Enhancing Analytical Sensitivity: New Complementary HPLC Phases for Versatile Separations in Capillary LC

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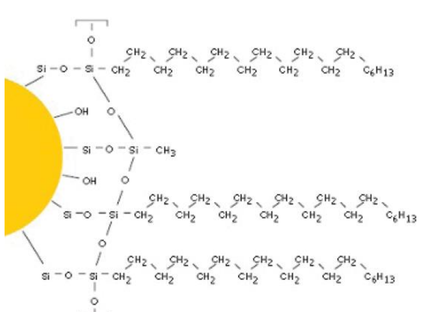
Introduction

Capillary High-Performance Liquid Chromatography (HPLC) columns are significantly improving sensitivity and sustainability in chemical analyses due to their narrow inner diameter (I.D.) and reduced flow rates. This design not only minimizes solvent usage and waste but also facilitates the detection of various analytes at trace levels. The small diameter of capillary columns enhances mass transfer, resulting in better separation and identification of complex mixtures.

Selecting the right chromatographic selectivity is also crucial for developing sensitive methods that can effectively resolve compounds of interest.

This poster showcases how different complementary phases in a capillary format - specifically, the Purospher® STAR RP-18 endcapped, SeQuant® ZIC®-HILIC, and ZIC®-cHILIC can address various analytical challenges.

Purospher® STAR RP-18e Capillary LC-Columns



Fully porous silica particles (FPP) have been a preferred choice in HPLC for decades due to their high efficiency, versatility, and compatibility with modern instrumentation. Ongoing advancements in particle technology continue to solidify their reputation as the gold standard in HPLC separations.

Purospher® STAR RP-18 endcapped (2 µm) capillary columns utilize ultra-pure fully porous silica particles (Type B) and are designed for universal application. They enable the effective separation of basic, neutral, and metal-chelating compounds using straightforward mobile phases while ensuring excellent peak symmetry. These columns exhibit remarkable stability across a pH range of 1.5 to 10.5 and over a broad temperature range, demonstrating superior retention characteristics for a variety of analytes.

2D LC-MS/MS Characterization of Trypsin-cleaved Proteins

Characterizing proteins is vital for understanding their roles in health and disease. Due to their complexity and high molecular mass, identifying intact proteins with a single analytical method is challenging.

Peptide mapping, which involves cleaving proteins into smaller fragments, enables amino acid sequence determination and protein identification through chromatographic separation against reference standards. This technique also evaluates protein quality by detecting alterations like misincorporation and modifications such as glycosylation.

Developing a peptide mapping identity test requires understanding the specific application and characteristics of the protein to ensure specificity and validation. Key considerations include sample pretreatment, cleavage techniques, separation, detection, and data analysis. The choice of cleavage method depends on the protein and involves various agents, each with specific pH and temperature requirements that affect effectiveness and reproducibility.

LC Conditions			
Instrument:	Capillary HPLC LC system with an Ultima QTOF mass spectrometer		
Columns:	RPLC: FPP C18, 5 µm (300 Å), 15 cm x 0.3 mm I.D. capillary column		
	HILIC: SeQuant® ZIC®-HILIC, 5 µm (200 Å), 15 cm x 0.3 mm I.D. (**150481-U**)		
Mobile phase:	[A] 100% water with 0.25% formic acid (FA)		
	[B] 100% acetonitrile with 0.25% formic acid (FA)		
Gradient:	**Time (minutes)**	**RPLC - A (%) HILIC - B (%)**	**RPLC - B (%) HILIC - A (%)**
	0	95	5
	4	95	5
	44	45	55
	46	20	80
	50	20	80
	52	95	5
	60	95	5
Flow rate:	7 µL/min		
Column temp.:	Room temperature (23 °C)		
Detector:	MS/MS (QTOF), scan range RPLC: m/z 300-1800, 1.5 s cycle time, HILIC: m/z 400-1800, 1.5 s cycle time		
Injection:	1 µL		
Sample(s):	10 pmol of each digested sample were injected via the autosampler using the instrument µL-pick-up technique.		

Table 1. Reversed Phase (RPLC) and HILIC method experimental conditions

Utilizing a zwitterionic HILIC column alongside a C18 RPLC column in a Two-Dimensional (2D) liquid chromatography setup enhances the characterization of trypsin-cleaved proteins by harnessing their complementary separation mechanisms.

The ZIC®-HILIC column effectively retains hydrophilic peptides and improves resolution for polar modifications, while the C18 column is adapted at retaining hydrophobic peptides, enabling a comprehensive analysis of protein digests.

By implementing tailored gradient separation methods, this approach efficiently separates a wider range of peptide properties, boosting sensitivity and specificity in LC-MS/MS characterization.

Notably, the SeQuant® ZIC®-HILIC column can identify some hydrophobic peptides that the RP column fails to detect due to excessive retention.

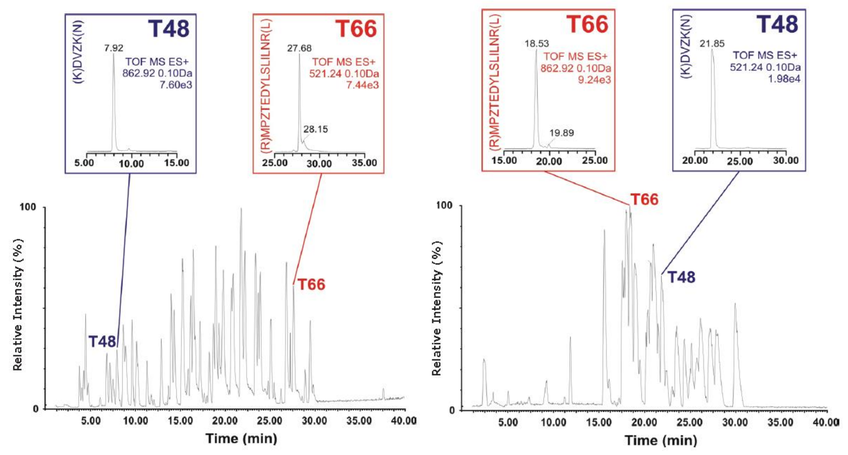
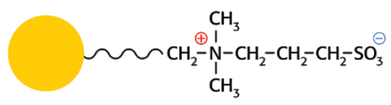


Figure 1a and 1b: Base peak intensity chromatograms (BPI's) show peptide maps of a BSA tryptic digest using RPLC (left) and ZIC®-HILIC (right) gradients. Inserts show the ion chromatograms of two peptide fragments T48 and T66, (K)DVZK(N) and (R)MPZTEDYLSLILNR(L) respectively. T48 is a smaller peptide fragment with a molecular weight of 520.23 whereas T66 has a molecular weight of 1723.23 (but detected as M+2R ion).

SeQuant® Capillary Columns

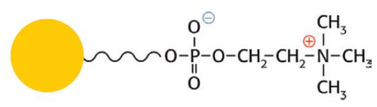
SeQuant® ZIC®-HILIC and ZIC®-cHILIC columns are advanced high-performance tools specifically engineered for the separation of polar hydrophilic compounds using hydrophilic interaction liquid chromatography (HILIC). Each column is equipped with densely bonded zwitterionic functional groups that maintain a 1:1 charge balance, facilitating separation through hydrophilic partitioning and weak ionic interactions. This design ensures optimal selectivity, high load capacity, and enhanced method optimization.

ZIC®-HILIC



Sulfobetaine

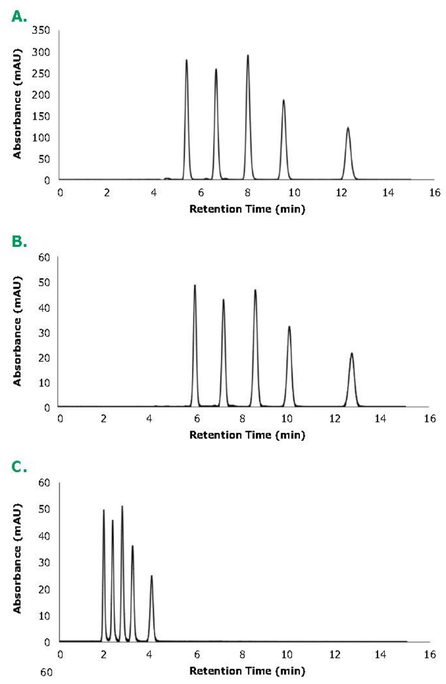
ZIC®-cHILIC



Phosphorylcholine

UHPLC -> Capillary LC

The separation of five nucleosides using ZIC®-cHILIC UHPLC and capillary columns of different lengths and diameters (150 x 2.1 mm, 150 x 1.0 mm, and 50 x 1.0 mm) showcases the effective scaling from UHPLC to capillary dimensions. The use of shorter columns not only facilitates rapid separations but also ensures baseline resolution. This approach contributes to sustainability by minimizing solvent consumption and reducing waste, making it an environmentally friendly choice for analytical applications.



Chromatographic Conditions:	
Column:	SeQuant® ZIC®-cHILIC (3 µm) A.) 150 mm x 2.1 mm I.D. B.) 150 mm x 1.0 mm I.D. C.) 50 mm x 1.0 mm I.D.
Mobile phase:	80:20 acetonitrile: 25 mM ammonium acetate pH unadjusted Isocratic
Flow rate:	A.): 0.095 mL/min B.) and C.): 0.020 mL/min
Temperature:	30 °C
Injection:	A.): 0.5 µL B.) and C.): 0.020 µL
Detector:	UV at 254 nm
Instrument:	A.): Dionex UltiMate™ 3000 B.) and C.): Thermo Vanquish™ Neo
Elution order:	1. thymidine 2. 5-methyluridine 3. uridine 4. 2-deoxycytidine 5. cytidine

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

Chromatogtaphic Conditions:

Instrument	Thermo Sincetific Vanquish Neo UHPLC system
Column	Purospher® STAR RP-18 endcapped (2 µm) 15 cm x 1.0 mm
Mobile Phase	70:30, Methanol:Water (Pre-mixed) Isocratic
Flow Rate	50 µL/min
Temperature	30 °C
Wavelength	UV, 254 nm
Injection Volume	0.25 µL
Test Mix	Uracil Acetophenone Benzene Toluene in 60:40 methanol:water

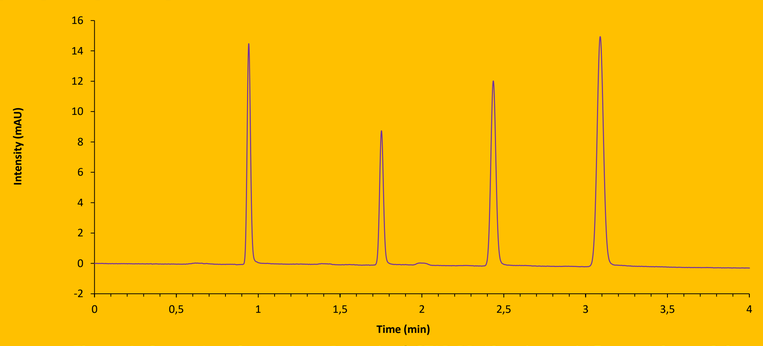
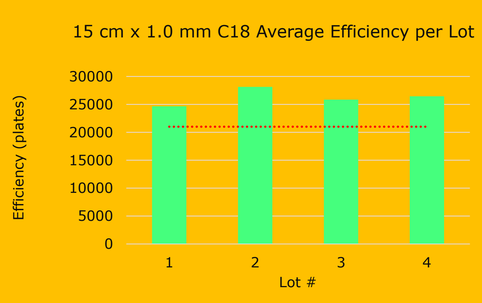


Figure 1: Separation of test mix on Purospher® STAR RP-18 endcapped 15 cm x 1.0 mm, 2.0 µm



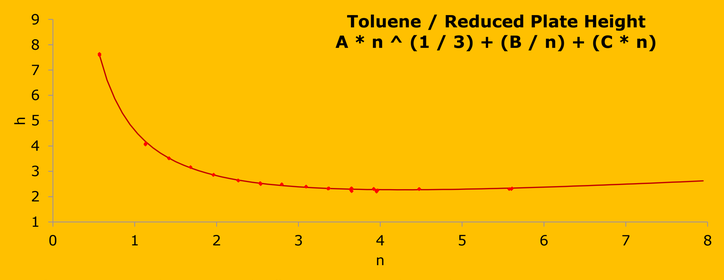


Figure 2: Knox plot on Purospher® STAR RP-18 endcapped 15 cm x 1.0 mm, 2.0 µm

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