Identifying the Increased Scope of Core-Shell Technology for HPLC and UHPLC Chromatographers

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Since their introduction in the late-2000s, core-shell particles (alternatively referred to as superficially porous particles), have become increasingly used across the HPLC/UHPLC landscape. Based upon our experience with both fully porous and core-shell based liquid chromatography products, we believe that, in the coming years, core-shell technology will continue to provide increasing benefits to separation scientists including greater sample throughput, improved sensitivity, and increased resolution as well as more compatibility across various instrumentation platforms. As of today, analysts, in virtually any industry that performs liquid chromatography can depend on core-shell columns because of their proven superior performance and wide reaching benefits compared to traditional and conventional HPLC, UHPLC and Preparative HPLC products. Even conservative pharmacopeia and compendial methods have begun accepting and labelling method updates with core-shell products. Most importantly, though, the recent development of new surface chemistries combined with innovations in core-shell media manufacturing have enabled core-shell products to keep pace with the market's separation needs and to overcome increasingly demanding challenges and obstacles. In order to gain a clear perspective into what the future might hold for core-shell particles, it is first important to quickly recap the evolution of core-shell technology in recent years.

The Solution for HPLC & UHPLC

Due to the technological hurdles of manufacturing core-shell particles, there were just three core-shell manufacturers in 2009. However, over time manufacturing improvements and technological advancements made core-shell particle development technology accessible to a broader range of media manufacturers, and now the number of core-shell suppliers has significantly increased (alongside an even larger increase in the number of core-shell distributors). The increased availability of core-shell media has resulted in a boon for chromatographers, as core-shell products now exist in an incredible amount of formats and selectivities to fit virtually any separation situation.

Initially, core-shell particles were only available in sub-3 µm (typically 2.6 or 2.7 µm) formats. Columns packed with these particles were able to provide column efficiency values that were significantly greater than those of conventional fully porous 3 µm particles, but operated at comparable pressures. In fact, as has been demonstrated in numerous publications [1], columns packed with 2.6 µm core-shell particles were able to generate efficiency values that were equivalent to columns packed with 1.7 µm fully porous particles. Thus, the sub-3 µm core-shell particles had the versatility to be compatible with both HPLC and UHPLC systems and, because of this flexibility; this particle size range became the most widely applicable for most analytical labs. With the sub-2 µm like efficiency at low backpressures, UHPLC scientists were given the ability to run faster and faster methods, while maintaining desired resolution and peak capacities. Meanwhile, HPLC chromatographers could upgrade their existing methods with ~2.6 µm core-shell columns that gave them a chance to see UHPLC results on HPLC systems, negating the need for expensive capital purchases of new instrumentation.

Expanding Particle Size Options

As the adoption of this mid-range core-shell particle steadily grew, it became apparent that core-shell particle technology had, in many cases, surpassed the performance limitations of typical HPLC instrumentation – system dwell volumes often actually placed a limitation on the performance of these ~2.6 µm particles. Additionally, some HPLC customers found that the mid-range core-shell particle generally provided slightly higher backpressures (greater than 3 µm fully porous) than they were willing to accept. This realisation drove the next major evolution in core-shell particles – the development and compartmentalisation of core-shell particles into sizes developed specifically for UHPLC (1.3 µm-1.7 µm) and standard HPLC systems (5 µm). Now, rather than trying to use one particle on all instruments, chromatographers could choose a specific core-shell particle best suited to their specific instruments and specific needs.

With 5 µm backpressure, but efficiencies equivalent to that of 3 µm fully porous media, the 5 µm core-shell particles allow HPLC chemists to simply drop-in this type of column in place of 3 µm or 5 µm fully porous products, with immediate benefits including increases in productivity, sensitivity, resolution, and in most cases in shorter analysis times. Additionally, with 5 µm core-shell particles packed in 21.2-50 mm ID AXIA columns, preparative and process chemists are now also seeing gains from core-shell science within their purification realm without negative effects on loadability [2].

On the UHPLC side, the sub-2 µm core-shell products represent the highest efficiency LC products currently commercially available and with which UHPLC scientists are currently able to achieve limits of detection that were previously unattainable. So now into 2015, scientists have become familiar with core-shell or superficially porous particles. Just about every chromatography

company has released a minimum of a mid-range ~2.6 µm particle along with multiple stationary phase options. But where does the core-shell particle go from here?

Novel Surface Chemistries to Overcome New Challenges

Dual Ring Core-Shell Biphenyl for Enhanced Polar Basic Retention

Most analysts will start with a C18 for their reversed phase work and it is the epitome of a workhorse within the chromatography community. However, the C18 is somewhat limited in terms of its selectivity as its retention mechanisms are limited predominantly to hydrophobic interactions (although various polar interactions with the underlying silica also play a role in the overall selectivity). For some chromatographic challenges, specifically the retention of highly polar compounds, hydrophobicity is often not enough to attain necessary retention for polar molecules. Take for instance a large therapeutic drug screen comprising acids, bases, neutrals, polars, and non-polars. The C18 may provide adequate retention and separation of most of the compounds based upon their hydrophobic properties, but even with a 100% aqueous mobile phase, many highly polar compounds will often be poorly retained and elute close to or in the void volume of the column, confounding accurate identification and/or quantitation. The inability of standard C18 phases to provide adequate retention for highly polar molecules drives the need for orthogonal selectivities as well as polar or hybrid modified C18 stationary phases.

Over thirty years ago, phenyl phases were developed to provide that alternative selectivity to complement that of a standard C18 phase. With the pioneering efforts involved with the development of phases such as a phenyl-hexyl stationary phase [3], customers were given the opportunity to begin exploring the versatile combination of hydrophobic and aromatic interaction mechanisms, and a reproducible alternative to a C18. In addition to standard hydrophobic mechanisms, these phenyl phases can also provide some degree of electrostatic interactions via the pi electron clouds of the aromatic ring. In effect, the phenyl ring could develop a slightly negative dipole and act as a weak ion-exchanger. With this extra retention mechanism and differing selectivity, phenyl phases gave customers an initial way to get more retention for the polar, basic analytes that were so often encountered in the pharmaceutical industry.

Now, other phases have been introduced that exhibit even greater 'polar selectivity'

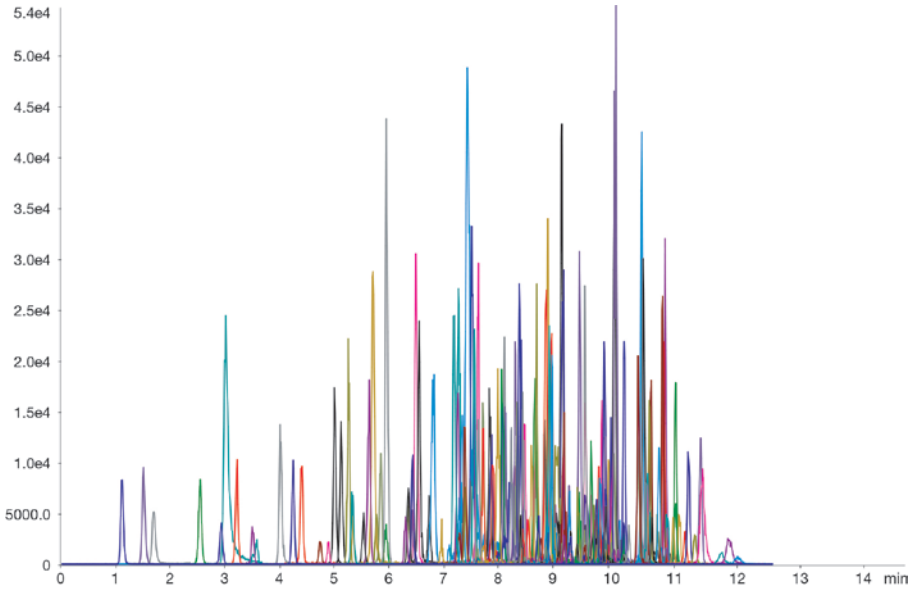


Figure 1. Analysis of 185 pesticides with Kinetex 5 µm Biphenyl

Dimensions: 100 x 2.1mm
Mobile Phase: A: 5 mM Ammonium formate in Water
B: 5 mM Ammonium formate in Methanol
Flow Rate: 0.5 mL/min
Temperature: 35°C
Detection: MS/MS (AB SCIEX 4500 QTRAP)
Sample: Mixture of 185 Pesticides

Gradient	Time (min)	% B
	0.01	10
	1.00	10
	10.00	90
	15.00	90
	15.10	10
	20.00	10

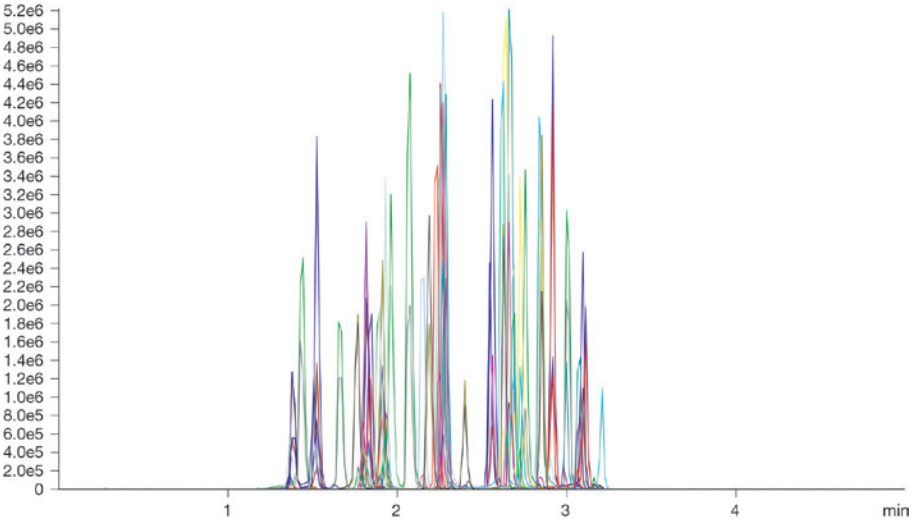


Figure 2. Fast pain management and illicit drug screen with Kinetex 2.6 µm Biphenyl column

Dimensions: 50 x 3.0 mm
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Methanol
Flow Rate: 0.7 mL/min
Column Temperature: 40°C
Detection: MS/MS (AB SCIEX API 5000)
Sample: Mixture of Benzodiazepines, Opiates, Synthetic Opioids, Amphetamines, Analgesics and Illicit Drugs

Gradient	Time (min)	% B
	0	10
	2.5	100
	3.5	100
	3.51	10
	5	10

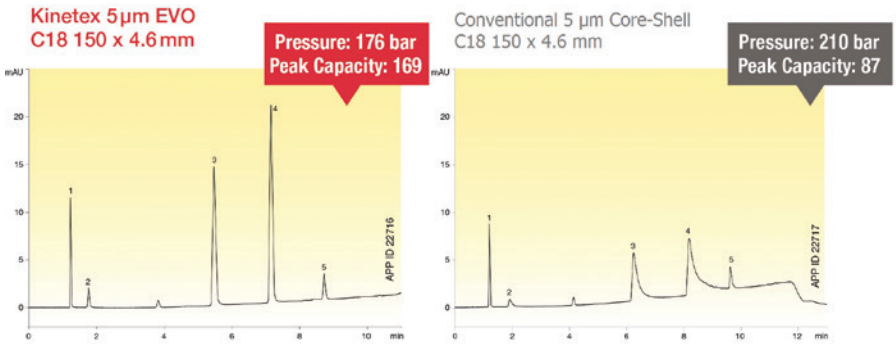


Figure 3. Basic drug analysis peak shape comparison
Dimensions: 150 x 4.6 mm
Mobile Phase: A: 20 mM Sodium phosphate dehydrate pH 7
B: Methanol
Gradient: 40% to 90% B over 10 minutes
Flow Rate: 1 mL/min
Temperature: Ambient
Detection: UV @ 254 nm
Sample: 1. Scopolamine
2. Pseudoephedrine
3. Doxylamine
4. Chlorpheniramine
5. Diphenhydramine

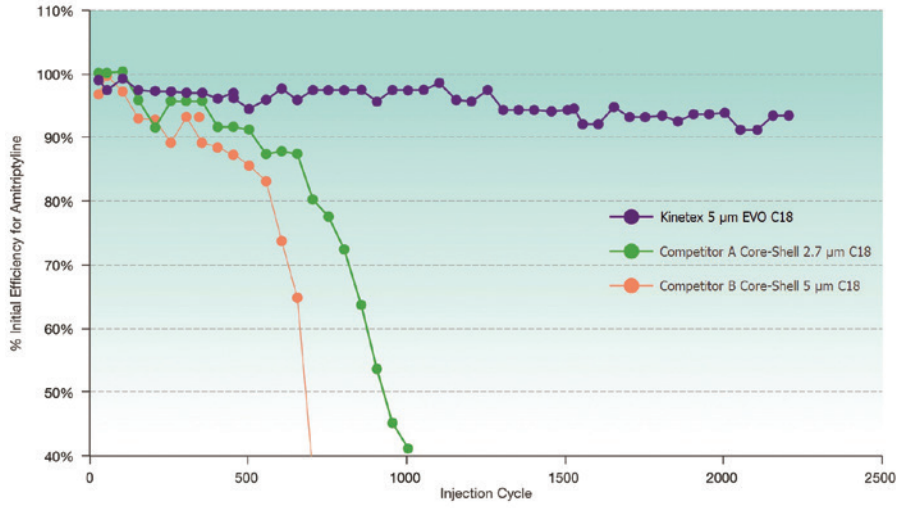


Figure 4. High pH Stability study of core-shell products
Dimensions: 50 x 2.1 mm
Mobile Phase: A: 10 mM Ammonium bicarbonate (pH 10.5)
B: Acetonitrile
Gradient: 5% to 95 % B in 2.3 min. Hold at 95% B for 0.7 min. Re-equilibrate at 5% B for 1.5 min.
Flow Rate: 0.8 mL/min
Temperature: 60°C
Detection: UV @ 254 nm
Sample: 1. Amitriptyline

characteristics than the initial phenyl phases. For instance, a core-shell biphenyl stationary phase, recently introduced by Phenomenex, was able to expand upon the unique selectivity properties of the phenyl phases while at the same time preserving the core-shell efficiency advantages. The great applicability of the –phenyl phase was magnified by increasing the intensity of the secondary interactions of the aromatic ring, by adding a second aromatic ring in series. This resulted in a stationary phase that had significantly more of the phenyl-base selectivity than was previously available. By replacing the carbon linker with a second ring, the dual ring structure of the biphenyl was born and provided greatly increased aromatic selectivity as well as a larger electron cloud to promote stronger dipole-dipole interactions. In addition, the dual ring structure provided 100% aqueous stability, further increasing the analyst's potential to retain challenging polar molecules.

In Figures 1 and 2 we can see the benefits of the core-shell biphenyl stationary phase. Figure 1 highlights a screen consisting of 185 multi-class pesticides run using typical LC/MS running conditions. With so many different classes and functional groups within this pesticide mix, the combination of hydrophobic, aromatic, and polar retention gives analysts the ability to widen their separation window and generate improved results over a traditional C18. Likewise, Figure 2 displays another example of these benefits. With a mixture of therapeutic drugs and drugs of abuse originating from biological matrices, enhanced retention of basic analytes is incredibly important to ensure that target analyte signals are not suppressed by matrix inference and that isobaric compounds are fully separated. Using the high performance and versatility of the Kinetex 2.6 µm Biphenyl phase, early eluting basic drugs are easily retained allowing for use of a steep gradient that potentially could minimise cycle time and significantly improve productivity.

A New pH Stable Core-Shell Particle

The overall success of core-shell particles in all types of laboratory environments has inevitably lead to some significant discoveries, as well as helping identify areas in which core-shell product improvements are needed. Many conventional C18 core-shell products have been shown to be sensitive to overloading of basic compounds [4], which can interfere with accurate quantitation. In addition, as with most silica-based media, the useful pH range of commercially available core-shell products prevented methods from taking advantage of the benefits of alkaline pH conditions. Recently Kinetex core-shell EVO C18 was released to address basic compound overloading (Figure 3) and lack of pH stability within core-shell product lines.

Kinetex EVO C18 columns incorporate a patented organo-silica grafting process (US Patent Nos. 7,563,367 and 8,658,038) that utilises uniform stabilising ethane cross-linking to create a unique selectivity and ultimately, pH stability from 1-12. As seen in Figure 4, other recent core-shell products demonstrate poorer longevity under the high pH stability test compared to the Kinetex EVO C18. With the advantage of high and low pH stability, method

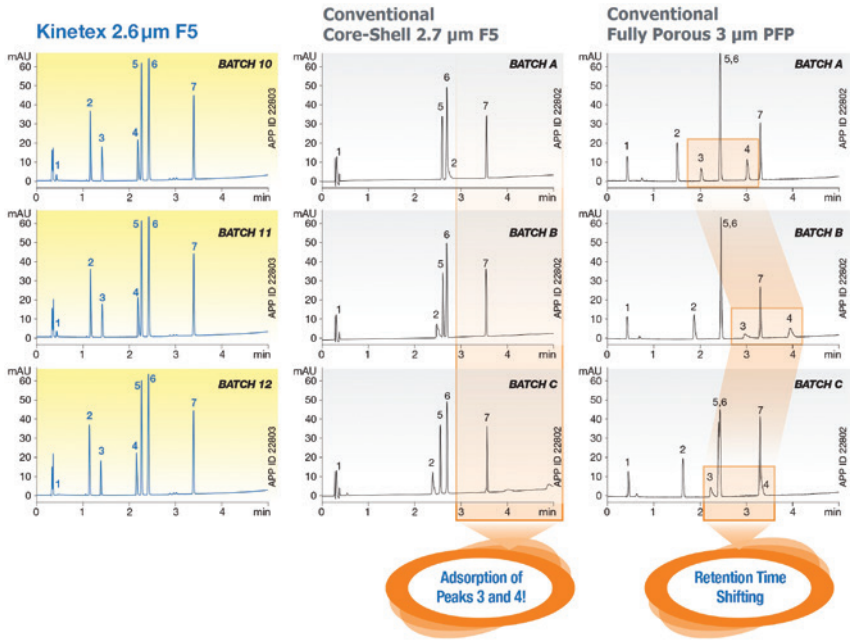


Figure 5. New and existing pentafluorophenyl (PFP) phase reproducibility
Dimensions: 50 x 4.6 mm
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Acetonitrile
Gradient: 5-95% B over 5 minutes
Flow Rate: 1.85 mL/min
Temperature: Ambient
Detection: UV @ 254 nm
Sample: 1. Uracil
2. Pindolol
3. Chlorpheniramine
4. Nortriptyline
5. 3-Methyl-4-Nitrobenzoic acid
6. 5-Methyl Salicyl Aldehyde
7. Hexaphenone

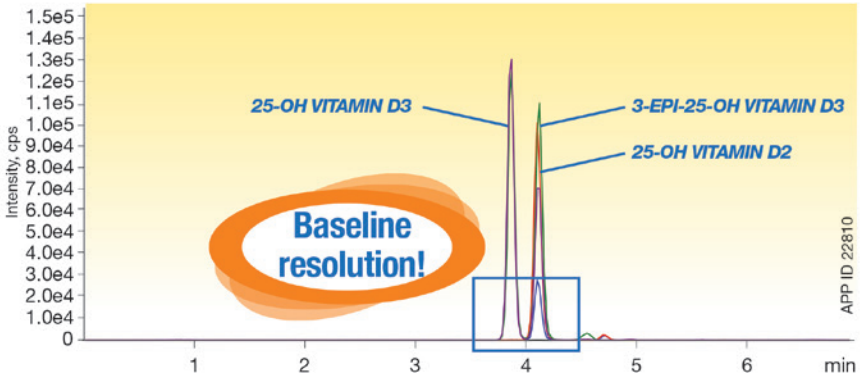


Figure 6. Vitamin D3 epimers separation with Kinetex 2.6 µm F5
Dimensions: 100 x 4.6 mm
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Methanol
Isocratic: A/B (15:85)
Flow Rate: 0.75 mL/min
Temperature: Ambient
Detection: MS/MS (AB SCIEX API 4000)
Sample: 1. 25-OH Vitamin D3
2. 25-OH Vitamin D2
3. 3-epi-25-OH Vitamin D3

developers can enhance selectivity and retention with pH manipulation. Alongside LC/UV analyses, the high performance and low pressure of the Kinetex 5 µm EVO C18 make it a applicable to LC/MS and LC/MS/MS work also. Increased polar basic retention at high pH provided by the Kinetex EVO C18 allows for greater use of organic solvent within the mobile phase, subsequently leading to improved ionisation and increased sensitivity.

Redesigned Pentafluorophenyl (PFP) Phase for Improved Reproducibility

Since its introduction prior to 1990 [5], the pentafluorophenyl (PFP) stationary phase has become a very versatile LC stationary phase. With its five interaction mechanisms (hydrophobic, aromatic, electrostatic, steric/planar and hydrogen bonding) and the ability to be used in five different separation modes (reversed phase, 100% aqueous, HILIC, SFC and 2D-LC) the PFP phase, like the biphenyl, is an orthogonal HPLC/UHPLC development choice. This wide applicability has resulted in LC column manufacturers choosing the PFP as an orthogonal selectivity choice to a C18 for reversed phase separations. While a C18 can differentiate between the small differences in the overall hydrophobicity of closely-related analytes, it is not as effective at separating compounds with structural differences, like positional isomers. The electrostatic and planar interactions of a PFP stationary phase allow for greatly improved resolution of structurally different compounds.

Despite its unique selectivity and versatility, a problematic feature of conventional PFP stationary phases was recently identified after extensive internal and external testing by Phenomenex. It was found that existing PFP phases (both fully porous and core-shell) from a range of vendors exhibited highly irreproducible adsorption of some basic compounds and also displayed shifts in analyte retention time that were batch-dependent. Potential causes of these unacceptable levels of batch-to-batch irreproducibility can be potentially attributed to differences in proprietary manufacturing and chemical bonding processes used during the creation of conventional pentafluorophenyl products. The new core-shell PFP product Kinetex F5 was designed and manufactured to provide consistently accurate chromatographic results that did not vary from batch-to-batch (Figure 5). The improved performance of the Kinetex F5 can be beneficial across a broad range of industries and analysis types. For instance, even tandem mass spectrometry (LC/MS/MS) users can take advantage of the performance

of Kinetex F5 and utilise the cross functionality and reliability as seen in Figure 6. With the same fragment ions originating from the Vitamin D3 epimers, reproducible chromatographic separation is mandatory. The unique combination of polar/non-polar selectivity of Kinetex F5 provides a highly sensitive assay with good resolution between isobaric components within a short analysis window.

Where to Next

With new and novel advancements in core-shell particle technology like those discussed above, chromatographers should continue to see performance gains and benefits such as higher sample throughput, better sensitivity, and increased resolution. As a manufacturer Phenomenex will provide new solutions and continue to focus on surface chemistry advancements and selectivities that solve existing and future obstacles.

References:

[1] Fekete, S., Fekete, J., Fast gradient screening of pharmaceuticals with 5 cm long, narrow bore reversed-phase columns packed with sub-3 µm core–shell and sub-2 µm totally porous particles, Talanta, 84(2), 2011, 416-423

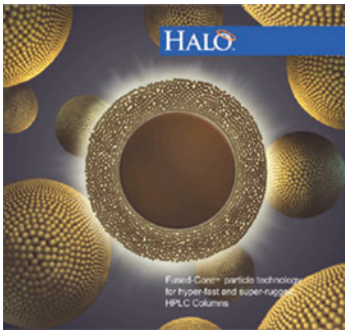
[2] Jacobs, M., Evaluation and Performance Comparison of New Core-Shell Media vs. Fully Porous Media for Preparative Purifications, International Symposium on Preparative and Process Chromatography, 2013

[3] Hanai, T., Ahmed, F., Rustamov, I., Babusis, D., Selectivity of phenylhexyl-bonded silica gel for liquid chromatography, J. Liq. Chromatogr. Rev., 22(4), 1999, 501-511

[4] McCalley, D.V., Some practical comparisons of the efficiency and overloading behaviour of sub-2 µm porous and sub-3 µm shell particles in reversed-phase liquid chromatography, J. Chromatogr. A, 1218 (2011), 2887–2897

[5] Csató, E., Fülöp, N., Szabó, G., Preparation and comparison of a pentafluorophenyl stationary phase for reversed-phase liquid chromatography, J. Chromatogr. A, 511 (1990), 79-88

HALO Glycan LC Columns for Oligosaccharides, Including Protein-linked Glycans and Glycopeptides



Advanced Materials Technology have introduced a new column line in the HALO BioClass family – **HALO Glycan columns**. The HALO Glycan stationary phase has a highly polar ligand containing 5 hydroxyl groups, which is attached to the 2.7micron Fused-Core silica particle via novel proprietary linkage chemistry. The Fused-Core particle used for HALO Glycan has a surface area of ~135 m2/g and an average pore size of 90Å. This high performance material provides a column that is specifically targeted for separations of highly polar oligosaccharides and particularly protein-linked glycans and glycopeptides by hydrophilic interactive liquid chromatography (HILIC). This latest column introduction joins the existing HALO BioClass column family, giving chromatographers a wide range of application specific solutions for the separation of bio-derived samples.

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