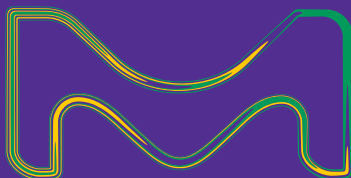


MERCK

Capillary and Nanoflow UHPLC Columns

Unravelling Complexity,
One Molecule at a Time



The Life Science business
of Merck operates as
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Analytical Products

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The Importance of Capillary HPLC Columns

Capillary High-Performance Liquid Chromatography (HPLC) columns hold significant importance in the field of analytical chemistry, offering a range of advantages that enhance the precision and efficiency of chemical analyses. One primary advantage is the columns' ability to operate at lower flow rates, leading to reduced solvent consumption. This feature not only makes capillary HPLC more environmentally friendly but also enables the analysis of trace-level compounds, where sample availability is limited. The decreased solvent usage contributes to cost-effectiveness and aligns with the growing emphasis on sustainable practices in analytical chemistry.

Additionally, the high efficiency and resolution provided by capillary HPLC columns play a crucial role in separating and identifying complex mixtures of compounds. The narrow, internal diameter of nano and micro capillary HPLC columns results in enhanced chromatographic efficiency and sensitivity due to reduced analyte dispersion leading to sharper and better-defined peaks. This feature is especially beneficial when dealing with samples containing closely related compounds or impurities that require precise separation. The improved resolution allows for accurate quantification and identification, supporting the reliability of analytical results.

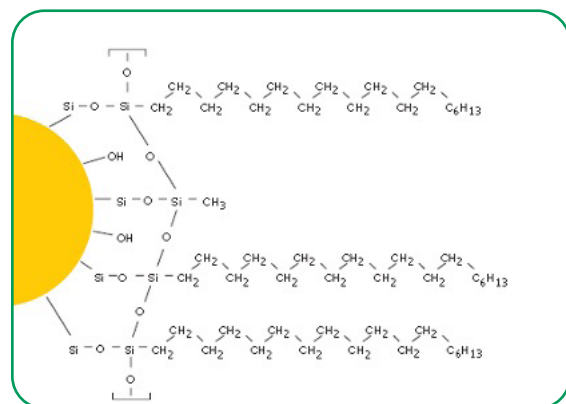


Purospher® STAR RP-18 endcapped (2 µm) Capillary Columns

Best in Class C18 Phase for Excellent Peak Symmetry, Performance and pH Stability

Fully porous silica particles (FPP) have been the go-to choice in HPLC for decades, offering high efficiency, versatility, compatibility with modern instrumentation, and advancements in particle technology, making them widely recognized as the state of the art in HPLC separations.

Purospher® STAR RP-18 endcapped capillary columns are based on ultra-pure fully porous silica particles (Type B). These columns are designed for universal use and allow the separation of basic, neutral, and metal chelating compounds with simple mobile phases and excellent peak symmetry. The columns offer an outstanding stability from pH 1.5 to 10.5 over a wide temperature range, and demonstrate best all-round retention characteristics.



Features and Benefits

- Accurate results with excellent peak shape for all types of analytes
- Outstanding resolution due to high separation efficiency
- Proven reliability and reproducibility from run to run and batch to batch
- Universal compatibility with best all-round performance according to Tanaka test
- Maximum flexibility in method development and choice of mobile phase
- pH stability from pH 1.5–10.5
- Highest sensitivity and suitability for LC-MS applications

Purospher® STAR RP-18 endcapped specifications:

Silica:	Type B (High purity silica)
Particle Platform:	Fully porous particles (FPP)
Phase Chemistry:	Octadecyl
USP:	L1
Particle Size:	2 µm
Pore Size:	120 Å
Carbon Load:	17%
Surface Area:	330 m ² /g
pH Range:	1.5 – 10.5
Max. Temperature:	60 °C
Endcapped:	Yes

High Column Efficiency for Robust Methods

Purospher® STAR RP-18 endcapped Capillary columns

The efficiency of an HPLC column, as described by the Knox equation, is significant in chromatographic separations, and directly affects resolution, sensitivity, analysis speed, peak capacity, selectivity, and method robustness.

The high separation efficiency of Purospher® STAR RP-18 endcapped (2 µm) capillary columns contributes to improved chromatographic performance and reliable analytical results.

The Knox equation is widely used to illustrate the dependence of the plate height (H) on the linear velocity (v) of the mobile phase. The relationship helps to analyze the efficiency of the column by evaluating the height equivalent to a theoretical plate (HETP) at different flow rates. A lower HETP indicates better column efficiency.

The Knox Plot results demonstrate the high separation power of the Purospher® STAR RP-18 endcapped (2 µm) 150 mm × 1.0 mm I.D. (**Figure 1**) and 150 mm × 300 µm I.D. (**Figure 2**) capillary columns.

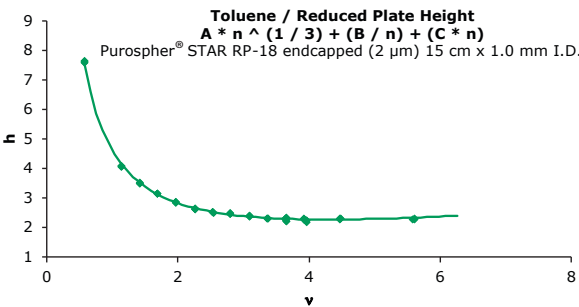
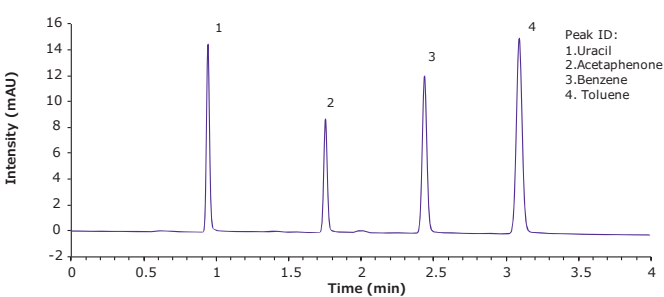


Figure 1: Knox Plot results of the Purospher® STAR RP-18 endcapped (2 µm) 150 mm × 1.0 mm I.D. capillary column

Peak ID:

- 1. Uracil
- 2. Acetaphenone
- 3. Benzene
- 4. Toluene

Parameter	Estimate
A	0.3237
B	4.1049
C	0.1840
$h = A * n^{1/3} + (B / n) + (C * n)$	

LC Conditions	
Column:	Purospher® STAR RP-18 endcapped (2 µm) 150 mm × 1.0 mm I.D. (50115-U)
Mobile Phase:	[A] Water; [B] Acetonitrile; A:B, 30:70
Flow:	65 µL/min
Column Temperature:	30 °C
Injection:	0.1 µL
Detection:	UV, 254 nm
Sampling Rate:	20 Hz
Flow Cell Volume:	45 nL
System Dispersion:	1 µL

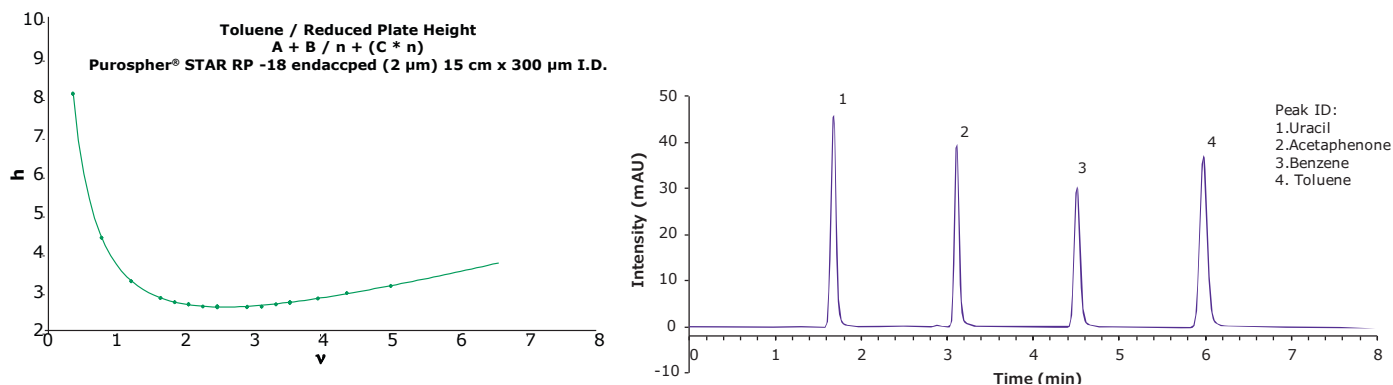


Figure 2: Knox Plot results of the Purospher® STAR RP-18 endcapped (2 µm) 150 mm x 300 µm I.D. capillary column

Parameter	Estimate
A	0.3641
B	3.3902
C	0.3480
$h = A * v + (B / v) + (C * v)$	

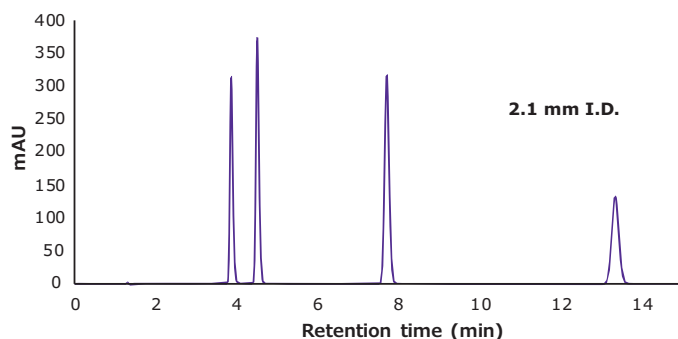
LC Conditions	
Column:	Purospher® STAR RP-18 endcapped (2 µm) 150 mm x 300 µm I.D. (50118-U)
Mobile Phase:	[A] Water; [B] Acetonitrile; A:B, 30:70
Flow:	6 µL/min
Column Temperature:	30 °C
Injection:	0.02 µL
Detection:	UV, 254 nm
Sampling Rate:	20 Hz
Flow Cell Volume:	45 nL
System Dispersion:	1 µL

Scaling from UHPLC to Capillary Column Dimension with Significant Solvent Savings

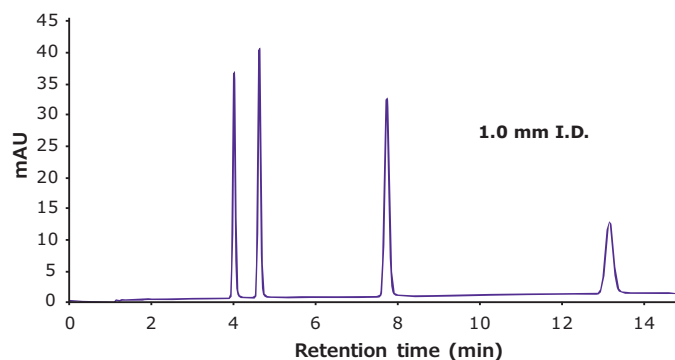
Purospher® STAR RP-18 endcapped Capillary Columns

Capillary columns typically have smaller internal diameters, resulting in a reduced column volume compared to UHPLC columns. This attribute translates to lower solvent requirements for each analysis, leading to significant solvent savings over time. In addition, the reduced column volume of capillary columns allows for higher analyte concentrations and increased sensitivity.

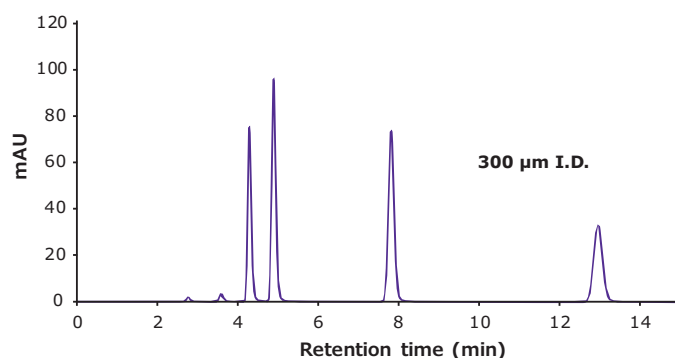
In this study, method scaling was evaluated from a 2.1 mm I.D. UHPLC column down to a capillary dimension of 1.0 mm I.D. and 300 µm I.D. **Figure 3** shows the method scale down for both flow rate, and injection volume to evaluate the overall method performance, and the resulting impact on solvent savings. In the case of the 300 µm I.D. capillary column, a slightly higher optimum linear velocity was used to maintain similar analysis times.



LC Conditions for 2.1 mm I.D. UHPLC Column	
Instrument:	Thermo Scientific™ Vanquish™ Horizon
Mobile Phase:	75% v/v acetonitrile in water (isocratic)
Column Temp.:	30 °C
Detector:	UV at 254 nm
Sampling Rate:	5 Hz
Flow Cell Volume:	13 µL
System Dispersion:	7 µL
Flow Cell Volume:	45 nL



LC Conditions for 1.0 mm I.D. and 300 µm I.D. Capillary Columns	
Instrument:	Thermo Scientific™ Vanquish™ Neo
Mobile Phase:	75% v/v acetonitrile in water (isocratic)
Column Temp.:	30 °C
Detector:	UV at 254 nm
Sampling Rate:	20 Hz
Flow Cell Volume:	45 nL
System Dispersion:	1 µL
Flow Cell Volume:	45 nL



- Peak ID:
1. Toluene
 2. Napthalene
 3. Anthracene
 4. 1,2 Benzantracene

Figure 3: Method scaling from a 2.1 mm I.D. UHPLC column down to a capillary dimension of 1.0 mm I.D. and 300 µm I.D

Column:	Instrument	Flow Rate	Injection Volume	1,2 Benzantracene r.t. (min)	Efficiency (plates/meter)	USP Tailing Factor	Peak Height	Solvent Consumption
Purospher® STAR RP-18 endcapped (2.0 µm) 150 mm x 2.1 mm I.D.	Thermo Scientific™ Vanquish™ Horizon	210 µL/min	1.0 µL	13.32	26967	1.07	118	2,940 µL
Purospher® STAR RP-18 endcapped (2.0 µm) 150 mm x 1.0 mm I.D.	Thermo Scientific™ Vanquish™ Neo	50 µL/min	0.250 µL	13.38	23661	1.04	11.7	700 µL
Purospher® STAR RP-18 endcapped (2.0 µm) 150 mm x 300 µm I.D.	Thermo Scientific™ Vanquish™ Neo	6 µL/min	0.1 µL	13.01	16661	1.06	33.9	84 µL

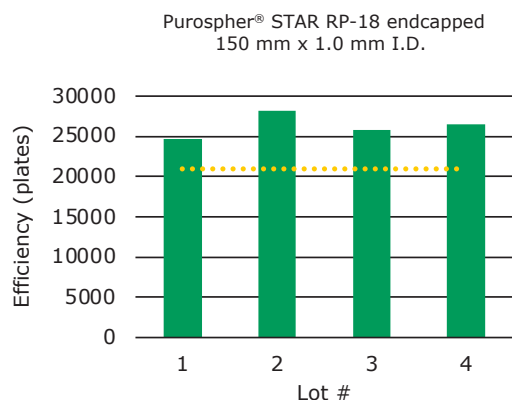
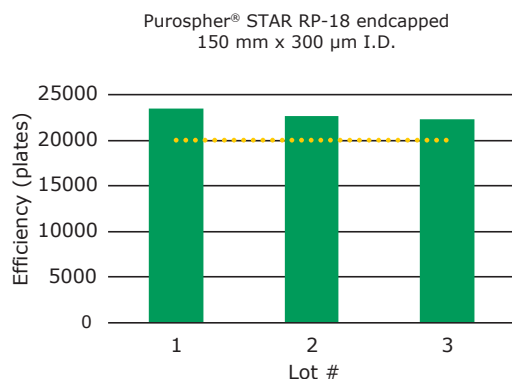
Column-to-Column and Lot-to-Lot Reproducibility

Purospher® STAR RP-18 endcapped Capillary Columns

The success of any method depends on the quality of the stationary phase. Precise, long-term reproducibility is a key factor in achieving reliable results.

Below, the lot-to-lot consistency of the Purospher® STAR RP-18 endcapped capillary columns is shown. Different lots of material are tested for column performance in both the 1.0 mm I.D. and 300 µm I.D. column dimension.

Furthermore, meticulous care is given to quality control over all aspects of silica structure and chemistry. These factors ensure that the columns will always perform consistently, resulting in method reproducibility you can trust.



LC Conditions	
Column:	Purospher® STAR RP-18 endcapped (2 µm) 150 mm x 1.0 mm I.D. (50115-U)
Mobile Phase:	70% Acetonitrile / 30% Water
Flow:	50 µL/min
Temperature:	30 °C
Injection:	0.25 µL
Detection:	UV, 254 nm
Sampling Rate:	20 Hz
Flow Cell Volume:	45 nL
System Dispersion:	1 µL

LC Conditions	
Column:	Purospher® STAR RP-18 endcapped (2 µm) 150 mm x 300 µm I.D. (50118-U)
Mobile Phase:	60% Acetonitrile / 40% Water
Flow:	6 µL/min
Temperature:	30 °C
Injection:	0.10 µL
Detection:	UV, 254 nm
Sampling Rate:	20 Hz
Flow Cell Volume:	45 nL
System Dispersion:	1 µL

Figure 4: Average column efficiency per lot. The yellow line marks the minimum specification for the column release test.

Long-term Column Stability

Purospher® STAR RP-18 endcapped Capillary Columns

The long-term HPLC column stability is crucial for consistent performance, method reproducibility, cost savings, time efficiency, and data integrity.

The study below evaluates the long-term stability of the Purospher® STAR RP-18 endcapped (2 µm) capillary column over a 500-injection series.

Purospher® STAR RP-18 endcapped (2 µm) capillary columns provide consistent and reliable separation performance over an extended period ensuring that the column retains its efficiency, resolution, and selectivity, allowing for accurate and reproducible analysis.

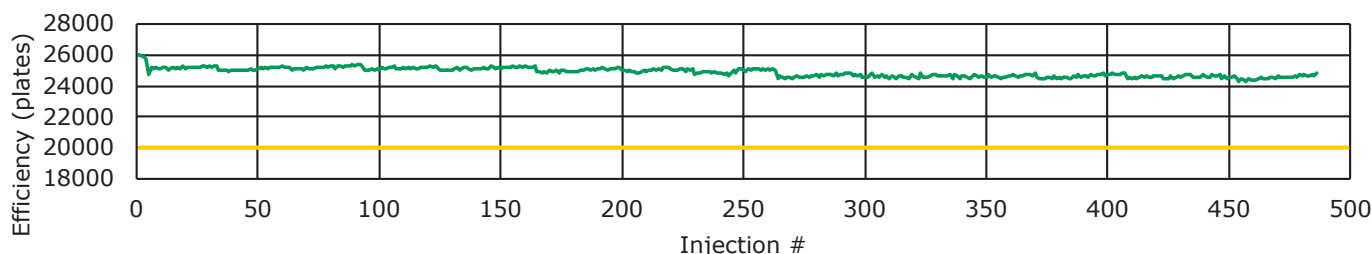
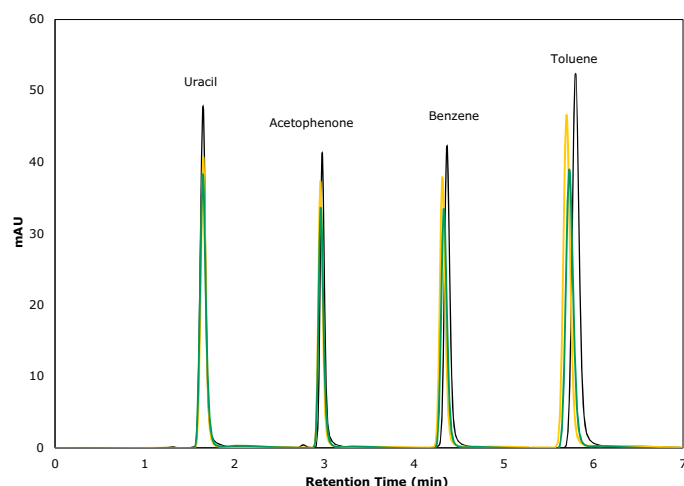


Figure 5: Injection Stability: Efficiency over 500 injections on Purospher® STAR RP-18 endcapped (2 µm) 150 mm x 300 µm I.D.

LC Conditions	
Column:	Purospher® STAR RP-18 endcapped (2 µm) 150 mm x 300 µm I.D. (50118-U)
Mobile Phase:	60% Acetonitrile / 40% Water
Flow:	6 µL/min
Temperature:	30 °C
Injection:	0.1 µL
Detection:	UV, 254 nm
Detector Sampling Rate:	20 Hz
Flow Cell Volume:	45 nL
System Dispersion:	1 µL

Injection Number	Pressure (bar)	toluene r.t. (min)	Efficiency (plates/meter)	USP Tailing Factor
Initial	250	5.799	25,876	1.17
250	255	5.699	24,482	1.14
500	266	5.709	24,783	1.14



Initial chromatogram

Chromatogram after 250 injections

Chromatogram after 500 injections

Ordering Information

Purospher® STAR RP-18 endcapped (2 µm)			
Length (mm)		I.D. (mm)	Part number
50	x	1.0	50111-U
150	x	1.0	50115-U
50	x	0.3	50114-U
150	x	0.3	50118-U

Figure 6: Long-term HPLC column stability of Purospher® STAR RP-18 endcapped (2 µm) capillary column 150 mm x 300 µm I.D. over 250 injections (yellow), 500-injections (green), in comparison to the Initial (black) chromatogram using a test mixture of 1. Uracil, 2. Acetophenone, 3. Benzene and 4. Toluene

SeQuant® ZIC®-HILIC and ZIC®-cHILIC Capillary Columns

The ideal solution for all classes of polar and hydrophilic compounds

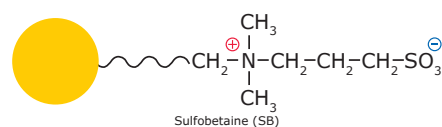
The SeQuant® ZIC®-HILIC stationary phase, based on ultra pure (Type B) fully porous silica particles (FPP), is designed to retain and separate all types of polar and hydrophilic compounds and for robust chromatography with high selectivity and reproducibility. The silica-based ZIC®-HILIC sorbent has a bonded stationary phase consisting of a highly polar, permanent zwitterion. Separation selectivity is favored by the 1:1 zwitterion charge balance, which makes the ZIC®-HILIC column overall neutral, with weak, but important, ionic interactions. Tuning of the selectivity on the ZIC®-HILIC column during method development is facilitated by the pH independent, permanent zwitterion, ensuring that only the analytes (and not the column) are affected during eluent optimization.

SeQuant® ZIC®-cHILIC is designed for excellent HPLC and LC-MS separation of polar, hydrophilic compounds. This zwitterionic stationary phase, with phosphorylcholine functional groups, provides you with complementary selectivity for easier method development for analytes that have been difficult to separate by previous types of HPLC columns operated in RP (reversed-phase) or HILIC (hydrophilic interaction liquid chromatography) mode.

Features and Benefits

- High-performance HPLC and LC-MS separations of polar hydrophilic compounds
- Zwitterionic stationary phase ensures reproducible retention
- Two complementary phase chemistries
- Maximum LC-MS compatibility with minimal column bleed
- Excellent reproducibility and robustness
- Available in a variety of lengths, particle sizes, and pore dimensions

SeQuant® ZIC®-HILIC



SeQuant® ZIC®-cHILIC

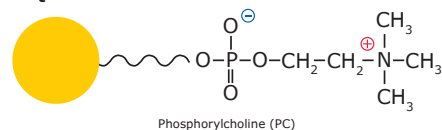


Figure 7: Complementary zwitterionic modifications of SeQuant® ZIC® HILIC and ZIC®-cHILIC

SeQuant®	Functional Group	USP	Features	Particle Size	Pore Size
ZIC®-HILIC	Sulfobetaine	L114	High-performance selectivity and robustness	3.5 µm, 5 µm	100 Å, 200 Å
ZIC®-cHILIC	Phosphoryl-choline		Complementary selectivity with favorable LC-MS performance	3 µm	100 Å

Best Overall Selectivity

Numerous stationary phase options exist for HILIC separations with varying versatility for different types of analyses. Understanding the properties of various HILIC stationary phase chemistries is essential for selecting the appropriate columns for specific applications. **Figure 8** illustrates a column classification approach, adapted from Ikegami [1], providing insights into the retention and selectivity characteristics of several HILIC phase chemistries. The Ikegami Method serves as an effective tool for assessing the selectivity of HILIC columns by evaluating attributes such as hydrophilic retention, selectivity, hydrogen bonding capacity, shape selectivity, and ionic properties. These attributes are measured using specific test probes under standardized conditions with results often represented as radar plots for easy visual comparison.

Details on the Ikegami Test results for various columns are described in the [Selectivity Study of Stationary Phases for Hydrophilic Interaction Chromatography \(HILIC\) and Multivariate Analysis for Material Classification](#).¹

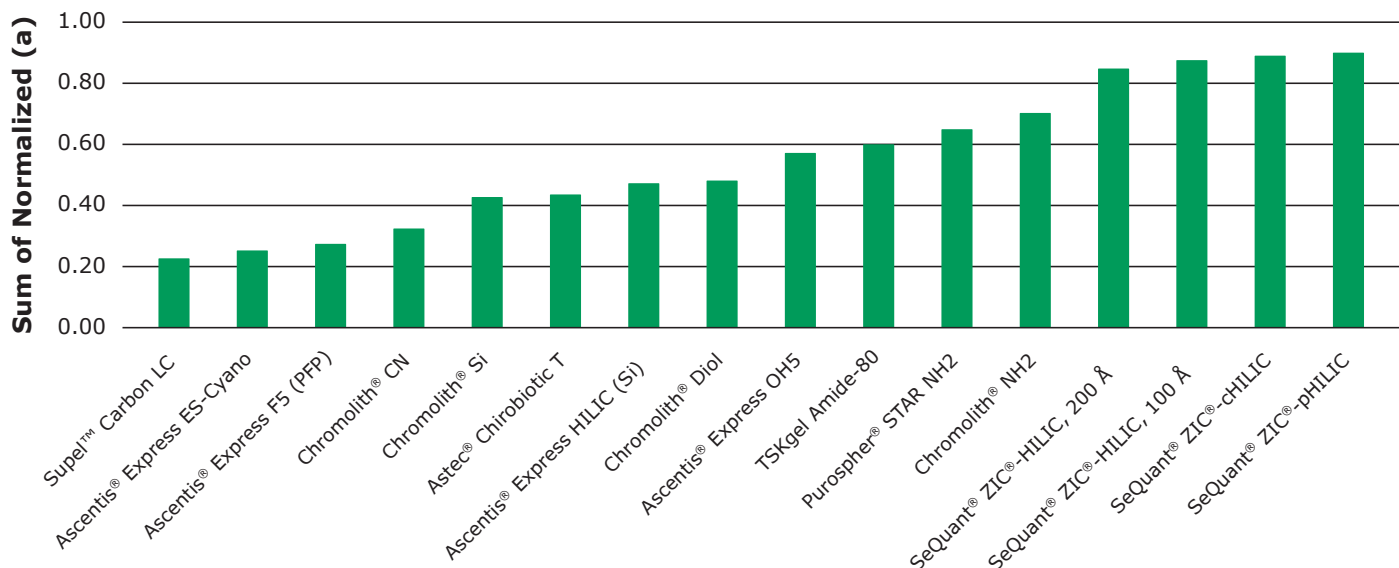


Figure 8: Overall selectivity for polar compound separation of various columns according to the Ikegami et al. [1] test conditions

Zwitterionic chemistries, characterized by oppositely charged ligands that yield a net neutral charge, differ significantly from traditional hydrophilic ligands. For example, zwitterionic phases like SeQuant® ZIC®-HILIC and SeQuant® ZIC®-chHILIC demonstrate superior hydrophilic retention compared to bare silica and other HILIC phases. The ZIC®-HILIC phase retains cation exchange properties, while ZIC®-chHILIC exhibits enhanced anion exchange characteristics. This variability underscores the unique chromatographic properties of ZIC®-HILIC phases, with the high water-layer thickness of zwitterionic phases providing greater flexibility in method development as demonstrated in **Figure 8**.

¹ Y. Kawachi, T. Ikegami, H. Takubo, Y. Ikegami, M. Miyamoto, N. Tanaka, J. Chromatogr. A 1218 (2011) 5903-5919

Overall stationary phase selectivity and retention behaviour for hydrophilic and electrostatic interactions of acidic (-) and basic (+) compounds

Phase Chemistry	Products	Overall Selectivity	Hydrophilic Retention	Electrostatic interaction (-)	Electrostatic interaction (+)
Stationary phases for polar compound separation operating in HILIC mode					
Zwitterionic	SeQuant® ZIC®-HILIC/cHILIC/pHILIC	Strongest	Strongest	Low (HILIC) Mild (cHILIC/pHILIC)	Mild (HILIC) Low (cHILIC/pHILIC)
Amine	Chromolith® NH ₂ , Purospher® STAR NH ₂	Strong	Mild	Strongest	None
Amide	TSKgel Amide 80	Strong	Strong	Low	Moderate
Alcohol	Ascentis® Express OH5, Chromolith® Diol	Mild	Mild	Low	Low
Bare Silica	Ascentis® Express HILIC (Si), Chromolith® Si	Low	Low	None	Strongest
Other stationary phases suitable for polar compound separation					
Cyano	Ascentis® Express ES-Cyano, Chromolith® CN	Low	None	None	Moderate
Fluoro	Ascentis® Express F5 (PFP)	Low	None	None	Strong
Teicoplanin	Astec® Chirobiotic T	Mild	Mild	Low	Strong
Carbon	Supel™ Carbon LC	Low	Low	Low	Low

The zwitterionic chemistries in SeQuant® ZIC®-HILIC and SeQuant® ZIC®-cHILIC are distinctive due to their use of doubly - charged ligands, resulting in a strong dipole moment. When these phases are exposed to water, solvation further separates the opposing charges, facilitating substantial water uptake compared to other stationary phase chemistries. Consequently, the ZIC® family of stationary phases stands out as the most selective HILIC stationary phases available.

Long-term Column Stability at High Efficiency for Polar Compound Separation

SeQuant® ZIC®-HILIC and ZIC®-cHILIC Capillary Columns

The long-term column stability at high efficiency for polar compound separation using SeQuant® ZIC®-HILIC and ZIC®-cHILIC capillary columns ensures consistent and reliable performance, enabling accurate and precise analysis of complex, polar samples.

Robust column stability enables the transferability of established separation methods between different instruments or laboratories. The consistent, high efficiency of these columns, as shown below for SeQuant® ZIC®-HILIC and ZIC®-cHILIC 150 x 300 µm I.D. capillary columns, allows for effective separation of closely related polar compounds, resulting in improved resolution.

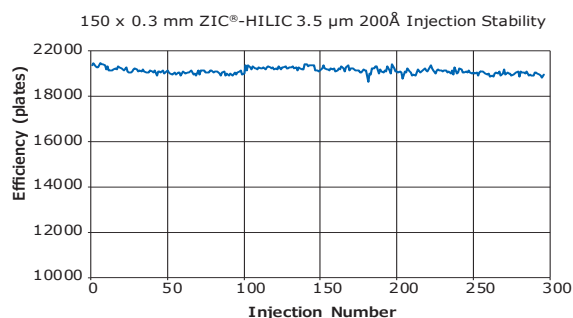
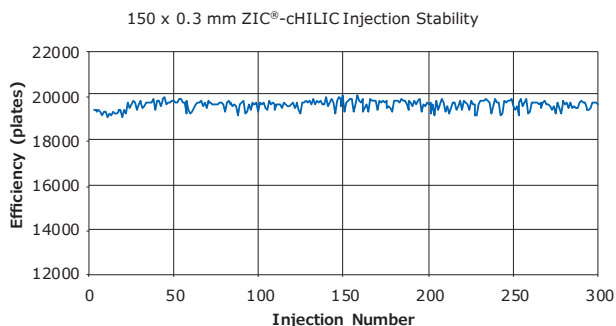


Figure 9: Long term column stability

Enhanced 2D-LC Separation Capabilities Combining Orthogonal Selectivities

SeQuant® ZIC®-HILIC Capillary Columns x Fused-Core® C18

HILIC and RP chromatography offer complementary separation mechanisms. Polar compounds, such as hydrophilic and ionizable compounds, exhibit increased retention on the HILIC column. In contrast, RP separates compounds primarily based on their hydrophobicity, with nonpolar or hydrophobic analytes having longer retention on the RP column. By utilizing HILIC and RP chromatography in a hyphenated or coupled mode, it is possible to obtain enhanced separation capabilities and obtain a more comprehensive analysis of complex samples by taking advantage of their orthogonal properties. This combination allows for a broader range of compound coverage and better separation of analytes that may be challenging to separate with a single chromatographic technique.

For the characterization of the polyphenolic content of berry juices, a hyphenated HILIC × RP-LC PDA-ESI-MS method was used.¹ The detailed qualitative profiling of the polyphenolic content of seven commercial berry juices was performed on a SeQuant® ZIC®-HILIC (3.5 µm) 150 × 1.0 mm I.D. capillary column for the first dimension, whereas an Ascentis® Express C18 (2.7 µm) 50 × 4.6 mm I.D. column was used for the second dimension. In the analysis of various berries, shown in **Figure 11**, elderberry exhibited the highest polyphenolic content (918 ± 1.10 mg/100 mL). This finding suggests that elderberry can be recommended as a prominent natural source of bioactive compounds for inclusion in food products. Furthermore, the outcomes obtained in this study could serve as valuable insights for conducting authenticity studies in the food industry.

Additionally, the detailed understanding of the polyphenolic profile across different berry species holds potential for conducting clinical studies to further assess the anticipated health benefits associated with consuming berries. Such knowledge can contribute to obtaining a more accurate estimation of the potential positive effects on human health derived from consuming berries.

This approach, combining HILIC and RP modes, has gained significant attention in recent years and has been successfully applied in various research groups for profiling polyphenols in food and natural products.

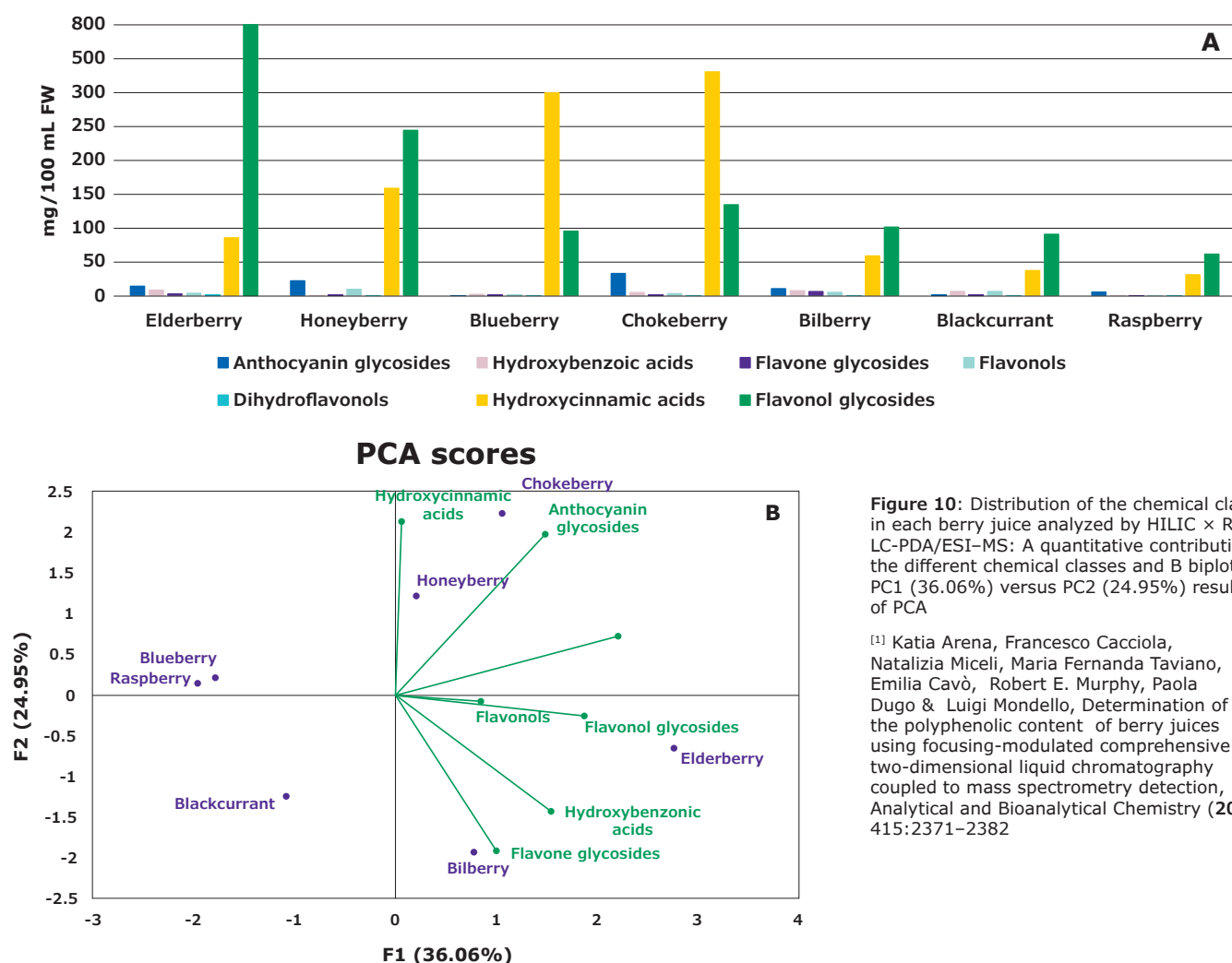


Figure 10: Distribution of the chemical classes in each berry juice analyzed by HILIC × RP-LC-PDA/ESI-MS: A quantitative contribution of the different chemical classes and B biplot of PC1 (36.06%) versus PC2 (24.95%) resulting of PCA

^[1] Katia Arena, Francesco Cacciola, Natalizia Miceli, Maria Fernanda Taviano, Emilia Cavò, Robert E. Murphy, Paola Dugo & Luigi Mondello, Determination of the polyphenolic content of berry juices using focusing-modulated comprehensive two-dimensional liquid chromatography coupled to mass spectrometry detection, *Analytical and Bioanalytical Chemistry* (2023) 415:2371–2382

Ordering information for SeQuant® ZIC®-HILIC and ZIC®-cHILIC Capillary Columns

Length (mm)		I.D. (mm)	ZIC®-HILIC (3.5 µm) 200 Å	ZIC®-HILIC (3.5 µm) 100 Å	ZIC®-HILIC (5 µm) 200 Å	ZIC®-cHILIC (3 µm) 100 Å
150	x	0.075			150465	
100	x	0.1	150466			
150	x	0.3	150479-U	150671-U	150481-U	150669-U
50	x	0.3	150473-U			150664-U
30	x	0.3	150489			
150	x	1.0	150480-U	150487-U	150482-U	150670-U
50	x	1.0	150476-U			150668-U
30	x	1.0	150478			

Supel™ Carbon LC Capillary HPLC Columns

Fully porous graphitic carbon capillary columns are ideal for separation of hydrophobic, polar or charged compounds even at extreme conditions

Porous graphitic carbon (PGC) particles, designed by a unique and patented synthetic process, constitute the packing of Supel™ Carbon LC U/HPLC columns. The key advantages of using PGC columns over silica, particle-packed columns include:

Elevated Temperature Stability: Columns can be readily operated at temperatures up to 250 °C, thereby allowing faster and more efficient separations.

pH Stability: Compatible with mobile phases in the pH range of 1–14 at any temperature without causing a decline in the column lifespan.

Unique Retention Mechanism: Porous Graphitic Carbon (PGC) is a unique stationary phase that significantly extends the range of polar analytes in reversed-phase mode, surpassing the capabilities of traditional polar embedded C18 phases. One of the standout features of PGC is its excellent analyte shape selectivity, particularly for flat and aromatic compounds. The retention behavior of PGC is influenced by the planar structure of the analytes; for instance, toluene exhibits retention times comparable to ethyl benzene due to steric hindrance caused by the hydrogen atoms in the ortho position, which twists the carboxylic acid group out of the plane, rendering ortho isomers less aromatic than their meta and para counterparts.

While PGC predominantly functions as a reversed-phase material, its retention properties overlap with

classical reversed-phase materials and exhibit strong π -interactions due to the high aromaticity of the PGC surface. This unique combination allows PGC to retain certain polar molecules that conventional reversed-phase materials cannot, which would typically require Hydrophilic Interaction Liquid Chromatography (HILIC). The retention characteristics of PGC are particularly pronounced for larger analytes or those with high molecular surface areas that can effectively interact with the graphitic planes of the PGC. This is especially true for aromatic compounds and those with multiple ring systems, which benefit from enhanced π - π interactions at the PGC surface.

Furthermore, PGC demonstrates exceptional shape selectivity for closely related isomeric compounds, making it a powerful tool for challenging separations. Although method development with PGC necessitates careful consideration due to its distinct characteristics, when employed correctly, it proves to be a highly versatile separation material for liquid chromatography. The innovative carbon adsorbent material developed for PGC applications empowers chromatographers to tackle complex separations with confidence.

Compatibility with any Solvent: Any polar or non-polar solvent can be used for the resolution of an analyte of interest.

Column Pressure Stability up to 700 bar.

Supel™ Carbon LC Specifications

Phase Bonding	Particle Size (µm)	Pore Size (Å)	Surface Area (m ² /g)	pH Stability	Max. Temperature	Endcapped	Shipping Solvent
N/A (porous graphitic carbon)	2.7	200	155	1 to 14	250 °C	No	Acetonitrile/Water

Particle Size Distribution of Supel™ Carbon LC HPLC Lots vs. Competitor

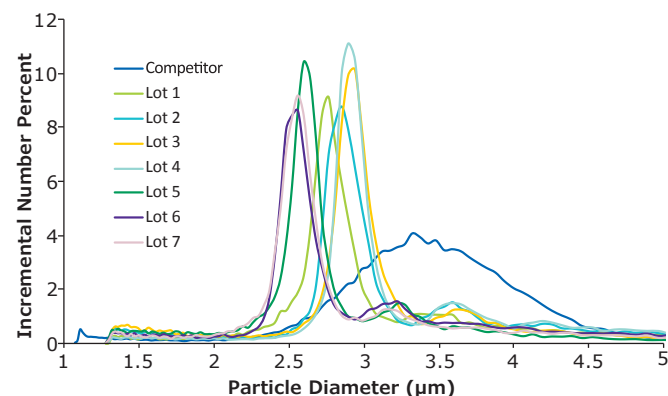
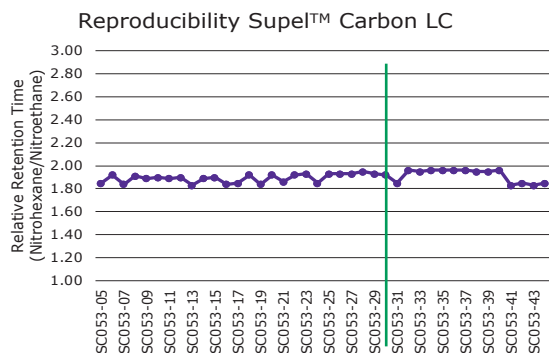


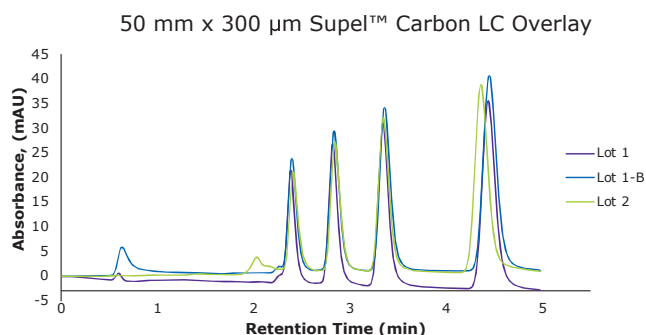
Figure 11: Narrower particle size distribution of Supel™ Carbon LC columns additionally improves separation efficiency and increases resolution.

Supel™ Carbon LC Column-to-Column and Lot-to-Lot Reproducibility



The capillary Supel™ Carbon LC column demonstrates remarkable reproducibility between columns and lots. Relative Retention Time (RRT) Relative Standard Deviation (RSD) was calculated to be only 2.4% between two lots of stationary phase, and the intercolumn RRT RSD was determined to be only 2.2% and 0.6% for lots 1 and 2, respectively. The red bar in the graph on the left divides columns from lot 1 vs. lot 2.

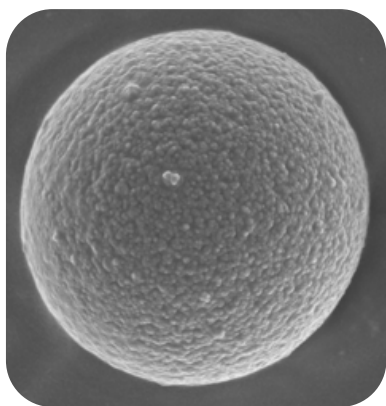
Figure 12: Reproducibility of the Supel™ Carbon LC capillary columns across different columns.



The chromatogram overlay, at left, showcases further the reproducibility of the capillary Supel™ Carbon LC column. Minimal differences were observed between two columns from Lot 1 and a column from Lot 2.

Conditions: Mobile Phase: 20:80 water:acetonitrile (isocratic); Flow rate: 3.0 µL/min; Column temp.: 30 °C; Detector: UV, 210 nm; Injection: 20 nL; Sample: Nitrohexane test mix, varied concentration in water.

Figure 13: Resolution of glycan isomers on Supel™ Carbon LC capillary columns. Two lots of the stationary phase are showcased here and each lot has similar chromatographic separation.



SEM Image of Supel™ Carbon LC Particle

The Supel™ Carbon LC adsorbents are generated by a specialized, in-house, hard-templating approach to create a mesoporous, hard carbon for Liquid Chromatography (LC). The particles have been designed with a tighter particle size distribution than other carbon adsorbents for LC with a cleaner surface profile. This attribute can be seen on the high resolution Scanning Electron Micrograph (SEM) image of an individual 2.7 µm Supel™ Carbon particle. The particles are highly spherical with a consistent aspect ratio and circularity. What can also be seen is the unique surface topography that is formed as a result of the specialized templating approach used in processing these particles.

Resolution of Glycan Structural Isomers Using Capillary Supel™ Carbon LC Columns

Glycans are important compounds to detect and monitor. These compounds play crucial roles as biomarkers for diseases, and if the incorrect glycans are expressed on therapeutic antibodies, it can lead to allergic responses in patients. Due to the same mass of structurally-similar glycans, chromatographic separation before the MS is a requirement.

This application demonstrates higher retentivity and the superior resolving power of Supel™ Carbon LC in separating a critical pair of glycans that a comparable PGC column cannot leading to a more accurate interpretation of the glycan composition of the sample.

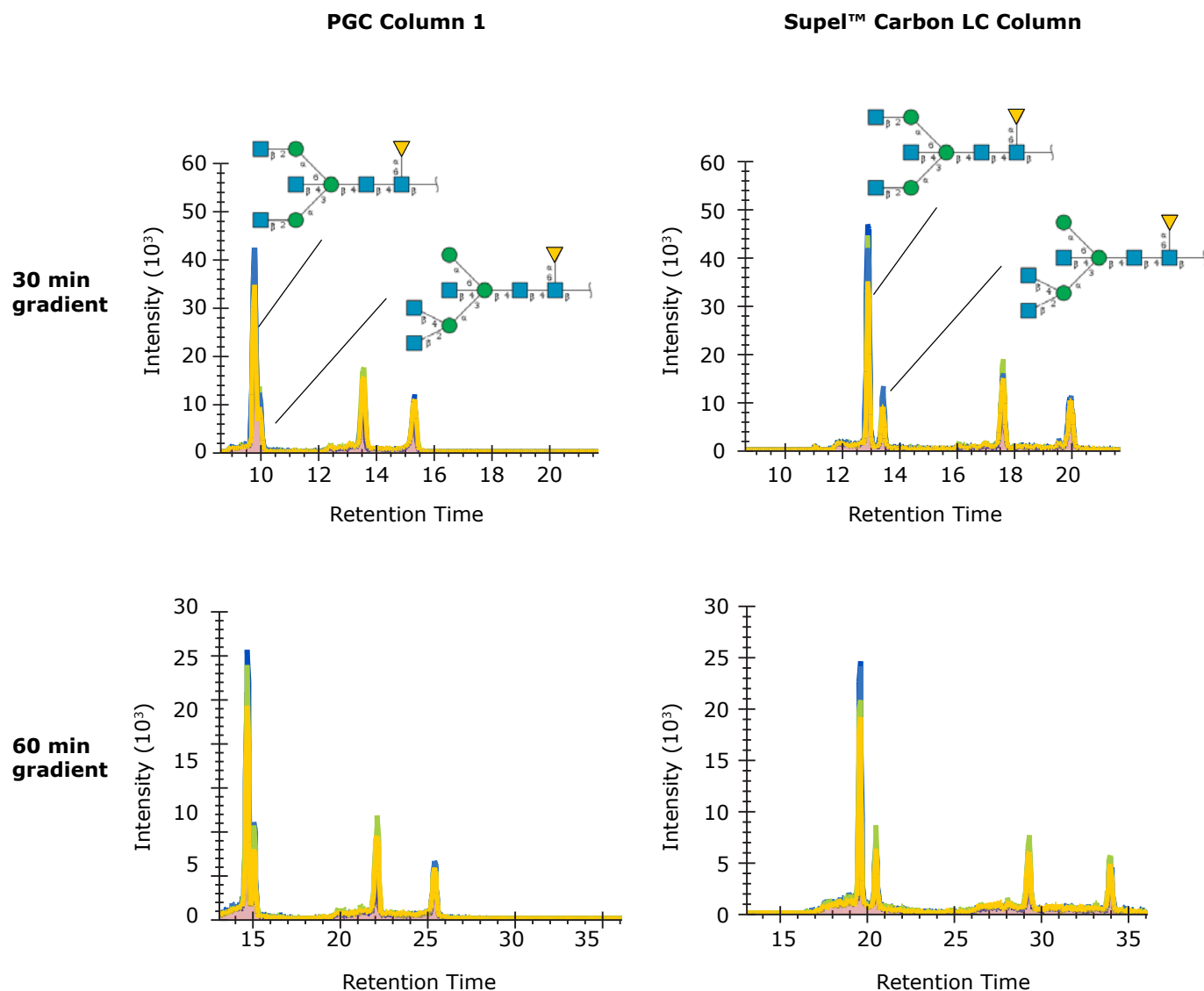


Figure 14: Comparison of different PGC columns in resolving glycans.

Conditions: Column: PGC, 150 mm x 1.0 mm I.D., 3.0 μ m (Column 1), 2.7 μ m (Supel™ Carbon LC); Mobile Phase: [A] Water*; [B] Acetone*; Gradient: 0% B to 26% B in 30 min or 60 min; Flow rate: 155 μ L/min; Column temp.: 90 °C; Detector: MSD, QQQ.

*Both solvents supplemented with undisclosed negative mode buffer. Data courtesy of Dr. Christopher Ashwood, Beth Israel Deaconess Medical Center, Harvard University.

Analysis of N-Acetyl-Hexosamine Isomers with Capillary Supel™ Carbon LC Columns

The retention behavior of two N-acetylhexosamine (HexNAc) epimers, critically important in human glycobiology i.e., N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc), in their reduced forms on a Supel™ Carbon LC column using a high-resolution Q-ToF mass spectrometer as the detector was investigated. It was demonstrated that the Supel™ Carbon LC column was able to consistently retain and separate reduced forms of the HexNAc isomers when analysed alone or in mixtures on a shallow LC gradient using conventional LC solvents. Particularly, the reduced forms of GlcNAc and GalNAc were well separated and exhibited excellent chromatographic behaviour including narrow peak width and symmetrical peak shape.

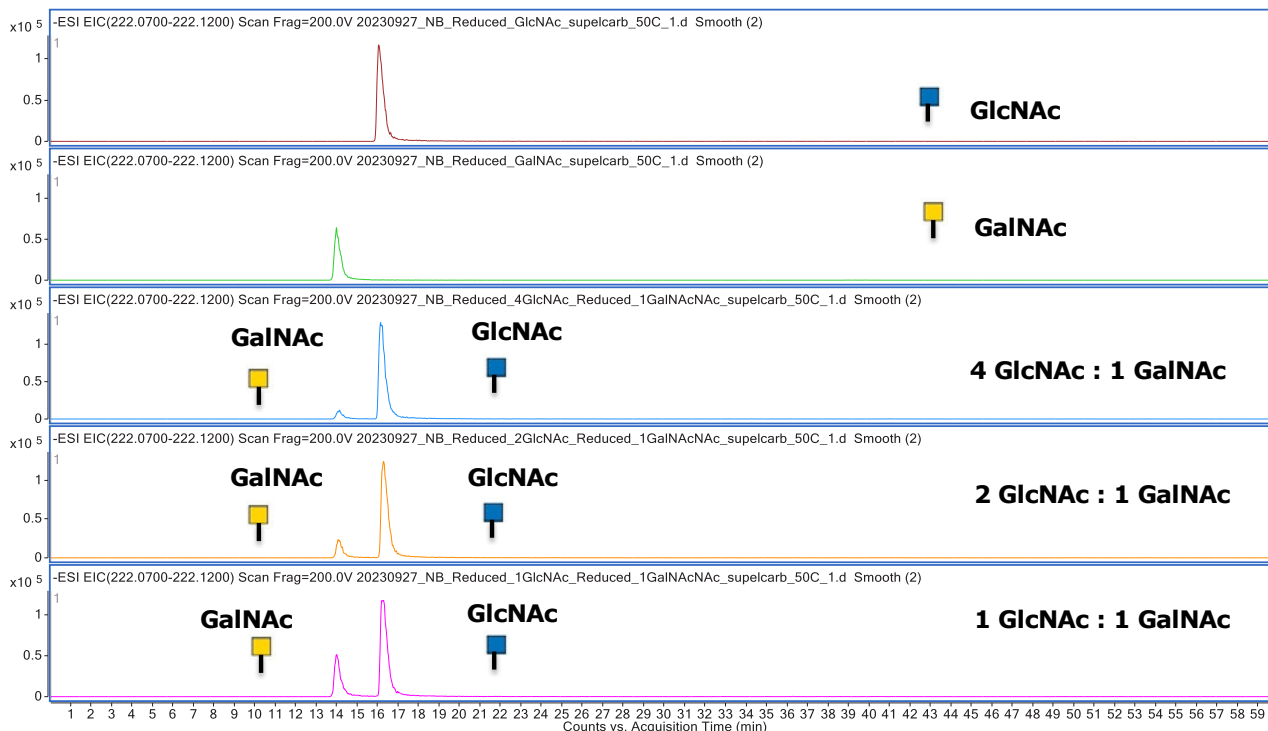


Figure 15: Separation of GalNAc and GlcNAc on Supel™ Carbon LC capillary columns. *Data courtesy of Dr. Morten Thaysen-Andersen, Macquarie University, Sydney, Australia.*

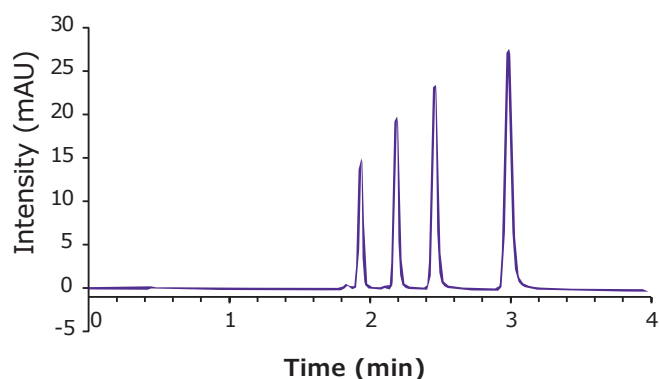
Table 1. Chromatographic Conditions

LC Conditions					
Instrument:	Agilent 1260 HPLC			MS Conditions	MS1
Column:	Supel™ Carbon LC, 150 x 1.0 mm I.D., 2.7 µm (59978-U)			Instrument:	Agilent 6538 UHD Accurate-Mass Q-ToF LC-MS system
Mobile Phase:	A: 10 mM ammonium bicarbonate in water, pH 8 B: 10 mM ammonium bicarbonate in 70% acetonitrile			Polarity:	ESI (-)
Gradient:	Time (min)	A%	B%	Spray Voltage:	4,300 V
	0	97	3		
	15	97	3		
	26	94	6		
	28	0.0	100		
	40	0.0	100		
	42	100	0.0		
	60	100	0.0		
Flow Rate:	20 µL/min			Capillary Temp.:	300 °C
Pressure:	86 bar (at 97% solvent A)			Sheath Gas:	20 p.s.i
Column Temp.:	50 °C			Aux. Gas:	3.5 L/min
Detector:	Q-ToF MS (high resolution, see below)			m/z Range:	150–2200 (a typical m/z range used in glycomics)
Injections:	1 µL (equivalent to 1.8 nmol for both reduced and non-reduced GlcNAc and GalNAc or dilution thereof of GalNAc for the mixtures)				
Sample(s):	GalNAc, GlcNAc (reduced and non-reduced forms)				

Supel™ Carbon LC Kinetic Studies

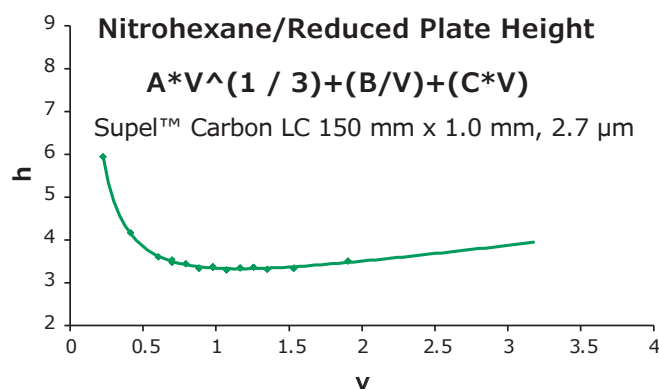
Knox Plot for 150 x 1.0 mm I.D. Supel™ Carbon LC, 2.7 µm

Understanding the kinetics of a separation enables the chromatographer to choose the appropriate flow rate to maximize the efficiency of their separation. Here, we display a Knox and van Deemter plot, respectively, for the 1.0 mm and 300 µm I.D. Supel™ Carbon LC columns.



LC Conditions	
Column:	Supel™ Carbon LC (2.7 µm), 150 mm x 1.0 mm I.D. (59978-U)
Mobile Phase:	[A] water; [B] acetonitrile; A:B, 30:70
Flow:	65 µL/min
Temperature:	30 °C
Injection:	0.1 µL
Detection:	UV, 254 nm
Sampling Rate:	20 Hz
Flow Cell Volume:	45 nL
System Dispersion	1 µL

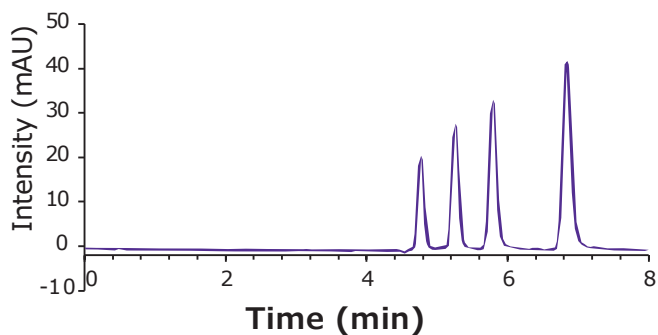
Figure 16: Typical chromatographic results for analysis of Knox probes on Supel™ Carbon LC capillary columns (150 x 1.0 mm I.D.)



Parameter	Estimate
A	0.9140
B	2.0929
C	0.1925
$h = A * n^{(1/3)} + (B / n) + (C * n)$	

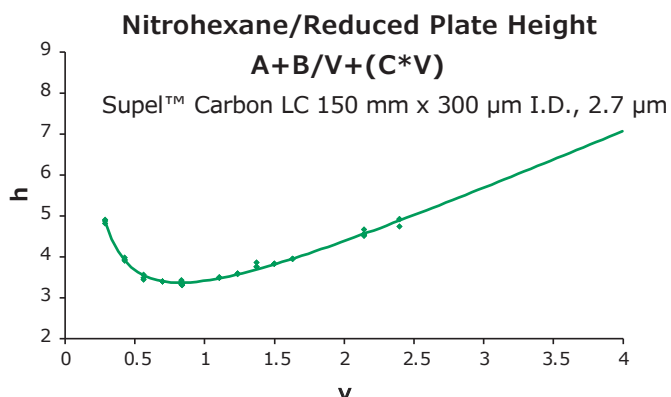
Figure 17: Reduced Knox Plot for Supel™ Carbon LC capillary column (150 x 1.0 mm I.D.)

van Deemter Curve for 150 x 300 µm I.D. Supel™ Carbon LC, 2.7 µm Column



LC Conditions

Column:	Supel™ Carbon LC (2.7 µm), 150 mm x 300 µm I.D. (59980-U)
Mobile Phase:	[A] water; [B] acetonitrile; A:B, 30:70
Flow:	6 µL/min
Temperature:	30 °C
Injection:	0.02 µL
Detection:	UV, 254 nm
Sampling Rate:	20 Hz
Flow Cell Volume:	45 nL
System Dispersion:	1 µL



Parameter	Estimate
A	0.9418
B	1.0007
C	1.4708
$h = A + B / n + (C * n)$	

A-term: multichannel flow or uniformity of the packed bed

B-term: molecular diffusion along the column axis

C-term: radial mass transfer into and out of particles

Figure 18: Knox Plot analysis of Supel™ Carbon LC capillary columns (150 mm x 300 µm I.D.)

Ordering information

Supel™ Carbon LC (2.7 µm)			
Length (mm)		I.D. (mm)	Part number
50	x	1.0	59975-U
150	x	1.0	59978-U
50	x	0.3	59977-U
150	x	0.3	59980-U

Chromolith® CapRod® Monolithic Silica Capillary Columns

Enhanced Robustness and Lifetime

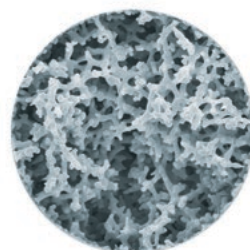
Monolithic capillary columns have gained significance in biomolecule separation, particularly when coupled with mass spectrometry. A Chromolith® CapRod® capillary column combines the speed of monolithic, silica technology with the sensitivity of nano-LC. This attribute enables superior productivity for high throughput, highly sensitive proteomics LC applications. Unlike particulate columns, these capillaries eliminate the need for frits and exhibit a significantly lower tendency to clog leading to extended column lifetime. This feature enables the use of higher flow rates, thereby enhancing the speed and precision of biomolecule characterization (faster separations). Separations can be achieved at 1–3 $\mu\text{L}/\text{min}$, compared to 200–400 nL/min for conventional media on a standard 100 μm I.D. capillary column. For more complex, biological samples, a trapping capillary can be used to protect the separation column and optimize separation efficiency. Even if the analytical column becomes clogged, there is a possibility to trim a few millimeters, rejuvenating it to a pristine state. The capillaries can even be slightly bent (up to 90° per 15 cm column) to fit any LC configuration or instrument without any negative effect on chromatographic separation.

In response to the increasing demand for micro and nano-HPLC, we provide an extensive selection of exceptional, monolithic, silica capillaries, offering various internal diameters, bonded phases, pore structures, and lengths to cater to diverse analytical needs. Chromolith® CapRod® columns are designed to work with various nano or capillary-LC systems. This attribute provides the highest efficiency and performance when coupled to mass spectrometers, both on-line (ESI, nanospray) and off-line (MALDI). On-line MALDI target spotting is possible using a 30 cm Chromolith® capillary column, allowing droplets to be collected directly from the column outlet. This eliminates the need for any additional connection capillary, helping to maintain high separation efficiency obtained in the separation column.

Chromolith® CapRod® analytical capillary columns are supplied complete with sleeves and standard 1/16" PEEK fittings to allow for direct coupling to a UV detector or mass spectrometer.



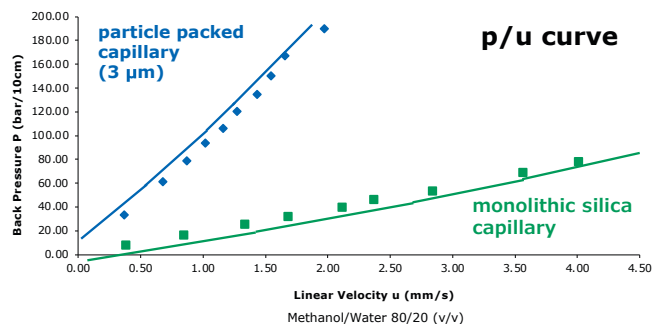
Cross section of the bimodal pore structure of Chromolith® CapRod® column with macropores at approximately 2 μm (1 μm for Chromolith® HighResolution CapRod® columns) and mesopores of 13 nm. The outer diameter of the capillary is 360 nm.



Chromolith® CapRod® Columns – Benefits

- Higher flow rates than particle-packed, capillary columns at low pressure
- Flat van Deemter curve allows wide range flow rate flexibility and flow gradients from 0.1–200 $\mu\text{L}/\text{min}$ ensure ideal compatibility with LC/MS systems, with both ESI and APCI interfaces or MALDI spotters
- Substantially longer column lifetime compared to particulate packed columns
- Longer columns could be used for higher resolution separations without back pressure consideration
- Robust and easy handling
- Higher efficiency and narrower peaks

Comparison of the relation between linear velocity and column back pressure for packed and monolithic silica columns.

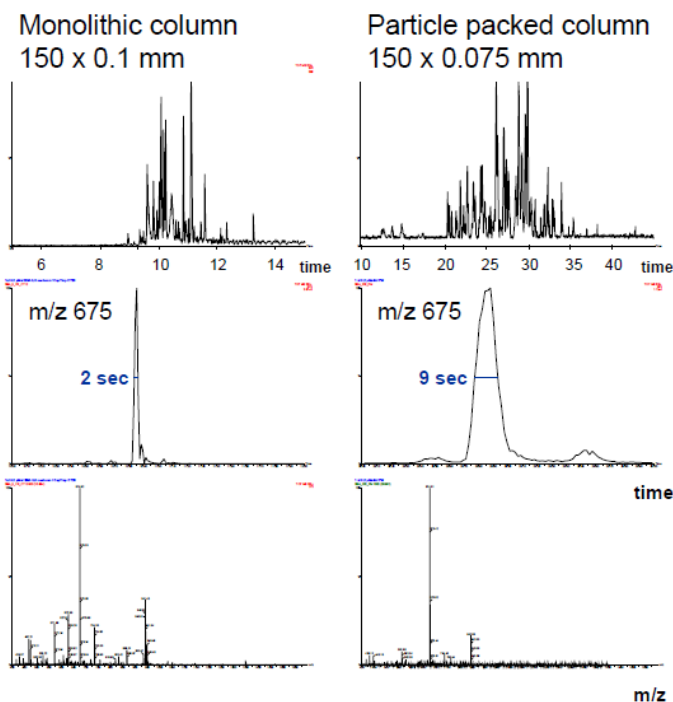


Recommended Use and Flow Rate Ranges

Recommended Use	1504010001 RP-8e HR 150-0.05	1504030001 RP-18e HR 150-0.05	1520310001 RP-8e Trap	1504000001 RP-8e HR 150-0.1	1504260001 RP-18e 50-0.1 Trap	1504020001 RP-18e 150-0.1	1504040001 RP-18e HR 150-0.1	1504240001 RP-18e HR 300-0.1	1504090001 RP-18e 50-0.2 Trap	1504050001 RP-18e 150-0.2	1504070001 RP-18e HR 150-0.2
Separation of small molecules		•			•	•	•	•	•	•	•
- of peptides	•	•	•	•	•	•	•	•	•	•	•
- of proteins	•		•	•						•	
Micro ESI				•		•	•	•		•	•
Nano ESI	•	•		•		•	•	•			
MALDI spotting						•				•	•
High Resolution	•	•		•			•	•			•
Flow rates (µL/min)	0.01 – 0.16	0.01 – 0.16	0.2 – 9	0.04 – 0.6	0.2 – 9	0.2 – 3	0.04 – 0.6	0.06 – 1	1 – 30	1 – 12	0.2 – 2.4
Max. back pressure (bar)*	218	218	218	218	218	218	218	218	218	218	218

* If the backpressure is too high, column material might detach from the column wall and move a few millimeters, creating an empty section of the capillary and a section with a denser, silica monolith. Consequently, the column would lose efficiency due to the presence of this empty capillary zone. In such cases, the capillary column can be trimmed to eliminate the empty and compressed sections, allowing it to be used further. However, trimming more than a centimeter of the column will result in a decrease in peak retention.

LC-MS separation of 200 fmol trypsin-digested BSA:



Particle packed capillary column:

Flow rate: 0.2 µL/min
Gradient: 2% to 50% B in 50 min

Monolithic silica capillary column:

Flow rate: 1.0 µL/min
Gradient: 2% to 50% B in 10 min

Mobile phase:

A: 0.1% FA in 2% Acetonitrile and Water

B: 0.08% FA in 80% Acetonitrile and Water

Monolithic capillary column:

Peak width of ~1/5th at 5-fold flow velocity.

Comparable sensitivity at

>3-fold increased throughput.

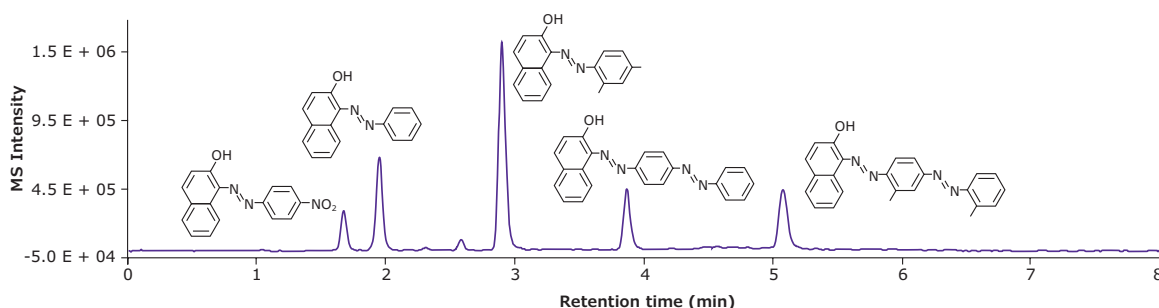
Chromolith® CapRod® Columns – Applications

Sudan dyes

Chromatographic Conditions

Column:	Chromolith® CapRod® RP-18 endcapped 150 x 0.1 mm I.D. (1.50402.0001)
Mobile Phase:	A: Water + 0.1 % formic acid B: Acetonitrile + 0.1 % formic acid
Gradient:	70 % B to 95 % B in 5 min
Flow Rate:	1.24 µL/min
Pressure Drop:	76 bar (1,100 psi)
Detection:	nano-ESI(+) 240–390 m/z
Temperature:	ambient
Diluent:	Acetonitrile
Injection Volume:	2.5 nL

Sample	Compound	Retention Time (min)	Monoisotopic mass (g/mol)	(M+H) ⁺ (m/z)
1.	Para red	1.67	293.08	294.01
2.	Sudan I	1.95	248.09	249.01
3.	Sudan II	2.9	276.13	277.09
4.	Sudan III	3.87	352.13	353.08
5.	Sudan IV	5.08	380.16	381.11

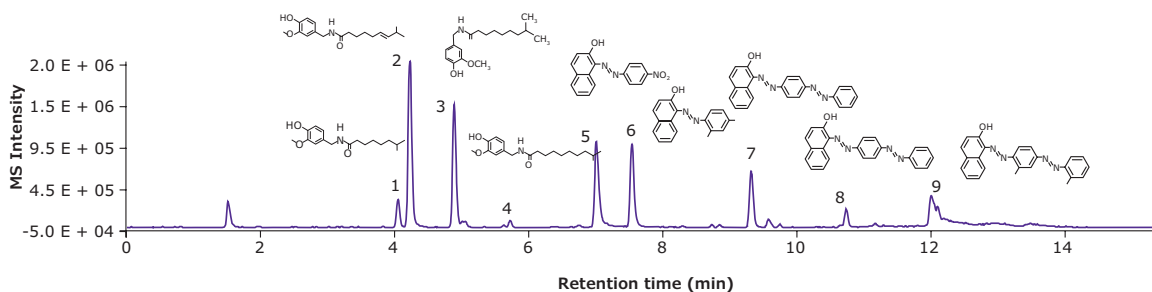


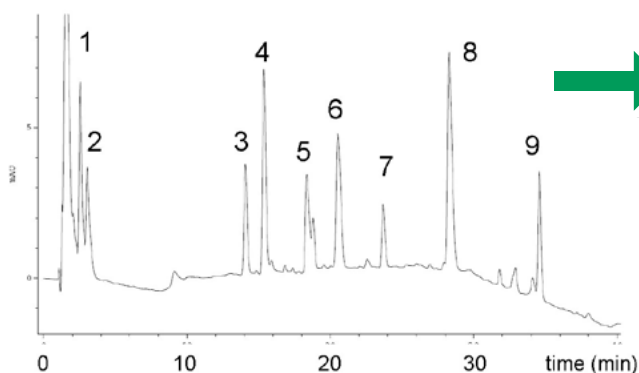
Sudan dyes and capsaicinoids in hot chili sauce

Chromatographic Conditions

Column:	Chromolith® CapRod® RP-18 endcapped 150 x 0.1 mm I.D. (1.50402.0001)
Mobile Phase:	A: Water + 0.1 % formic acid B: Acetonitrile + 0.1 % formic acid
Gradient:	35 % B to 95 % B in 12 min
Flow Rate:	1.24 µL/min
Pressure Drop:	80 bar (1,160 psi)
Detection:	nano-ESI(+) 100–600 m/z
Temperature:	ambient
Diluent:	Acetonitrile
Injection Volume:	2.5 nL

Sample	Compound	Retention Time (min)	Monoisotopic mass (g/mol)	(M+H) ⁺ (m/z)
1.	Nordihydrocapsaicin	4.05	293.2	294.11
2.	Capsaicin	4.23	305.2	306.18
3.	Dihydrocapsaicin	4.9	307.21	308.19
4.	Homodihydrocapsaicin	5.73	321.23	322.11
5.	Para red	7.02	293.08	294.05
6.	Sudan I	7.55	248.09	249.05
7.	Sudan II	9.33	276.13	277.1
8.	Sudan III	10.74	352.13	353.11
9.	Sudan IV	12.11	380.16	381.21





LC-MS Application:

Analysis of peptide sequence
with a Mass Spectrometer
coupled to the Chromolith®
CapRod® capillary

Flow Rate: 3 µL/min

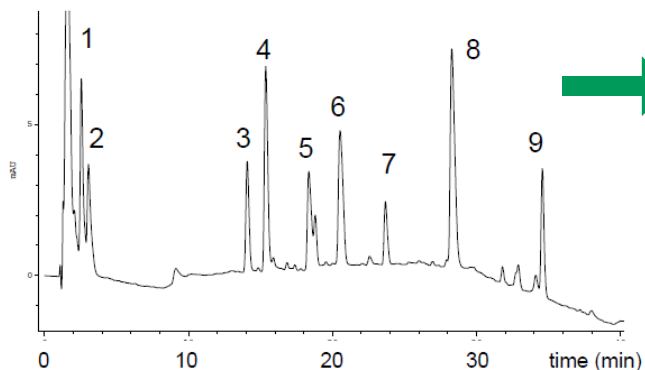
Solvent A: 2% Acetonitrile; 0.1% FA

Solvent B: 80% Acetonitrile; 0.08% FA

Gradient: 2% to 40% B in 35 Min

Detection: UV 214 nm

Analysis 12.5 ng tryp. digested Cytochrome C



Peak	m/z	Sequence
1	677,37	YIPGTK
2	633,38	IFVQK
3	1583,76	KTGQAPGFSYTDANK
4	1455,66	TGQAPGFSYTDANK
5	1167,61	TGPNLHGLFGR
6	778,44	MIFAGIK
7	963,53	EDLIAYLK
8	1632,81	IFVQKCAQCHTVEK
9	2137,04	GITWGEETLMEYLENP KK

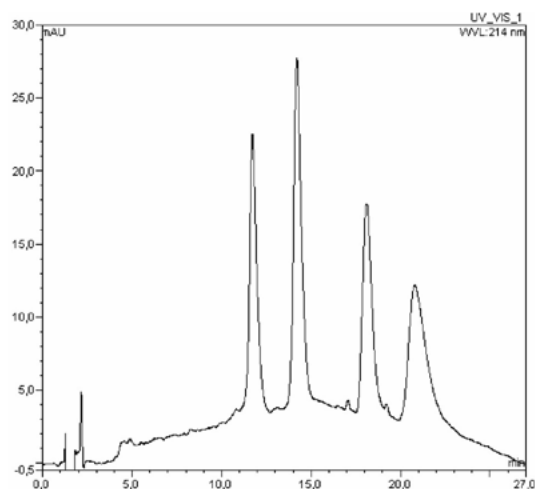
Monolithic silica
capillary column: Chromolith® CapRod®, RP-18e,
150 mm x 0.1 mm

Flow Rate: 3 µL/min

Gradient: 2% to 40% B in 35 Min

Mobile phase:
A: 0.1% Formic acid in
2% Acetonitrile
B: 0.08% Formic acid in
80% Acetonitrile

**Fast separation. Sharp
separation pattern.**



Column: Chromolith® CapRod®, RP-8e
150 mm x 0.1 mm (1.50400.0001)

Flow rate: 3 µL/min

Sample: Cytochrome C, Ribonuclease A,
BSA, APO-Transferrin (12
kDa–85 kDa)

Mobile phase A: 2% Acetonitrile, 0.01% FA

Mobile phase B: 80% Acetonitrile, 0.08% FA

Gradient: 5% to 60% B in 25 min

Ordering Information

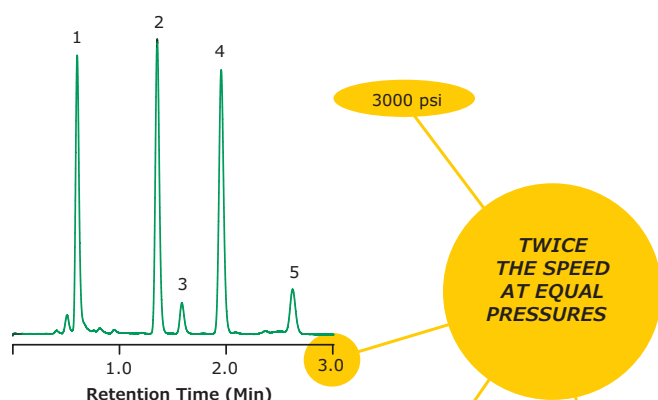
Column dimension						
Length (mm)		I.D. (mm)		HR RP-18e	RP-18e	RP-8e
Chromolith® CapRod® HPLC capillary columns [1 unit]						
50	x	0.1	Trap		1.50426.0001	1.52031.0001
50	x	0.2	Trap		1.50409.0001	
150	x	0.05		1.50403.0001		1.50401.0001 HR
150	x	0.1		1.50404.0001	1.50402.0001	1.50400.0001 HR
150	x	0.2		1.50407.0001	1.50405.0001	
300	x	0.1		1.50424.0001		

Ascentis® Express / BIOshell™ Fused-Core® Capillary LC-Columns

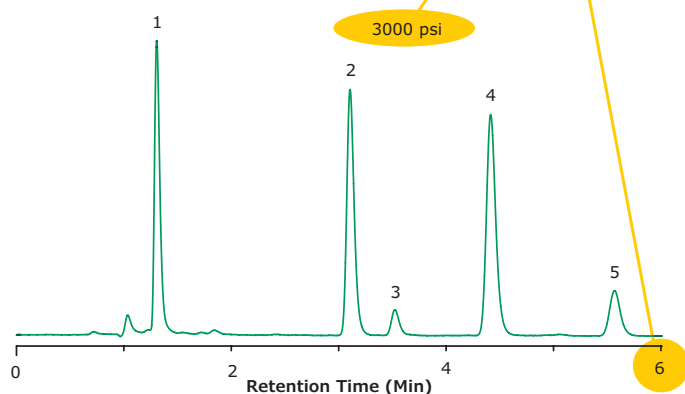
Maximum Resolution at High Speed

Ascentis® Express HPLC and UHPLC columns are based on Fused-Core® particle technology enabling fast results with highest resolution. Ascentis® Express columns provide about 40% more efficiency in comparison to columns with fully porous particles (FPP) of the same size. This performance enhancement is applicable to all HPLC instruments (in addition to UHPLC systems).

Ascentis® Express C18
0.4 mL/min flow rate



Sub-2 µm Competitor 2
0.2 mL/min flow rate



Twice the Speed at Equivalent Pressure vs. sub-2 µm

Compared to fully porous sub-2 µm particles typically used in UHPLC, Ascentis® Express Fused-Core® 2.7 µm particles generate approximately half the backpressure while providing the same high resolution. This attribute permits both longer columns, for more resolving power, and faster flow rates, for higher throughput.

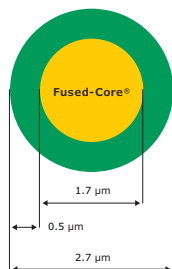
Demonstrating this point, the separation on the left shows a steroid mixture on Ascentis® Express (top) and a sub-2 µm UHPLC column (bottom) of the same dimensions. Due to the lower backpressure of the Ascentis® Express column, an increased flow rate (double in this case) can be applied providing the same backpressure, separation efficiency and resolution as on a sub-2 µm UHPLC, just with a 50% shorter runtime, increasing sample throughput.

Chromatographic Conditions:

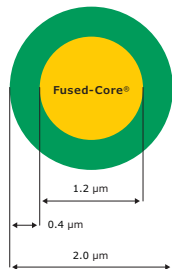
Columns:	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U) and sub-2 µm particle column (same dimensions)
Mobile Phase:	water/acetonitrile 49:51 (for Ascentis® Express); water/acetonitrile 55:45 (for sub-2 µm)
Column Temp.:	ambient
Detector:	UV, 200 nm
Injection:	1 µL

1. Estradiol
2. β-Estradiol
3. Impurity
4. Estrone
5. Estrone degradant

Benefits From the Exceptional Advancements of Fused-Core® Technology



A practical solution that delivers UHPLC performance from any HPLC
2.7 µm: 600 bar



An optimized solution for high throughput small molecule analysis
2 µm: 1000 bar

Fused-Core® particles consist of a solid silica core and a porous silica shell allowing a shorter diffusion path compared to conventional, fully porous particles.

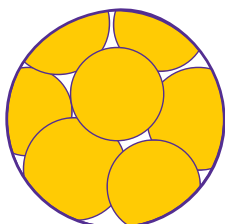
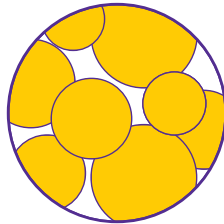
Features of Fused-Core® Particles Over Fully Porous Particles

- Narrower particle size distribution
- More consistently packed bed
- Shorter diffusion path

Fused-Core® (Superficially Porous Particles, SPP)

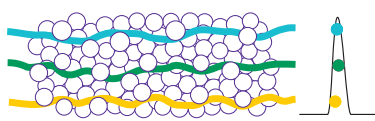
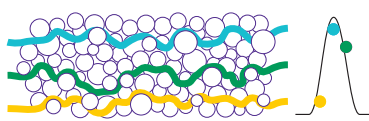
Fully Porous Particles (FPP)

Narrow Particle Size Distribution



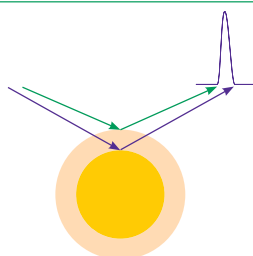
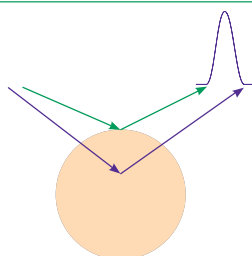
The innovative manufacturing process for Fused-Core® particles produces a very narrow particle size distribution. This attribute allows for the use of larger porosity frits that clog less, resulting in a more rugged column. Traditional, fully porous particles provide a larger particle distribution, requiring smaller pore frits, that clog more easily.

More Consistent Bed

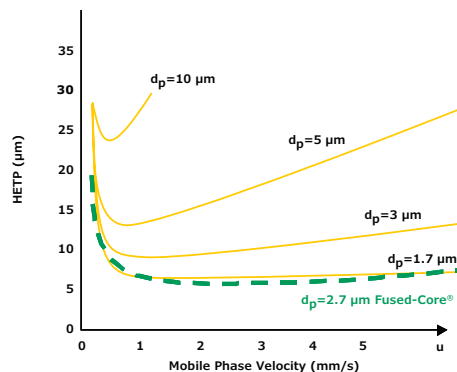


The "A" term in the van Deemter equation accounts for the effects of heterogeneities in the packed bed of an HPLC column. Narrow particle size distributions form a more consistently packed bed and a more consistent path length, minimizing analyte dispersion (peak broadening) through the column. This eddy diffusion is effectively independent of mobile phase velocity.

Shorter Diffusion Path



The short diffusion path of the Fused-Core® particle yields sharper peaks than on traditional, fully porous particle columns. The minimized resistance to mass transfer, the "C" term in the van Deemter equation, of the Fused-Core® particle, provides sharper peaks than traditional porous particles. The short diffusion path also permits the use of higher flow rates without significant peak broadening / loss in efficiency.



The factors that affect chromatographic efficiency are eddy diffusion, longitudinal diffusion, and resistance to mass transfer, the A, B and C terms, respectively, from the van Deemter equation.

$$H = A + \frac{B}{u} + Cu$$

- H Height equivalent to a theoretical plate (column length/efficiency)
- A Eddy diffusion
- B Longitudinal diffusion
- C Resistance to mass transfer
- u Mobile phase linear velocity

A New Column Dimension Emerges: 1.5 mm I.D.

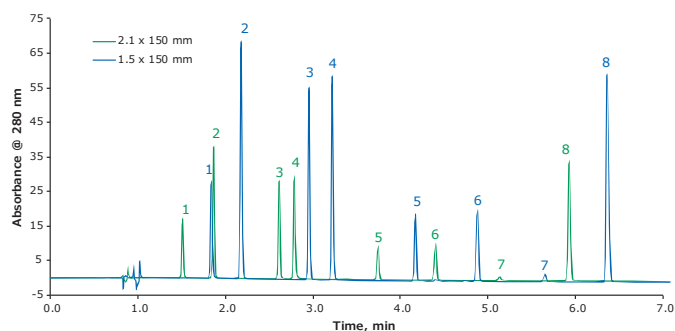
Ascentis® Express and BIOshell™ break new ground again with a 1.5 mm internal diameter column to push the boundaries of adopted UHPLC systems. Founded on all the benefits of Fused-Core® particles, these 1.5 mm I.D. columns deliver increased sensitivity and reduced solvent consumption, allowing scientists to experience the benefits of capillary columns without the pains of specialized, microflow systems.

Delivering More Performance

- More sensitivity from conventional UHPLC Systems
- Higher ionization efficiencies from LC-MS systems
- Reduced solvent consumption compared to 2.1 mm I.D. columns

Through newly designed, specialized, and manufactured fluidic hardware, the new Ascentis® Express 1.5 mm I.D. capillary columns deliver increased signal response in comparison to 2.1 mm I.D. columns and demonstrate higher efficiencies compared to 1 mm I.D. columns on optimized UHPLC systems.

Wall effects play a crucial role in small volume columns. Without an optimized UHPLC system, the effects garnered by small volume columns can be negated by instrument dispersion.



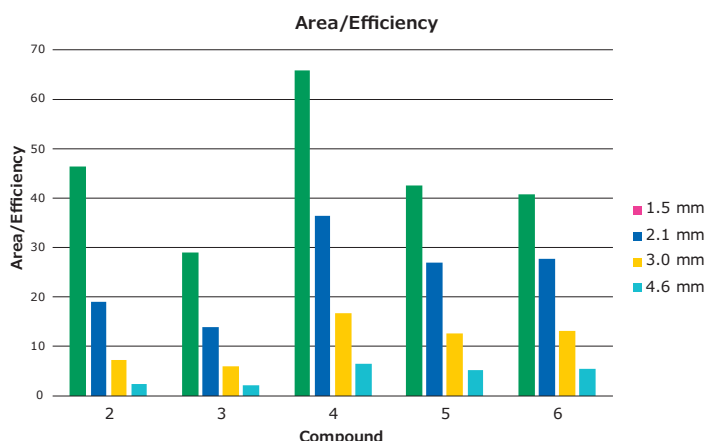
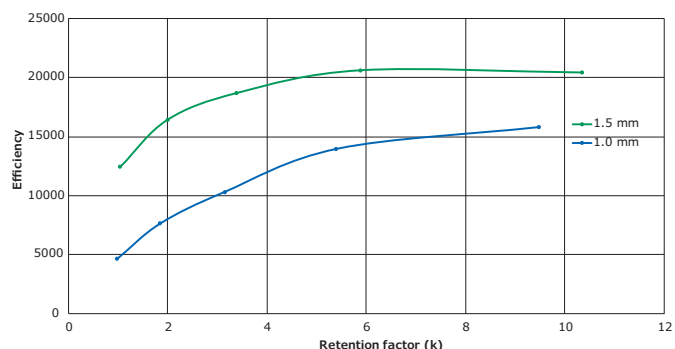
Peak #	Compound
1	Uracil
2	Acetophenone
3	Propiophenone
4	Butyrophenone
5	Valerophenone
6	Hexanophenone

Using a 1.5 mm I.D. column compared to a 2.1 mm I.D. column, the peak areas are doubled, and the sensitivity is improved using the same injection volume on both columns.

Test Conditions	
Column 1:	Ascentis® Express C18 (2.7 µm) 100 mm x 1.5 mm I.D. (50630-U)
Column 2:	Ascentis® Express C18 (2.7 µm) 100 mm x 2.1 mm I.D. (53823-U)
Mobile phase A:	Water
Mobile phase B:	Acetonitrile; Isocratic: 50/50 A/B
Flow Rate:	0.2 mL/min (1.5 mm); 0.39 mL/min (2.1 mm)
Pressure:	236 bar (1.5 mm I.D. column); 310 bar (2.1 mm)
Temperature:	35 °C
Detection:	UV 254 nm, PDA
Injection Volume:	0.5 µL
Instrument:	Shimadzu Nexera X2

Demonstration of Improved Efficiency with Ascentis® Express 1.5 mm I.D. Capillary Columns

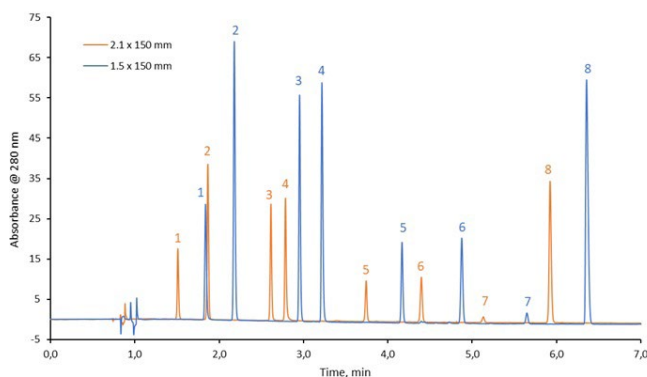
Using a 1.5 mm I.D. column compared to a 2.1 mm I.D. column, the peak areas are doubled, and the sensitivity is improved using the same injection volume on both columns. Through newly designed, specialized, and manufactured fluidic hardware, the new Ascentis® Express 1.5 mm I.D. capillary columns deliver increased signal response in comparison to 2.1 mm I.D. columns and demonstrate higher efficiencies compared to 1 mm I.D. columns on optimized UHPLC systems. A mixture of alkylphenones was run on 1.0, 1.5, 2.1, 3.0, and 4.6 mm I.D. columns. The area/efficiency of the 1.5 mm I.D. column outperforms all the analytical I.D. columns. While the 1.0 mm I.D. should perform better than the 1.5 mm I.D., even on an optimized UHPLC system, the dispersion is too great for a 1.0 mm I.D. column, thus causing lower efficiencies at all retention factors.



The 1.5 mm I.D. Advantage

Gradient Separation of OTC Cough and Cold Medicines

With extra column dispersion minimized, the 1.5 mm I.D. column shows taller peaks compared to 2.1 mm I.D. column providing the greatest benefit for minor components.



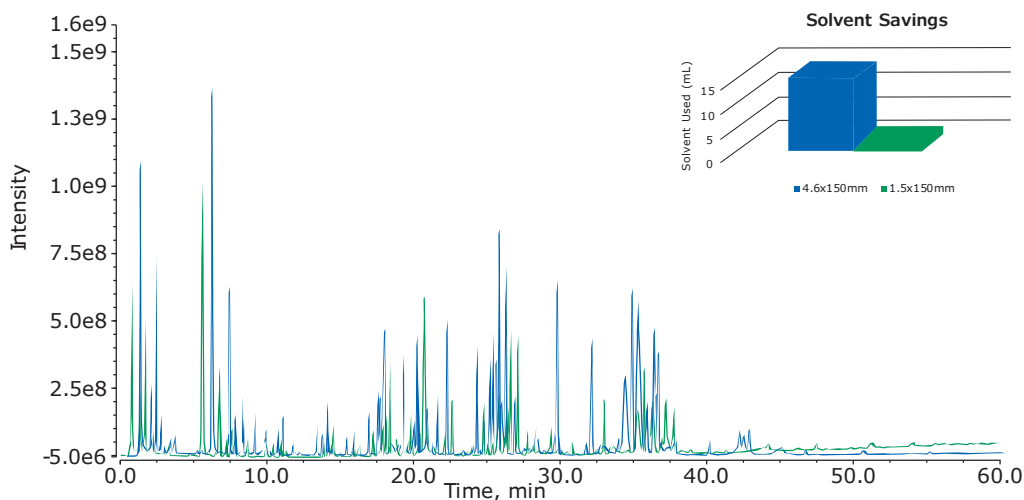
Peak #	Compound
1	Phenylephrine
2	Acetaminophen
3	Caffeine
4	Doxylamine
5	Guafenesin
6	Aspirin
7	Salicylic Acid
8	Dextromethorphan

Test Conditions	
Mobile Phase A:	Water/0.15% TFA
Mobile phase B:	Acetonitrile/0.1% TFA
Gradient :	5-50 % B in 8 min
Column A:	Ascentis® Express C18, 2.7µm, 15 cm x 1.5 mm
Part Number:	50636-U
Column B:	Ascentis® Express C18, 2.7µm, 15 cm x 2.1 mm
Part Number:	53825-U
Flow Rate :	0.2 mL/min for 1.5 mm I.D. column 0.4 mL/min for 2.1 mm I.D. column
Pressure:	425 bar/1.5 mm I.D. column 470 bar/2.1 mm I.D. column
Temperature:	35 °C
Detection:	UV 280 nm, PDA
Instrument:	Shimadzu Nexera X2

Solvent Reduction

The costs associated with solvents continue to grow from both a purchase price perspective and price of waste removal. In step with efforts to decrease the amount of environmental impact generated from hazardous chemicals, companies are seeking solutions to go greener. A decrease in column I.D. is optimized with lower flow rates resulting in an overall solvent savings. Moving to the BIOshell™ A160 Peptide C18, 1.5 mm I.D. capillary column helps analysts and companies reduce their solvent consumption. This attribute is an advantage of both BIOshell™ and Ascentis® Express columns in the 1.5 mm I.D. column geometry.

Peptide Map Solvent Savings Using BIOshell™ A160 Peptide C18 1.5 mm I.D. capillary columns



When a 1.5 mm I.D. column is used in comparison to a 2.1 mm I.D. column for a peptide map of trastuzumab, 50% of the solvent is saved since the 1.5 mm I.D. column is run at 0.2 mL/min compared to 0.4 mL/min for the 2.1 mm I.D. column. For a 60-minute analysis, only 12 mL of solvent is used vs. 24 mL of solvent for the 2.1 mm I.D. column. Not only is solvent saved, but the cost of waste disposal is also reduced.

MS Conditions	
Spray Voltage (kV):	3.8
Capillary Temperature:	320 °C
Sheath Gas:	35
Aux Gas:	10

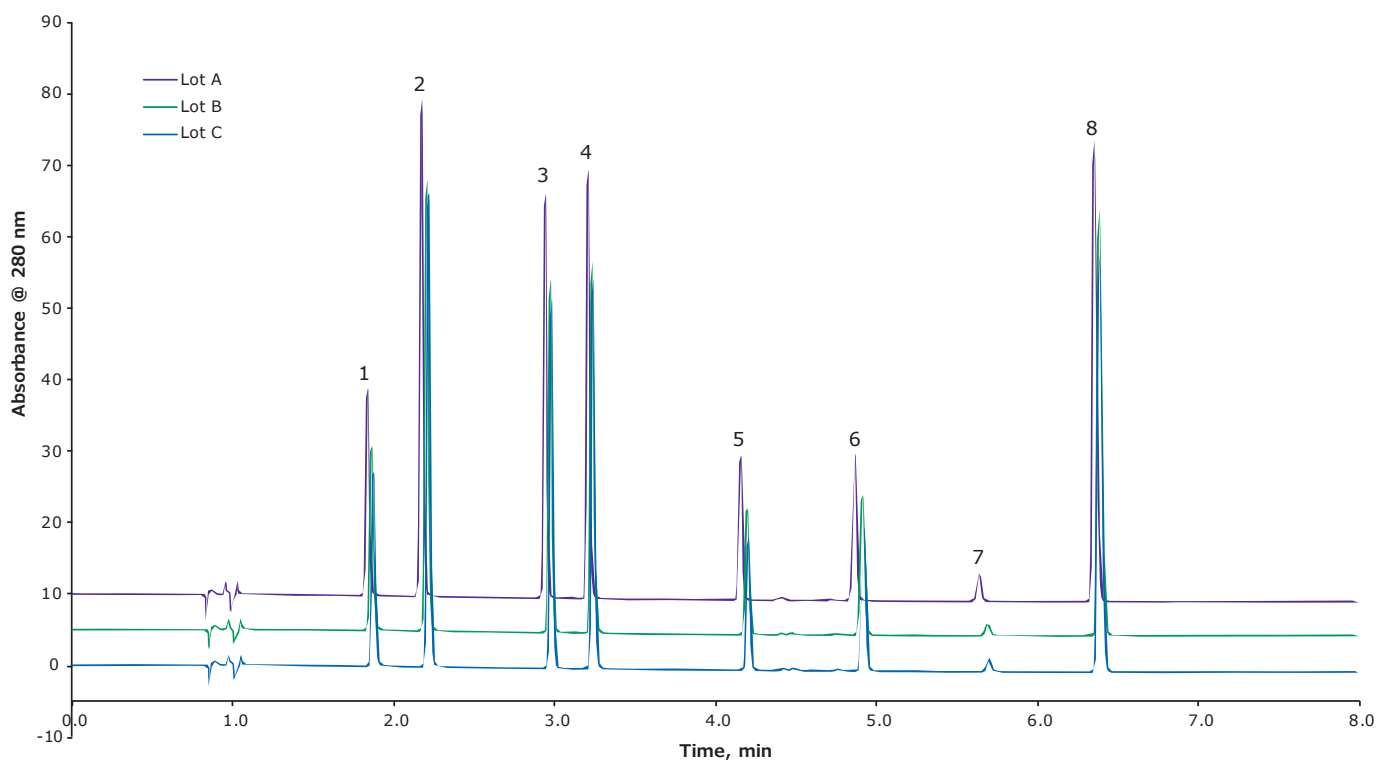
Test Conditions	
Column:	BIOshell™ A160 Peptide C18 (2.7 µm) 150 mm x 1.5 mm I.D. (66922-U)
Mobile Phase A:	Water/0.1% DFA B: Acetonitrile/0.1% DFA
Gradient:	2–50 %B in 60 min
Flow Rate:	0.2 mL/min for 1.5 mm I.D.; column 0.4 mL/min for 2.1 mm I.D. column
Back Pressure:	310 bar (1.5 mm I.D.) 444 bar (2.1 mm I.D.)
Detection:	ESI +
Injection Volume:	2 µL of 1.25 mg/mL Trastuzumab tryptic digest
Sample Solvent:	1.5 M Guanidine HCl/0.5% Formic Acid
LC System:	Shimadzu Nexera X2
MS System:	ThermoFisher Q Exactive

Stability

The new Ascentis® Express C18 1.5 mm I.D. capillary columns meet the same rigorous quality standards of all Ascentis® Express products. Multi-lot testing on the new hardware design and a QA report on every column produced from our ISO certified facility results in reliability you can depend on, column-to-column, analysis to analysis for the lifetime of the method.

Excellent Lot-to-Lot Reproducibility with the 1.5 mm I.D. Hardware

Separation of OTC cough and cold medicines using three different lots of the 1.5 mm I.D. column-hardware shows confidence in reproducibility.



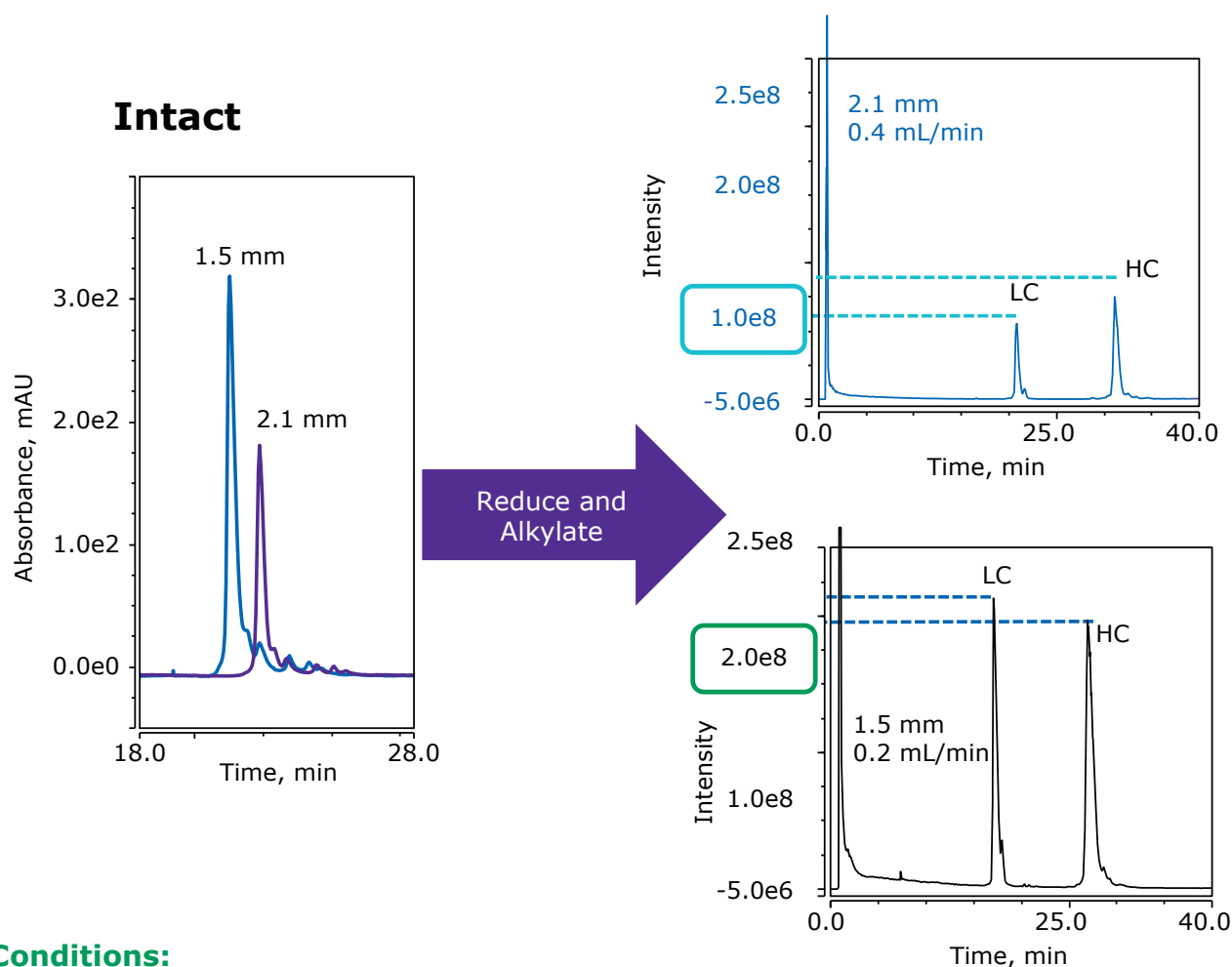
Test conditions for the above chromatogram are the same as separation reported on page 29.

Middle-Up Analysis of Trastuzumab: Sensitivity Gain Using 1.5 mm I.D. Columns

Sensitivity and recovery are two desirable traits in any method, especially in methods for characterizing monoclonal antibodies (mAbs). Having a method that can provide sharp, well-resolved peaks with sufficient area counts allows the analyst to better quantitate the analyte of interest and determine if any degradation products are in the sample.

In the intact analysis of trastuzumab, double the area for the mAb was obtained on the 1.5 mm I.D. column as compared to the 2.1 mm I.D. column.

In addition, in the reduced and alkylated assay, the area counts and sensitivity were improved significantly (2.7-fold increase in area count for the light chain (LC) and 2.3-fold increase in area count for the heavy chain (HC)) when using a 1.5 mm I.D. column as compared to a 2.1 mm I.D. column.



Conditions:

Column:	BIOshell™ IgG 1000 Å Diphenyl, 15 cm x 2.1 or 1.5 mm I.D., 2.7 µm (577450-U, 577421-U)
Mobile Phase:	[A] Water (0.1% (v/v) DFA); [B] 50:50 Acetonitrile (0.1% (v/v) DFA): n-Propanol (0.1% (v/v) DFA)
Gradient:	27–36% B in 40 min
Flow Rate:	As indicated
Column Temp.:	60 °C
Detector:	MSD, ESI-(+)
Injection:	3 µL
Sample:	Trastuzumab, 1.0 mg/mL, 100 mM Ammonium bicarbonate

Ascentis® Express

Ascentis® Express (2.7 µm)									
Length (mm)		I.D. (mm)	C18 pH+	C18	ES-C18	PCS-C18	Peptide ES-C18	C8	PCS-Phenyl-Hexyl C30
50	x	0.3		53992-U			53546-U	53997-U	
150	x	0.3		54271-U			53554-U	54272-U	
50	x	0.5		53998-U			53547-U		
150	x	0.5		54273-U					
50	x	1.0					53548-U		
150	x	1.0							577141-U
50	x	1.5	70012-U	50629-U	50582-U	50637-U			50686-U
100	x	1.5	70013-U	50630-U	50584-U	50638-U			50688-U
150	x	1.5	70014-U	50636-U	50586-U	50639-U			50689-U

BIOshell™

BIOshell™ U/HPLC Columns												
Length (mm)		I.D. (mm)	120 Å 2.7 µm	160 Å 2.0 µm	160 Å 2.7 µm			400 Å 3.4 µm		1000 Å 2.7 µm		
			Oligo	C18	C18	CN	Phenyl-Hexyl	PCS-C18	C4	C18	C4	C18
150	x	0.2						67037-U				
50	x	0.3				67159-U	577546-U	67038-U	67496-U	63336-U	581367-U	577436-U
150	x	0.3			67093-U	67160-U	577545-U	67039-U		63335-U	581366-U	577435-U
50	x	0.5				67161-U	577548-U			63338-U	581381-U	577438-U
100	x	0.5			67096-U							
150	x	0.5			67097-U	67163-U	577547-U	67041-U		63337-U	581380-U	577437-U
50	x	1.0				67164-U	577550-U			63340-U	581383-U	577440-U
150	x	1.0			67099-U	67165-U	577549-U	67045-U		63339-U	581382-U	577439-U
50	x	1.5	70000-U	67284-U	66923-U			50653-U		581385-U		577451-U
100	x	1.5	70001-U					50654-U				
150	x	1.5	70002-U	67283-U	66922-U			50655-U		581384-U		577450-U

Installation / Care and Use for Packed Capillaries Along with Chromolith® CapRod® Columns

Guidelines for Capillary Columns

High performance columns with small internal volumes (shorter lengths, internal diameters <1 mm) are being increasingly used for high-speed separations, especially with mass spectrometers. These low-volume columns generate peaks having considerably less volume than those eluting from columns of larger dimensions (e.g., 15 cm x 2.1 mm I.D.). The efficiency of separations performed in low volume columns is highly dependent on the U/HPLC system having components designed to minimize band spreading. All low volume columns perform best when used with proper attention being paid to the following factors:

- **UV Detector:** For optimum performance, depending on flow rate, we recommend the following detector cell volumes:

Flow Rate (µL/min)	Flow Cell Volume (nL)
Nano, 0.05–1	3
Capillary, 0.5–50	45
Micro, ≥50	180

To properly sense and integrate the fast peaks that can elute from low volume columns, the detector response time should be set to the fastest level (~0.1 s) to allow integration of signal by software of at least 20 points across the narrowest peak.

- **Injector:** The injection system should be of a low volume design. Autosamplers will often cause band spreading but may be used for convenience with the expectation of some loss in column efficiency. Recommendation for injection parameters is to keep injection loop inline, and to ensure needle wash solvent is weaker than mobile phase to prevent mobile phase mismatch. Draw speed and draw delay should be set to lowest setting and dispense speed set to highest setting. It is also recommended to minimize sample loop size to minimize baseline interference. Smaller injection volumes are normally injected with lower accuracy; therefore, it is important to check instrument limits.
- **Connection Tubing:** The shortest possible lengths of connection tubing with small internal diameters must be used for both the connection between the injector and the column, and for the connection between the column and the detector (flow cell). The recommended tubing length is <50 cm for connection between injector and column inlet; recommended tubing I.D. is listed below. Tubing connections that cause excessive dead volume will significantly deteriorate efficiency and peak shape and should be avoided. Flow cells of small volumes (nL) may have preassembled fused silica lines to attach directly to the column with the use of a ferrule

and nut. A minimal dead volume connection will be achieved if care is taken to make sure the fused silica is completely inserted into the column outlet prior to tightening of the nut. When tightening the nut to the column outlet, use two wrenches (one on column outlet fitting and one on nut) and apply pressure to the fused silica to ensure the fused silica does not slip out of column outlet. Tighten the nut to the column outlet until the ferrule holds the fused silica snug in the column outlet. For the tubing between the injector and column inlet, fused silica tubing is not required, but it is necessary to ensure that the tubing is completely seated into the injector, as well as the column inlet. For optimum performance, we recommend the following:

- **Recommended flow rates and injection volumes:** To maximize chromatographic performance and resolution, it is crucial to use small capillary columns with the optimal flow rate as indicated by the van Deemter plot. Ensuring the flow rate is within this optimal range minimizes band broadening and maximizes efficiency. Additionally, avoiding column overloading is essential; therefore, the injection volume and sample concentration must be kept within optimal intervals. For optimum performance, we recommend the following:

Column Dimension (mm)	Typical Flow Rates	Max Sample Amount	Sample Volume
100 x 0.075	0.15–0.5 µL/min	9 ng	0.5–9 nL
100 x 0.1	0.25–1 µL/min	16 ng	1–16 nL
100 x 0.2	1–4 µL/min	60 ng	0.01–0.06 µL
100 x 0.3	2–9 µL/min	140 ng	0.01–0.14 µL
100 x 0.5	5–25 µL/min	0.4 µg	0.05–0.4 µL
100 x 1	20–100 µL/min	2 µg	0.1–2 µL
100 x 1.5	40–200 µL/min	4 µg	0.2–4 µL
100 x 2	0.08–0.4 mL/min	6 µg	0.4–6 µL

- **Sample Solvent:** For isocratic separations, the volume of sample injected should be kept as small as possible in a solvent weaker than the mobile phase. Sample volumes are less critical for gradient separations, and a larger volume is possible if the sample is dissolved in a solvent weaker than the mobile phase flowing through the column at the moment of injection.
- **Mobile Phase:** Ultra-pure (MS Grade) solvents and buffer salts are recommended for use with capillary UHPLC columns. It is generally recommended that all mobile phases and samples be filtered using a 0.22 µm membrane filter. When utilizing gradient conditions, and/or inline solvent mixing please consult instrument vendor guidance for appropriately sized mobile phase mixing chamber.

Notes

[illegible]

Supelco®

Analytical Products

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Frankfurter Strasse 250
64293 Darmstadt, Germany

SigmaAldrich.com



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