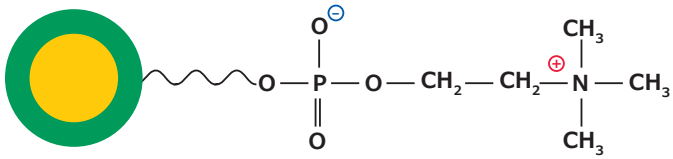
Supelco®
Analytical Products

MilliporeSigma

Ascentis® Express 160Å ZIC®-cHILIC

Superior selectivity for the separation of polar compounds

The Ascentis® Express ZIC®-cHILIC column is designed for the effective retention and separation of polar and hydrophilic compounds, delivering robust chromatography with high selectivity and reproducibility. Its zwitterionic phosphorylcholine functional groups provide superior hydrophilic retention compared to bare silica and other HILIC phases, facilitating easier method development for difficult-to-separate analytes. The column's high water-layer thickness enhances partitioning interactions, offering flexibility in method development and showcasing the unique properties of zwitterionic HILIC phases. Additionally, the Fused-Core® particle technology enables faster results and significantly improved separation resolution, achieving 40% greater efficiency than fully porous particulate columns of the same size.



Key Benefits

- Exceptional separation efficiency and chromatographic performance for optimal separation of polar hydrophilic compounds
- Ensures excellent reproducibility and robustness
- Pore size of 160Å meets the demands for biopharmaceuticals
- Fully inert INERTProve™ column hardware featuring coated SST

Ascentis® Express ZIC®-cHILIC Specifications:

Silica:	Type B (High purity silica)
Particle platform:	Superficially porous particles (SPP)
Phase chemistry:	Phosphorylcholine
USP:	-
Particle size:	2.7 µm
Pore size:	160Å
Surface area:	90 m^2/g
pH range:	2–8
Max temperature:	60 °C
Column hardware:	INERTProve™

Maximum resolution in HILIC

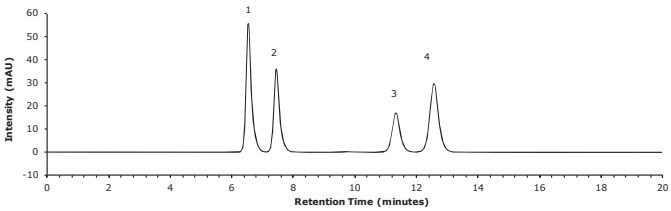
The following separation examples compare the performance of a fully porous particulate (FPP) column, SeQuant® ZIC®-cHILIC, with a superficially porous particulate (SPP) Ascentis® Express ZIC®-cHILIC column in separating four nucleosides and nucleobases. Both columns were evaluated under identical chromatographic conditions, utilizing a mobile phase of acetonitrile and 25 mM ammonium acetate buffer in an 80/20 ratio, with a flow rate of 0.1 mL/min and an injection volume of 0.2 µL.

The results demonstrate a significant enhancement in separation efficiency and peak shape, highlighting the exceptional chromatographic performance of the Ascentis® Express ZIC®-cHILIC columns for optimal separation of polar hydrophilic compounds.

Separation of Nucleosides

FPP

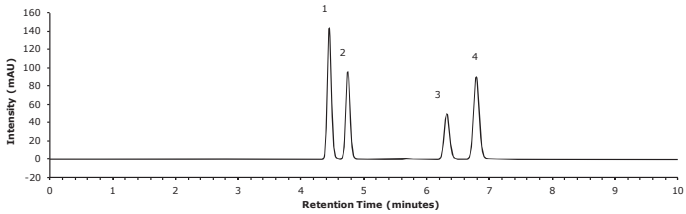
SeQuant® ZIC®-cHILIC (3 µm) 100Å, 150 x 2.1 mm



No.	Compound	Retention Time (min)	Plates (USP)	Tailing Factor (USP)
1	Adenosin	6.53	56400	1.45
2	Uridin	7.44	61487	1.37
3	Cytidin	11.32	67653	1.13
4	Guanosin	12.56	66060	1.11

SPP

Ascentis® Express ZIC®-cHILIC (2.7 µm) 160Å, 150 x 2.1mm

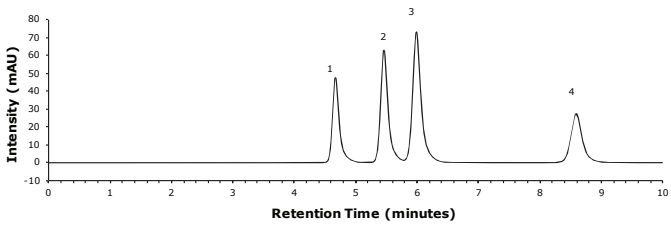


No.	Compound	Retention Time (min)	Plates (USP)	Tailing Factor (USP)
1	Adenosin	4.45	147573	1.14
2	Uridin	4.74	153726	1.12
3	Cytidin	6.32	175133	1.09
4	Guanosin	6.79	177573	1.08

Separation of Nucleobases

FPP

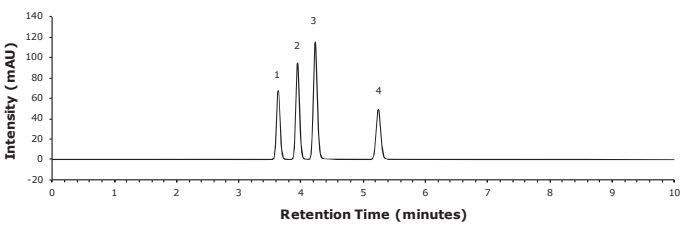
SeQuant® ZIC®-cHILIC (3 µm) 100Å, 150 x 2.1 mm



No.	Compound	Retention Time (min)	Plates (USP)	Tailing Factor (USP)
1	Thymine	4.67	68493	1.49
2	Uracil	5.46	74180	1.40
3	Adenine	5.99	68333	1.36
4	Cytosine	8.59	77427	1.20

SPP

Ascentis® Express ZIC®-cHILIC (2.7 µm) 160Å, 150 x 2.1mm



No.	Compound	Retention Time (min)	Plates (USP)	Tailing Factor (USP)
1	Thymine	3.63	134953	1.12
2	Uracil	3.94	146406	1.11
3	Adenine	4.23	146093	1.13
4	Cytosine	5.24	164147	1.09

Unsurpassed inertness – INERTProve™ column hardware

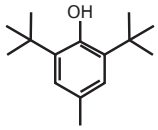
The Ascentis® Express ZIC®-cHILIC column features fully inert hardware, which is essential for effective HILIC applications. The inert nature of the hardware enhances the separation of polar and hydrophilic compounds, significantly improving the overall efficiency of chromatography.

Inert materials minimize unwanted interactions between the stationary phase and analytes, leading to better peak shapes and resolution. This is particularly important for compounds with high polarity, such as amino acids, carbohydrates, and polar metabolites, which are susceptible to interactions that can adversely affect their retention and separation.

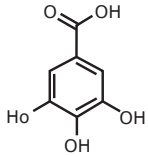
Phosphorylated compounds, known for their high polarity, are especially prone to engaging in unwanted interactions with the reactive surfaces of standard column materials. Such interactions can result in variability in retention times and peak shapes, complicating the analysis.

To evaluate the inertness of the column hardware, a test was designed using butylated hydroxytoluene (BHT), gallic acid, and norepinephrine. These compounds are highly sensitive to metal content, which can lead to pronounced tailing in chromatographic peaks.

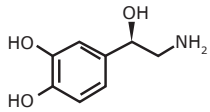
A comparison between the Ascentis® Express ZIC®-cHILIC column with INERTProve™ hardware and competing inert columns reveals significant differences in chromatographic performance. The results demonstrate the superior inertness of the Ascentis® Express ZIC®-cHILIC column as evidenced by improved peak shapes and reduced tailing for these sensitive compounds.



BHT

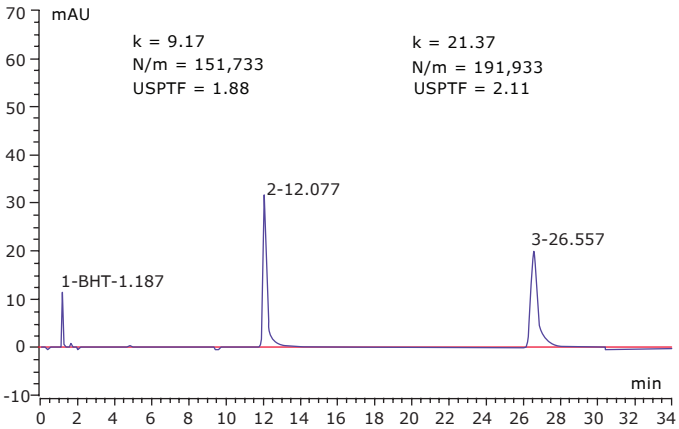


Gallic Acid

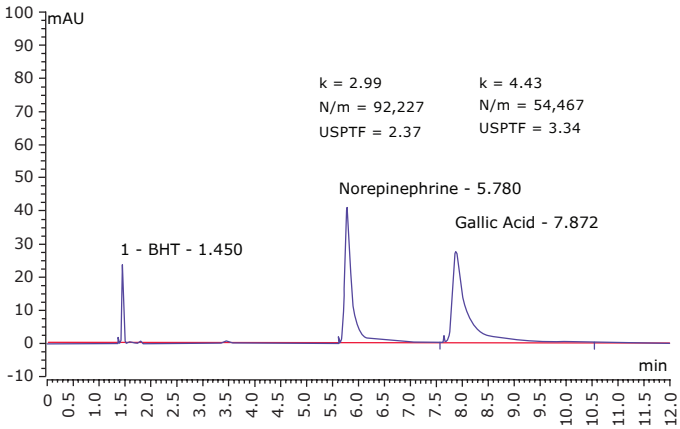


Norepinephrine

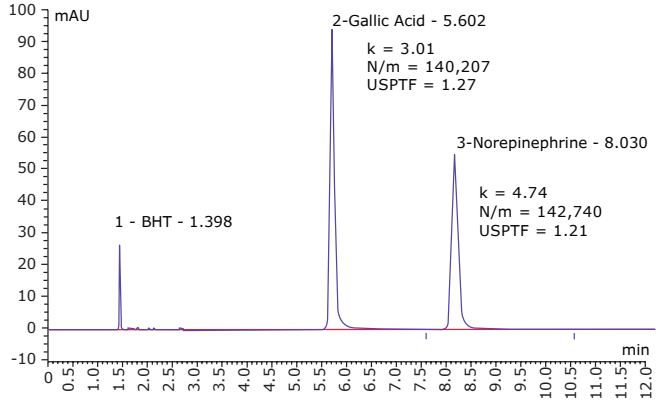
Competitor W, 1.7 µm/95Å Z-HILIC, Inert Hardware



Competitor A, 2.7 µm/120Å HILIC-Z, PEEK-lined



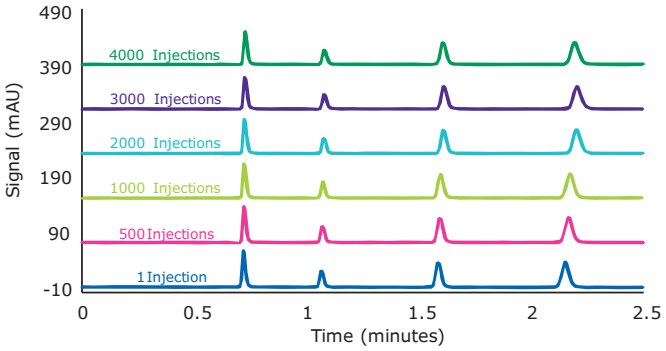
Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ Hardware



Chromatographic Conditions	
Mobile phase:	Acetonitrile/50 mM Ammonium acetate pH 5.5, 80/20 (v/v)
Flow rate:	0.2 mL/min
Temperature:	23 °C
Injection:	0.1 µL
Detector:	UV @230 nm
Instrument:	ThermoFisher Scientific Vanquish Flex Bio-Inert LC system
Sample:	1. BHT 2. Gallic acid 3. Norepinephrine

Excellent reproducibility and robustness for reliable results

The stability and reproducibility of HPLC columns are essential for achieving accurate, reliable, and efficient analytical results. The following results highlight the exceptional consistency of injection performance for the Ascentis® Express 160Å ZIC®-cHILIC columns with INERTProve™ column hardware, which ensures dependable and reproducible outcomes. To demonstrate its outstanding stability, the flow rate was increased to four times the optimal rate, enhancing throughput while subjecting the column to slightly elevated conditions. Additionally, the injection volume was raised by 2.5 times to create a more realistic stress scenario at the column head.



Chromatographic Conditions	
Column:	Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ 15 cm x 2.1 mm I.D.
Mobile phase:	85% Acetonitrile: 15% 25 mM Ammonium Acetate Buffer
Flow rate:	0.4 mL/min
Column temp.:	30 °C
Injection:	0.5 µL
Detection:	UV-220 nm

Peak Identification	
1	Dodecylbenzene
2	Uracil
3	Cytosine
4	Cytidine

Length x I.D. (mm)	Part Number	Description
50 x 2.1	**50502-U**	Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ 5 cm X 2.1 mm I.D.
100 x 2.1	**50503-U**	Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ 10 cm X 2.1 mm I.D.
150 x 2.1	**50504-U**	Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ 15 cm X 2.1 mm I.D.
50 x 3.0	**50505-U**	Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ 5 cm X 3.0 mm I.D.
100 x 3.0	**50506-U**	Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ 10 cm X 3.0 mm I.D.
150 x 3.0	**50510-U**	Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ 15 cm X 3.0 mm I.D.
50 x 4.6	**50513-U**	Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ 5 cm X 4.6 mm I.D.
100 x 4.6	**50514-U**	Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ 10 cm X 4.6 mm I.D.
150 x 4.6	**50515-U**	Ascentis® Express 160Å ZIC®-cHILIC, 2.7 µm, INERTProve™ 15 cm X 4.6 mm I.D.

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