffusion
B/*u* = Analyte longitudinal / axial diffusion
C.*u* = Analyte mass transfer between stationary & mobile phases

Plotting HETP against the mobile phase linear velocity (i.e. flow rate) generates the composite plot shown in Figure 2A. By considering this plot, it is possible to understand the influence of each of the three terms on column efficiency.

A: Eddy Diffusion
The A-term relates to band broadening that occurs as a result of the uniformity of the column packed bed (i.e. the quality of the packed bed) and resulting flow unevenness. An analyte molecule may take any one of many different flow paths through the column packed bed, due to packed bed heterogeneity, ultimately leading to a broadening of the analyte band as it passes through the column. The A term can be reduced by using well packed columns and using smaller particle sizes with a narrow particle size distribution.

B: Longitudinal (Axial) Diffusion
Within an analyte band, analyte concentration is greatest at the centre. Therefore, a concentration gradient exists and as the band migrates through the column, analyte molecules tend to disperse outwardly from the peak centre with time, causing the band to broaden. The B term is reduced significantly at higher mobile phase linear velocities (i.e. higher flow rate) (Figure 2A). Importantly, longitudinal diffusion also occurs within any system dead volume. It is therefore important to minimise connecting tubing where possible and ensure that it is correctly installed with good fittings.

C: Resistance to Mass Transfer
Resistance to mass transfer is an artefact of insufficient equilibration of the concentration gradient of the analyte band as it moves across the surface of a pore, and involves a combination of contributions. This includes mass transfer between the mobile phase and the stationary phase particle via diffusion across a stationary film of mobile phase at the particle surface; analyte diffusion through the porous structure and adsorption-desorption kinetics. All these contributions operate on finite timescales and so contribute to band broadening. The mass transfer term is more dominant at higher flow rates (Figure 2A). Mass transfer can be reduced by using a smaller particle size or by heating the column to increase the rate of diffusion.

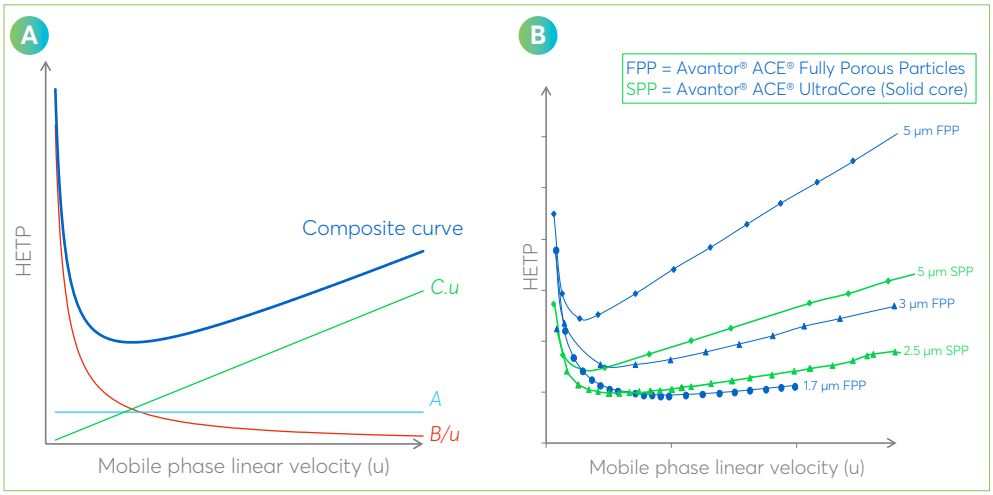


FIGURE 2: **A.** Composite van Deemter curve and relative contributions from the three terms described in equation 2. - **B.** Experimentally determined van Deemter plots for Avantor® ACE® fully porous particles (FPP) and solid core particles (SPP).

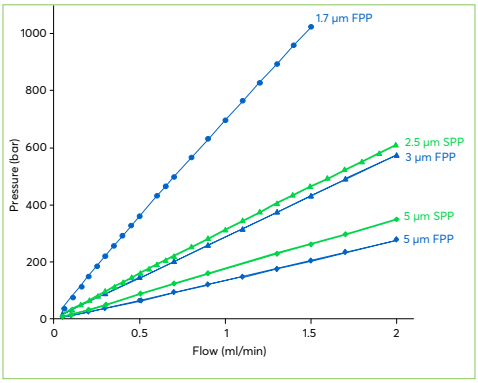


FIGURE 3: Plot of flow rate vs total pressure for 50 x 2.1 mm columns packed with ACE solid core and fully porous particles. Isocratic analysis of Naproxen using MeCN/H_2O + 0.1% TFA, k = 10, 40 °C, λ = 265 nm.

In practical terms, maximum column efficiency is achieved at a specific linear velocity or flow rate, at the minimum of the curve in Figure 2. It is therefore important to perform separations at a suitable flow rate to minimise both the B and C terms, operating at too high or too low flow rate will result in a loss in efficiency. In order to minimise the A term, the use of well packed columns containing high quality packing materials, such as the Avantor® ACE® range, is vital.

Experimentally generated van Deemter plots are valuable for comparing particles of differing size and morphology. Figure 2B demonstrates columns packed with smaller particles deliver higher efficiency (i.e. lower HETP). In addition, for smaller particles, the optimum flow rate is higher than for larger particles. The curve is also flatter at higher flow rates for smaller particles, which means that even higher flow rates can be utilised to achieve ultra-fast UHPLC separations.

Considering the curves for the solid core particles (Figure 2B), these particles clearly offer performance advantages compared to their fully porous counterparts. Comparing the 5 µm solid core and 5 µm FPP, a dramatically lower HETP value is obtained for the solid core particle. Solid core particles can therefore be utilised on any standard HPLC system to improve chromatographic performance over traditional 5 µm fully porous particles, without encountering back pressure issues. Additionally, the 2.5 µm solid core particles can deliver significantly higher performance than a 3 µm FPP and is similar to the performance of a fully porous 1.7 µm UHPLC particle. However, as shown by Figure 3, this increased performance is obtained at more modest pressures. The higher efficiencies of solid core particles mean that shorter column lengths can be used, leading to reduced run times and improvements in laboratory efficiency.

It was previously widely stated that improved mass transfer (i.e. the C-term) was responsible for the higher performance of solid core particles. However, more recent studies have shown that for small molecules, this contributes less than expected and that it is reductions in both the A and B-terms that are primarily responsible.[6, 31, 32] A reduction of the A-term provides the largest contribution to the improved efficiency, potentially due to a more homogenously packed bed for columns packed with solid core particles.[32] A reduced B-term contribution also improves performance as the solid core reduces the internal dead volume of

the column (i.e. reduced internal porosity) and hence reduces the longitudinal diffusion coefficient term.[6] The inverse relationship to linear velocity does however mean that improvements in the B-term are more impactful at low flow rates (Figure 2A). Although the C-term is reduced, it provides a minor contribution to reduced band broadening for small molecules (<1,000 Da).[33]

For large molecules, the situation is somewhat different. The influence of the C-term on overall band broadening is much more impactful, due to the much lower diffusion coefficients of large molecules. A reduction in the C-term through introduction of the solid core into the particle is therefore highly beneficial for large molecules, in theory reducing the shell thickness and hence minimising mass transfer effects can provide further benefits.[34]

THE AVANTOR® ACE® ULTRACORE RANGE FOR SMALL MOLECULES

Columns packed with solid core particles have been successfully utilised for the analysis of small molecules in a wide range of application areas. Small molecule LC analysis is typically performed using stationary phases with pore sizes of approximately 100 Å. Table 1 provides details of all the solid core phases available for small molecule analysis in the Avantor® ACE® UltraCore range. A separate range of 300 and 500 Å wide pore UltraCore BIO phases are additionally available for the analysis of large biomolecules but are beyond the scope of this paper.[35, 36]

Stationary phase chemistry is a powerful parameter for varying the overall chromatographic selectivity of an LC separation and is therefore an important method development tool.[37] Assessing a variety of different stationary phase chemistries during method development (along with different organic modifiers, pH etc.) maximises the chances of successfully separating all analytes in a sample. A variety of stationary phases are therefore available in the Avantor® ACE® UltraCore range, which provide different analyte/stationary phase retention mechanisms under reversed-phase conditions.

Stationary phase selectivity can be empirically compared through column characterisation tests, for example, the Tanaka[38] and PQRI (Product Quality Research Institute)[39] approaches. These use well defined molecular probes, to assess specific analyte-stationary phase retention mechanisms. It has been extensively demonstrated that such approaches allow meaningful and reliable comparisons of stationary phase chemistries.[40]

Figure 4A shows Tanaka characterisation data for four Avantor® ACE® UltraCore solid core stationary phases. A comprehensive explanation of individual Tanaka parameters is provided in reference 40. This data clearly confirms the different selectivity offered by these columns, indicating that each stationary phase can provide substantially different selectivity for a given separation. Figure 4B shows a simple test mix injected on the four stationary phases using the same analytical conditions. While all four stationary phases were able to separate these components, clear differences in the elution order and peak spacing was observed.

In addition, further method development capability is provided by the SuperC18 and SuperPhenylHexyl stationary phases, which are manufactured using unique encapsulation technology. These two stationary phase chemistries are compatible with a wider range of mobile phase pH's, which permits separations to be run at

Phase	USP Listing	Stationary phase chemistry	End-capping	Particle size (µm)	Pore size (Å)	Surface area (m^2/g)	Carbon load (%)	pH range
UltraCore SuperC18	L1	Octadecyl	Encapsulated	2,5	95	130	7,0	1.5-11
				5		100	5,4	
UltraCore SuperPhenylHexyl	L11	Phenyl-Hexyl	Encapsulated	2,5	95	130	4,6	1.5-11
				5		100	3,6	
UltraCore C18	L1	Octadecyl	Yes	3,5	95	115	8,2	2-9
UltraCore Phenylhexyl	L11	Phenyl-Hexyl	Yes	3,5	95	115	6,2	2-9
UltraCore Biphenyl	L11	Biphenyl	Yes	3,5	95	115	6,5	2-9
UltraCore C18-Amide	L60	C18-Amide	Yes	3,5	95	115	5,5	2-9

TABLE 1: Avantor® ACE® solid core columns for small molecule analysis.

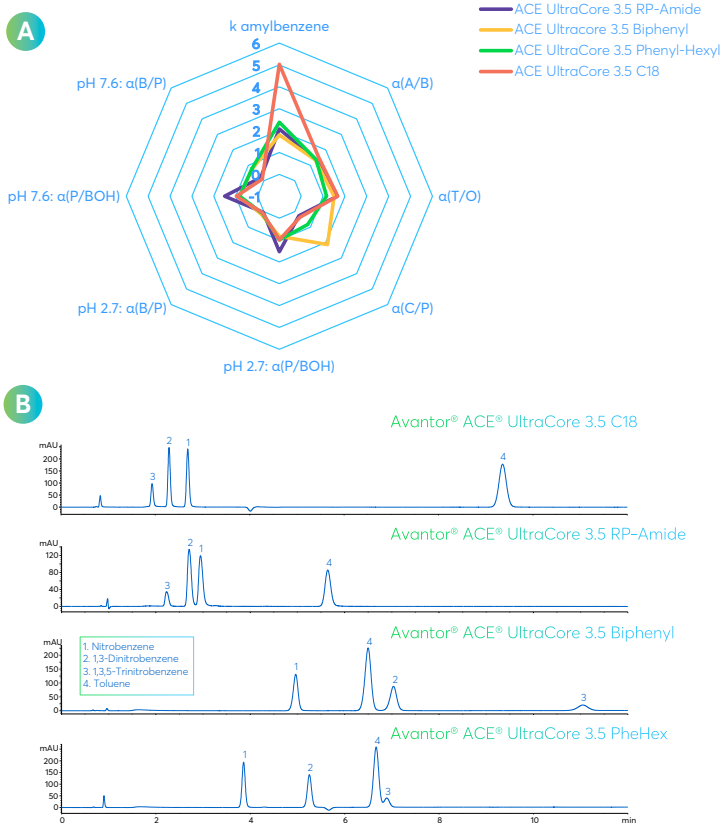
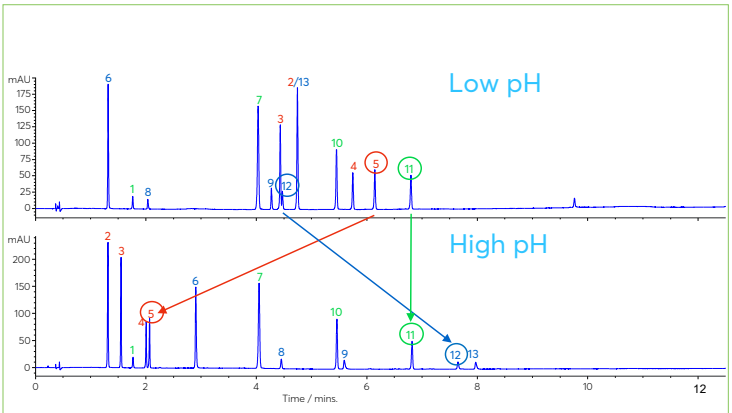


FIGURE 4: **A.** Tanaka characterisation data for four Avantor® ACE® UltraCore stationary phase chemistries, showing the relative contributions to overall stationary phase selectivity. **B.** Separation of nitro compounds on the same four columns under identical conditions, demonstrating the impact of stationary phase chemistry on the separation obtained.



1. Methylphenyl sulfoxide, 2. Ketoprofen, 3. Sulindac, 4. Diclofenac, 5. Mefenamic acid, 6. N-Acetylprocainamide, 7. 1,4-Dinitrobenzene, 8. Prilocaine, 9. Carvedilol, 10. Piperine, 11. Heptanophenone, 12. Trimipramine, 13. Clomipramine

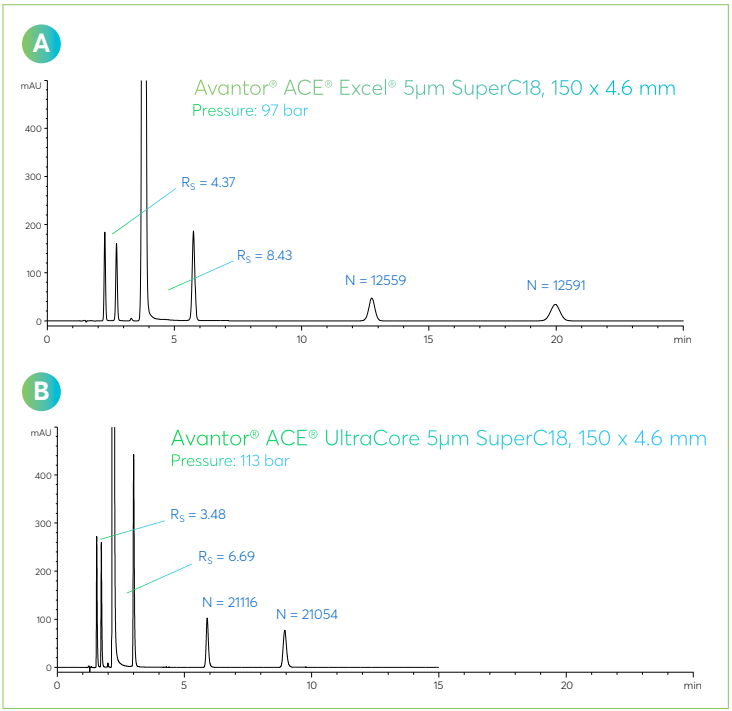
Column:	Avantor® ACE® UltraCore 2.5 SuperPhenylHexyl, 100 x 3.0 mm
Mobile phase (low pH):	A: 15 mM ammonium formate pH 3.0 (aq) B: 15 mM ammonium formate pH 3.0 in MeCN/H_2O 9:1 v/v
Mobile phases (high pH):	A: 0.1% NH_3 (aq) B: 0.1% NH_3 in MeCN/H_2O 9:1 v/v
Gradient:	5 to 100% B in 10 minutes
Flow Rate:	1.2 ml/min
Injection Volume:	1 µL
Temperature:	40 °C
Detection:	UV, 260 nm

FIGURE 5: Separation of a range of acidic, basic and neutral analytes on an alkali-compatible solid core column with novel encapsulated bonding.

both high and low pH. For samples containing ionisable analytes, it can be highly beneficial to assess separation selectivity at both pH extremes.[41] Figure 5 demonstrates how dramatically different selectivity can be obtained for such samples by varying mobile phase pH. The range of column selectivities available, coupled with the extended pH stability of the SuperC18 and SuperPhenylHexyl phases highlights the applicability of the Avantor® ACE® UltraCore range as an excellent method development platform.

APPLICATIONS FOR SMALL MOLECULE ANALYSIS

Over the last 15 years, solid core particles have rapidly gained wide acceptance in a large variety of small molecule application areas, ranging from pharmaceutical to environmental and clinical testing. Reversed-phase is still the most widely used form of liquid chromatography, therefore development and application of solid core stationary phases has primarily been focussed in this area. The solid core particle has been widely demonstrated to be highly



Method A

Mobile phase	H_2O/MeCN/MeOH/H_3PO_4 (60:35:5:0.2 v/v/v/v)
Temperature:	25°C
Flow Rate:	1 ml/min
Injection Volume:	5 µL
Detection:	UV, 237 nm (2.5 Hz)

Method B

Mobile phase	H_2O/MeCN/MeOH/H_3PO_4 (60:35:5:0.2 v/v/v/v)
Temperature:	25°C
Flow Rate:	1 ml/min
Injection Volume:	3.9 µL
Detection:	UV, 237 nm (20 Hz)

Sample:	1. 4-hydroxybenzoic acid, 2. 4-hydroxyisophthalic acid, 3. acetylsalicylic acid (aspirin), 4. salicylic acid, 5. acetylsalicylsalicylic acid, 6. salsalate
Impurities were spiked at:	0.5% w/w

FIGURE 6: Improving performance/throughput of an isocratic method for the analysis of aspirin and related impurities on 3-400 bar HPLC systems.

suited to reversed-phase small molecule analysis and is able to deliver fast, high-efficiency and high-resolution separations. This section aims to present a non-exhaustive overview of the types of analytical applications to which the Avantor® ACE® UltraCore range of solid core columns can provide practical benefits.

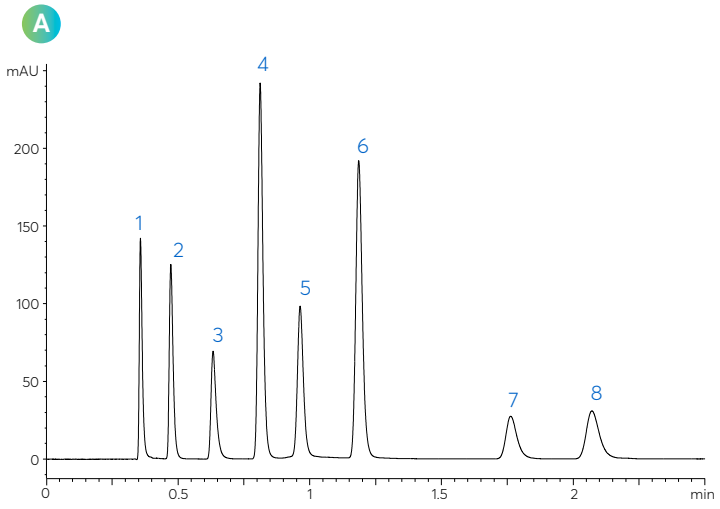
A major benefit is that by switching from fully porous to solid core columns, higher efficiencies can be obtained on standard 400 bar HPLC instruments. In addition, due to the presence of the solid core, these particles have a lower surface area and are consequently less retentive. Many laboratories have therefore been able to utilise solid core columns to obtain higher performance and improved sample throughput from their existing HPLC systems, as an alternative to investing in expensive upgrades to UHPLC instrumentation. Figure 6 demonstrates this approach. In this example, an existing method for the analysis of aspirin and related substances run on a 150 x 4.6 mm 5 µm fully porous column was moved to a 5 µm UltraCore solid core particle. The column dimensions and stationary phase chemistry were identical and all method parameters, except for injection volume and detector rate (which were reduced to account for the lower internal volume of the solid core column and to accurately record the lower peak volume respectively) were kept constant. The efficiency gains from switching to the equivalent UltraCore column are clearly demonstrated, with the two final peaks showing a near doubling of efficiency. Notably, this increase in efficiency is delivered despite a substantial reduction in retention for all analytes. This does result in a slight loss of resolution for poorly retained analytes in this example, however, although the UltraCore column is less retentive, it maintains acceptable resolution of the peaks, with less than half the analysis time and equivalent back pressure. The resulting separation was entirely compatible with the existing 400 bar HPLC system, therefore delivering a doubling in sample throughput and instrument utilisation.

As discussed earlier, UltraCore columns deliver increased efficiencies compared to fully porous particles of similar size. In the case of 2.5 µm solid core particles, performance comparable to sub-2 micron fully porous UHPLC particles is achievable, without the associated high back pressure. High separation

efficiencies can therefore be obtained using shorter columns lengths, leading to reduced run times. Additionally, the somewhat flatter van Deemter curves means that higher mobile phase flow rates can be utilised, providing further improvements in run time and sample throughput.

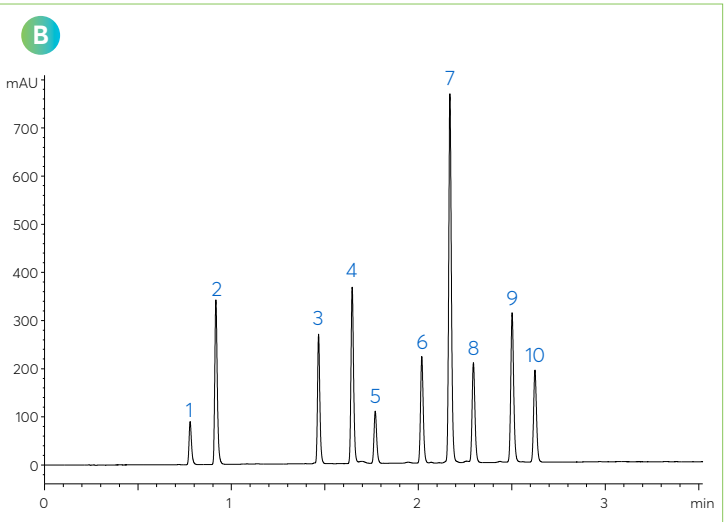
Figure 7 demonstrates the use of Avantor® ACE® UltraCore SuperC18 particles packed in small format columns, to obtain fast separations in both isocratic and gradient reversed-phase analyses. In both cases, the target analytes are fully separated in less than 3 minutes. These two applications utilise elevated linear velocities (equivalent to 2 ml/min on a 4.6 mm ID column), yet these separations were achieved at relatively modest back pressures of 426 bar and 203 bar respectively and are therefore compatible with 4-600 bar HPLC systems.

The ability to obtain rapid, high resolution separations makes solid core particles ideal for use in high throughput applications,



1. Maleic acid, 2. Norephedrine, 3. Doxylamine, 4. Salicylamide, 5. Guaifenesin, 6. Guaiacol, 7. Chlorpheniramine, 8. Triprolidine

Column:	Avantor® ACE® UltraCore 2.5 SuperC18
Dimensions:	75 x 3.0 mm
Mobile Phase:	30 mM KH_2PO_4 pH 2.7 in H_2O/MeOH (60:40 v/v)
Flow Rate:	0.85 ml/min
Injection:	0.9 µL
Temperature:	30 °C
Detection:	UV, 214 nm



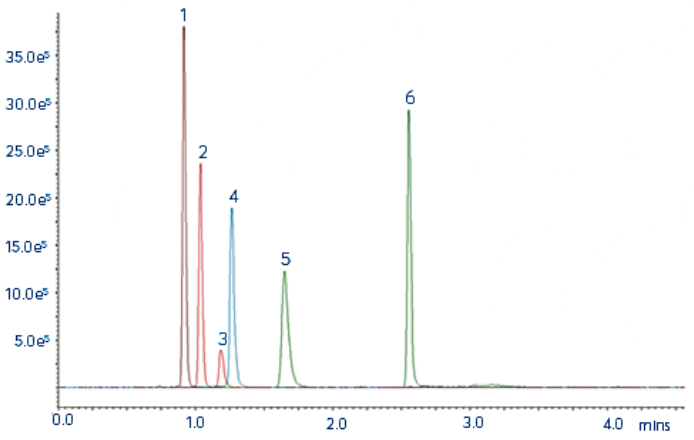
1. Aspirin, 2. Phenacetin, 3. Sulindac, 4. Tolmetin, 5. Naproxen, 6. Nimesulide, 7. Flurbiprofen, 8. Diclofenac, 9. Phenylbutazone, 10. Meclofenamic acid

Column:	Avantor® ACE® UltraCore 2.5 SuperC18
Dimensions:	50 x 3.0 mm
Mobile Phase:	A: 0.1% formic acid in H_2O B: 0.1% formic acid in MeCN
Gradient	**Time (min)** / **% Mobile phase B** 0.2 / 20 2.71 / 70 3.39 / 70 3.52 / 20
Flow Rate:	0.85 ml/min
Injection:	1.04 µL
Temperature:	40 °C
Detection:	UV, 254 nm

FIGURE 7: Applications demonstrating rapid, high efficiency separations achievable with solid core particles. **A:** Isocratic separation of antihistamines and **B:** Gradient separation of non-steroidal anti-inflammatory drugs, on Avantor® ACE® UltraCore SuperC18 columns.

such as clinical analyses. Additionally, due to the larger particle size and higher porosity frits used in column packing, solid core columns can be less prone to plugging than equivalent sub-2 µm UHPLC particles. This makes their use potentially advantageous for applications involving complex or "dirty" sample matrices, such as plasma or urine. Two common clinical applications are shown in figure 8: the separation of vitamins D2 and D3 active forms, and catecholamines and their metabolites. These are both fast analyses that combine the resolving power of the UltraCore stationary phases with the sensitivity of tandem mass spectrometry. The high efficiency of the UltraCore column achieves the necessary chromatographic separation in fast run times, whilst the narrow, low volume chromatographic peaks aid in providing high sensitivity via MS/MS detection.

A



1. Norepinephrine (m/z 170 → 107), 2. Epinephrine (m/z 184 → 166), 3. Normetanephrine (m/z 184 → 166), 4. Dopamine (m/z 154 → 91), 5. Metanephrine (m/z 198 → 180), 6. 3-Methoxytyramine (m/z 181 → 91)

Column:	Avantor® ACE® UltraCore 2.5 SuperPhenylHexyl
Dimensions:	100 x 2.1 mm
Mobile Phase:	A: 2 mM ammonium formate + 0.05% formic acid in H_2O B: 2 mM ammonium formate + 0.05% formic acid in MeOH
Gradient	**Time (min) / % Mobile phase B** 0.00 / 0 1.00 / 70 1.10 / 70 1.11 / 0 4.50 / 0
Flow Rate:	0.3 ml/min
Injection:	10 µL
Temperature:	30 °C
Detection:	Shimadzu LCMS-8040 (ESI, positive ion mode)
Sample:	Standard 100 ng/ml in urine (after SPE purification)

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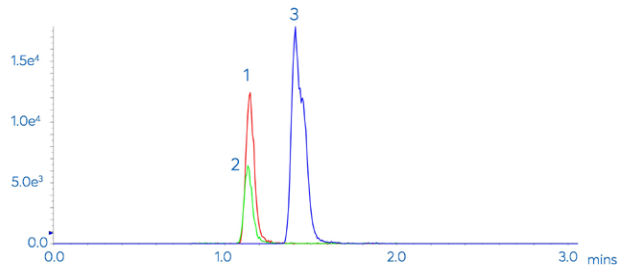
B



1. 1,25-diOH vitamin D3-PTAD-methylamine complex (m/z 623.4 → 314.1), 2. D3-1,25-diOH vitamin D3-PTAD-methylamine complex (I.S.) (m/z 626.4 → 317.1), 3. 1,25-diOH vitamin D2-PTAD-methylamine complex (m/z 635.4 → 314.1)

Column:	Avantor® ACE® UltraCore 2.5 SuperC18
Dimensions:	50 x 2.1 mm
Mobile Phase:	MeCN/H_2O (50:50 v/v) containing 30 µL methylamine per 500 ml
Flow Rate:	0.5 ml/min
Injection:	20 µL
Temperature:	40 °C
Detection:	AB Sciex 5500 triple quad MS - ESI, positive ion mode - IonSpray Voltage: 5500 V
Source temperature:	550 °C
Sample:	High calibration standard (500 pg/ml) in vitamin D stripped serum. 1,25-diOH vitamin D2 and 1,25-diOH vitamin D3 metabolites extracted from serum using SLE. LC-MS/MS sensitivity maximised through use of PTAD (9-phenyl-1,2,4-triazole-3,5-dione) derivatisation and methylamine complexation

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FIGURE 8: Clinical applications, **A:** LC-MS/MS determination of catecholamines and metanephrines spiked into urine and **B:** LC-MS/MS method for analysis of serum levels of the active forms of vitamin D.

The high efficiencies provided by solid core columns also make them ideally suited to other, highly complex LC-MS/MS applications, such as environmental monitoring, which may require the determination of hundreds of target analytes in a single analytical run. Figure 9 shows the application of an Avantor® ACE® UltraCore SuperC18 column to the analysis of pesticide residues in the environment. The high performance of the column allows sufficient chromatographic resolution to permit monitoring of all 300 components, via their respective MRM transitions, in one run.

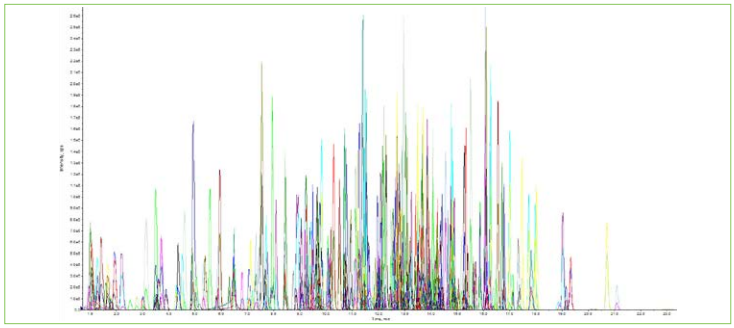
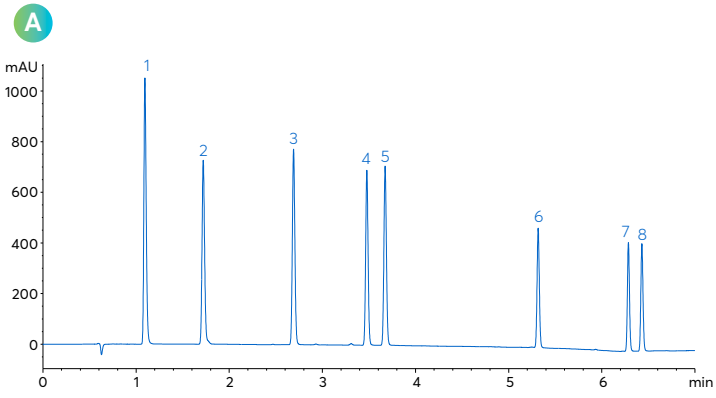
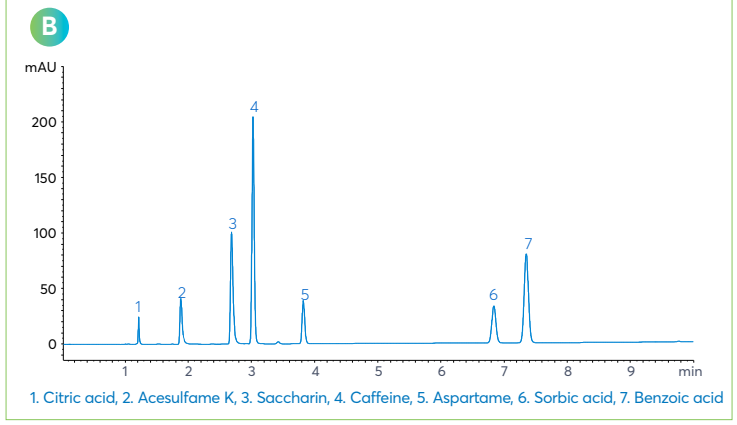


FIGURE 9: LC-MS/MS method for the analysis of 300 pesticides on an Avantor® ACE® UltraCore 2.5 SuperC18 column (100 x 2.1 mm). For full application details, please refer to reference 42. Reproduced with permission of National Food Chain Safety Office, Directorate of Plant Protection, Soil Conservation and Agri-Environment, Hungary.



1. Dimethyl phthalate, 2. Diethyl phthalate, 3. Di-n-propyl phthalate, 4. Benzyl butyl phthalate, 5. Di-n-butyl phthalate, 6. Di-n-hexyl phthalate, 7. Bis(2-ethylhexyl) phthalate, 8. Di-n-octyl phthalate

Column:	Avantor® ACE® UltraCore C18
Dimensions:	100 x 3.0 mm
Mobile Phase:	A: 0.1% Formic acid in H_2O B: 0.1% Formic acid in MeCN
Gradient	**Time (min) / % Mobile phase B** 0 / 20 5 / 70 10 / 70 10.5 / 20 18 / 50
Flow Rate:	0.6 ml/min
Injection:	2 µL
Temperature:	40 °C
Detection:	UV, 200 nm



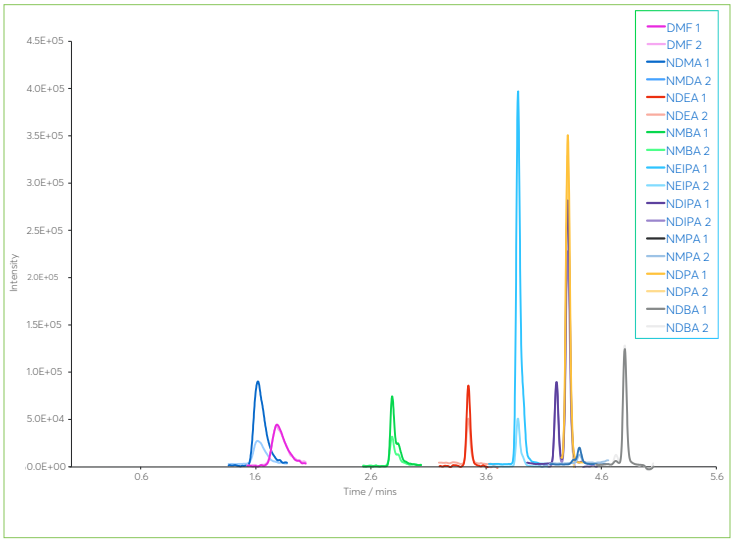
1. Citric acid, 2. Acesulfame K, 3. Saccharin, 4. Caffeine, 5. Aspartame, 6. Sorbic acid, 7. Benzoic acid

Column:	Avantor® ACE® UltraCore C18-Amide
Dimensions:	100 x 3.0 mm
Mobile Phase:	A: 20 mM KH_2PO_4 pH 2.0 in H_2O B: 20 mM KH_2PO_4 pH 2.0 in MeCN/H_2O 75:25 v/v
Gradient	**Time (min) / % Mobile phase B** 0 / 10 10 / 40 12 / 40
Flow Rate:	0.43 ml/min
Injection:	1 µL
Temperature:	50 °C
Detection:	UV, 214 nm

FIGURE 10: Applications relevant to food analysis. **A:** Separation of a range of phthalates on an Avantor® ACE® UltraCore C18 phase and **B:** Separation of caffeine and a range of sweeteners and preservatives utilising the alternative selectivity and enhanced retention of polar compounds provided by the Avantor® ACE® UltraCore C18-Amide phase.

Another important application area is food analysis. Figure 10A shows a simple hydrophobic separation of phthalates that is readily achieved using the UltraCore C18, which could be used for monitoring phthalate content in foodstuffs. In many applications, C18 stationary phases are not able to provide adequate retention or selectivity of target sample components. In these situations, the availability of a range of alternative selectivity stationary phase chemistries is highly beneficial. Figure 10B shows an example where the retention of more polar compounds is needed, a separation that is difficult to achieve on a C18 phase. In this case, the alternative selectivity provided by the UltraCore C18-Amide was found to be suited to the separation of caffeine, sweeteners and preservatives.

A final example of the solutions that can be provided by using different stationary phase chemistries is provided by nitrosamine analysis. The detection and quantification of nitrosamines in pharmaceutical drug substances and products, such as sartans, has become an important topic in recent years. The rapid separation of key nitrosamines identified by regulatory authorities can be achieved using solid core C18 stationary phases. However, given the low molecular weight of the nitrosamines that are typically monitored, interference from co-eluting matrix components can potentially cause problems. One such example is isobaric interference from DMF, which can co-elute with NDMA on C18 phases, potentially resulting in over-estimation of NDMA content in the sample. By using an Avantor® ACE® UltraCore Biphenyl column, enhanced retention and resolution of these two components was achieved, sufficient to avoid this issue (Figure 11). [43]



Column:	Avantor® ACE® UltraCore Biphenyl
Dimensions:	100 x 2.1 mm i.d.
Particle size:	3.5 µm
Mobile Phase:	A: 0.1% formic acid in H_2O B: 0.1% formic acid in MeOH
Gradient:	**Time (min)** / **% Mobile phase B** 0 / 1 1 / 1 5 / 100 5.4 / 100 5.5 / 1 7.8 / 1
Flow rate:	0.5 ml/min
Temperature:	40 °C
Inj. volume:	40 µL
MS Source Parameters:	**Parameter** / **Optimised value** Ionisation mode / APCI, positive mode Source temperature / 300 °C Curtain gas / 33 psig Ionspray™ source voltage / 5500 V Ion Source Gas 1 / 30 psig Ion Source Gas 2 / - Needle current / 2 µA

FIGURE 11: LC-MS/MS separation of nitrosamines spiked into valsartan drug substance at 0.1 ng/ml on an Avantor® ACE® UltraCore Biphenyl column. Overlayed traces represent the quantifier and qualifier transitions for each nitrosamine and DMF.

CONCLUSIONS

Solid core particles have a different particle morphology compared to the fully porous silica particles widely used in liquid chromatography and offer distinct performance advantages. The key benefit is that significantly higher efficiencies can be achieved than with equivalently sized fully porous particles. These higher efficiencies can be achieved without the higher backpressure trade-offs associated with a reduction in particle size. As a result, in the case of 2.5 µm particles, UHPLC like performances can be achieved at HPLC pressures, allowing for higher performance and increases in sample throughput to be realised on standard HPLC instrumentation. The advantages of solid core particles were effectively demonstrated by assessing their van Deemter plots. Solid core particles show improvements in the A, B and C terms, although for small molecules, it is improvements in the A and B terms that are most impactful.

In the 15 years since the introduction of the current generation of solid core particles, they have gained wide acceptance for small molecule analysis, particularly by reversed-phase and have been utilised in a wide variety of application areas. The Avantor® ACE® UltraCore range of columns combines the advantages of solid core particles with a novel range of stationary phase chemistries and particle sizes to provide solutions for the full range of analyses typically encountered in RPLC. Applications presented in this article have demonstrated the successful use of UltraCore columns for RPLC analyses in a wide range of analytical fields. The high performance of these columns, along with the flexibility provided by stationary phase chemistries, including phases that can be used at high pH, mean that this range of columns can provide solutions for the challenging separations encountered in the modern analytical laboratory.

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