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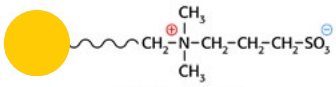
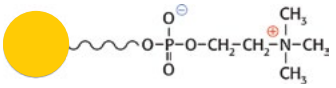
Analytical Products

MilliporeSigma

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

Superior separation of polar hydrophilic compounds

SeQuant® ZIC®-HILIC and ZIC®-cHILIC columns are high-performance tools designed for separating polar hydrophilic compounds under hydrophilic interaction liquid chromatography (HILIC) conditions. Each column features densely bonded zwitterionic functional groups with a 1:1 charge balance, allowing separation through hydrophilic partitioning and weak ionic interactions for optimal selectivity, high loadability, and method optimization.

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

	ZIC®-HILIC	ZIC®-cHILIC
Silica:	Type B (high purity silica)	Type B (high purity silica)
Particle Platform:	Fully porous particles (FPP)	Fully porous particles (FPP)
Phase Chemistry:	Sulfobetaine	Phosphorylcholine
Particle Size:	3.5 µm and 5 µm	3 µm
Pore Size:	100 Å and 200 Å	100 Å
pH-Range:	2-8	2-8
Max. Temperature:	70 °C	70 °C
Pressure Stability:	550 bar	550 bar

Features and Benefits:

- High-performance separations for polar hydrophilic compounds in HPLC and LC-MS
- Zwitterionic stationary phase ensuring reproducible retention
- Two complementary phase chemistries
- Low column bleed for maximum LC-MS compatibility
- Excellent reproducibility and robustness

The **NEW SeQuant® ZIC®-HILIC and ZIC®-cHILIC capillary columns** are housed in modernized glass-lined capillary column hardware, enhancing their ability to separate a broad range of hydrophilic bio-related molecules, including phosphorylated compounds and peptides, and provide improvements for applications in OMICs fields such as proteomics, metabolomics, and glycomics. These columns have demonstrated exceptional performance in these areas.

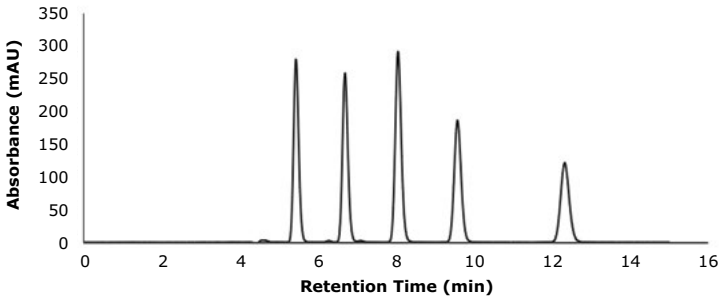
Separation of nucleosides on SeQuant® ZIC®-cHILIC (3 µm) using 2.1 mm I.D. and 1.0 mm I.D. columns

Nucleosides are vital polar components of nucleic acids, essential for encoding and expressing genetic information. This application analyzes five nucleosides: thymidine, 5-methyluridine, uridine, 2-deoxycytidine, and cytidine. Thymidine and uridine are crucial for DNA and RNA, while 2-deoxycytidine and cytidine serve as precursors for their synthesis, with cytidine also involved in metabolic pathways.

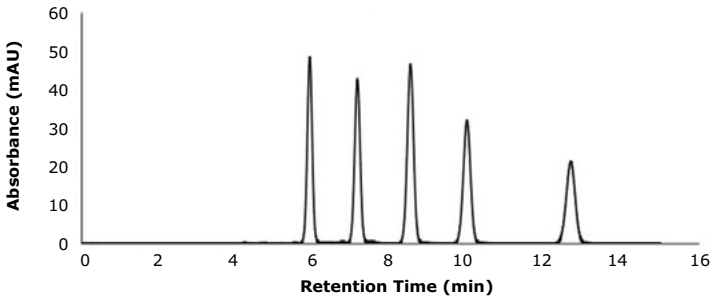
Hydrophilic interaction chromatography (HILIC) is preferred for polar compounds but requires high acetonitrile concentrations, posing health and environmental risks. Reducing acetonitrile consumption with narrow inner diameter columns is both cost-effective and more eco-friendly.

The separation of five nucleosides using ZIC®-cHILIC UHPLC and capillary columns of varying lengths and diameters (150 x 2.1 mm, 150 x 1.0 mm, and 50 x 1.0 mm) demonstrates the efficient scaling from UHPLC to capillary dimensions. Additionally, very short columns enable rapid separations while maintaining baseline resolution.

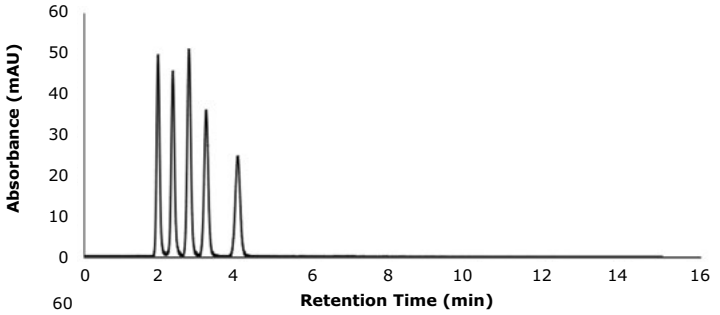
A.



B.



C.



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

By using a narrow column I.D. (A to B), solvent consumption dropped significantly by a factor of 4.75 in the shown separation example while maintaining the same separation pattern. Additionally, the use of a short column (C) reduces the analysis time from 15 minutes to 5 minutes.

LC-MS/MS characterization of trypsin-cleaved proteins

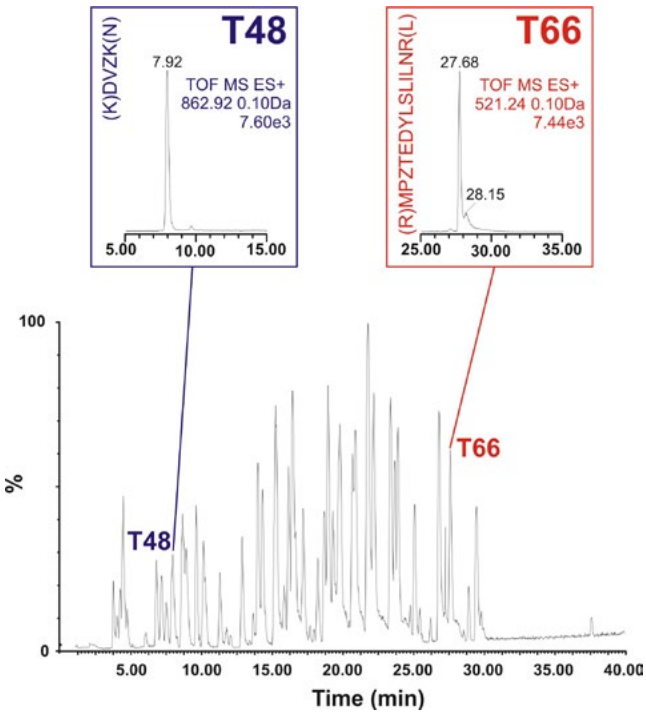
Characterizing proteins is vital for understanding their health-related roles. Peptide mapping, which breaks proteins into smaller fragments, aids in amino acid sequencing and protein identification.

HILIC columns are an ideal complement to RPLC for 2D-LC. The zwitterionic (ZIC®) HILIC column effectively retains hydrophilic peptides and enhances resolution for polar modifications, while the C18 column is excellent for retaining hydrophobic peptides, enabling a thorough analysis of protein digests.

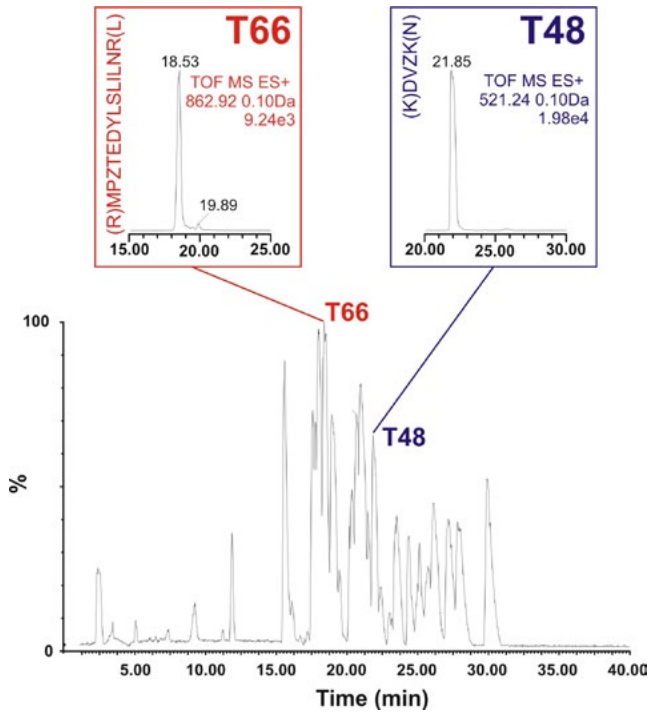
The separation example below analyzes trypsin-cleaved proteins like BSA using capillary LC and TOF-MS for enhanced resolution and sensitivity.

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.		

RPLC

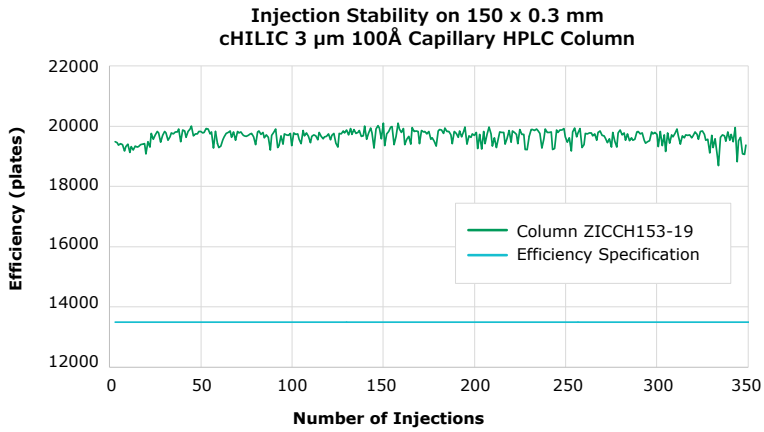


HILIC

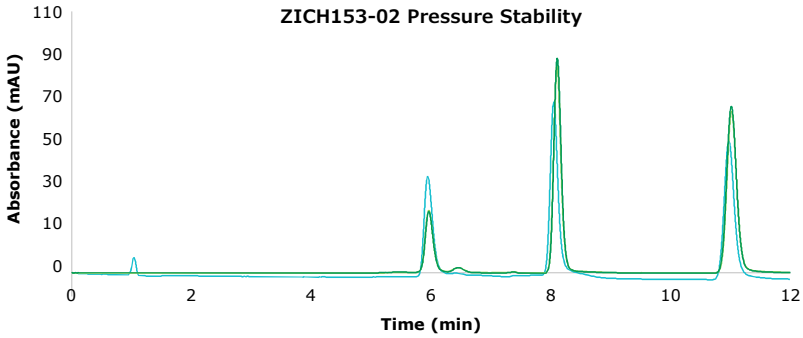


Base peak intensity chromatograms (BPI's) show peptide maps of a BSA tryptic digest using RPLC (left) and ZIC®-HILIC (right) gradients. Inserts show the ion chromatograms of two peptide fragments T48 and T66, (K)DVZK(N) and (R)MPZTEDYLSLILNR(L) respectively. T48 is a smaller peptide fragment with a molecular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

The **new hardware**, combined with an **improved** packing procedure, provides **superior reproducibility** and **stability** compared to existing capillary columns.



The improved packing results in an extremely good stability of the NEW SeQuant® ZIC®-HILIC and ZIC®-cHILIC capillary columns.



The very good pressure stability is proven with a pressure-shock test showing excellent stability.

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			**1.50465**	
100	x	0.1	**1.50466**			
50	x	0.3	**150473-U**			**150664-U**
150	x	0.3	**150479-U**	**150671-U**	**150481-U**	**150669-U**
50	x	1.0	**150476-U**			**150668-U**
150	x	1.0	**150480-U**	**150487-U**	**150482-U**	**150670-U**

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