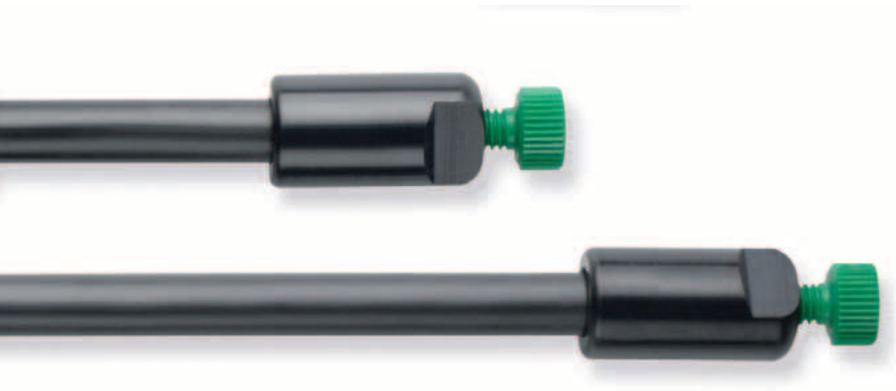
It's hot!

SeQuant® ZIC®-cHILIC

Complementary selectivity
for HPLC and LC-MS separation
of polar hydrophilic compounds

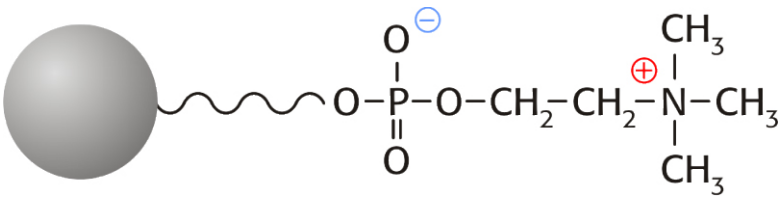


Merck Millipore is a division of MERCK

SeQuant® ZIC®-cHILIC

Your benefits

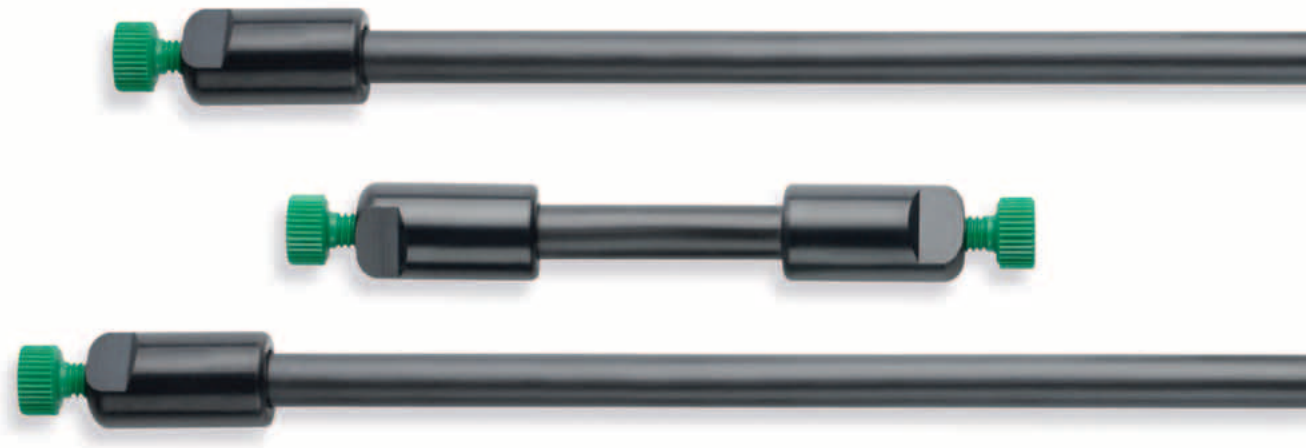
- High-performance HILIC column
- Complementary selectivity for polar hydrophilic compounds
- Maximum LC-MS compatibility with minimized column bleed
- Excellent reproducibility and robustness



SeQuant® ZIC®-cHILIC is designed for excellent HPLC and LC-MS of polar hydrophilic compounds. This new zwitterionic stationary phase with phosphorylcholine functional group provides you with complementary selectivity for easier method development for substances that have been difficult to separate by previous types of HPLC columns operated in reversed-phase or HILIC mode.

The selectivity features of SeQuant® ZIC®-cHILIC are especially beneficial for negatively charged polar compounds such as nucleotides and organic acids, but do also provide advantageous separations of positively charged hydrophilic molecules; for example aminoglycosides and cations. The column is also very suitable for zwitterionic and neutral polar hydrophilic substances.

Benefit from easier method development with complementary selectivity from hydrophilic partitioning and weak ionic interactions

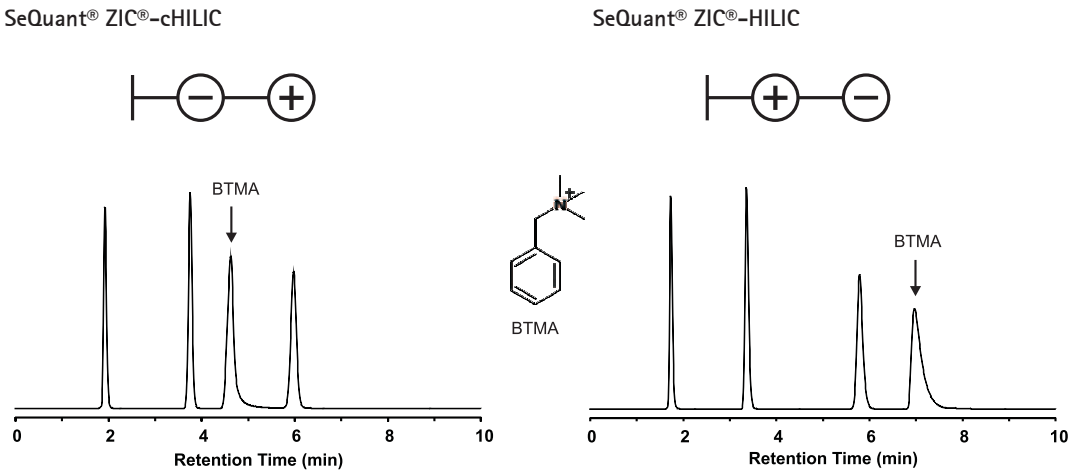


As a truly zwitterionic stationary phase, SeQuant® ZIC®-cHILIC shows weak electrostatic interactions (attraction and repulsion) for favorable selectivity thanks to its 1:1 charge balance in close proximity. The weak electrostatic interactions enable separation tuning and optimization at low concentrations of buffer in mobile phase without interfering with detection techniques, including mass spectrometry (MS).

SeQuant® ZIC®-cHILIC shows maximum mass spectrometry compatibility by its totally minimized column bleed. The very long column lifetime and superb batch-to-batch reproducibility make it a powerful choice for LC-MS and HPLC of numerous polar hydrophilic compounds.

Complementary selectivity of ZIC®-cHILIC

The selectivity of ZIC®-cHILIC is complementary to ZIC®-HILIC and other types of HILIC columns. Charged polar hydrophilic compounds typically experience significant selectivity differences on ZIC®-cHILIC versus ZIC®-HILIC due to their differently oriented zwitterionic functional groups. The outermost, more accessible, charged moiety, tend to dominate the interaction with solutes. Positively charged compounds thus typically have lower retention on ZIC®-cHILIC whereas negatively charged compounds have higher retention. Neutral compounds tend to experience more similar retention on the two zwitterionic columns.



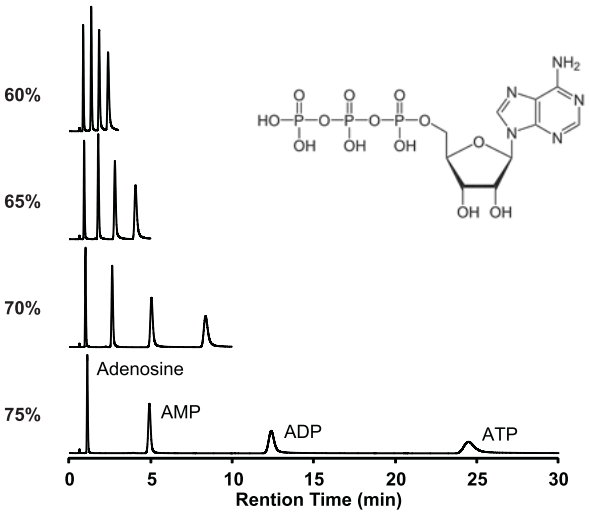
Isocratic separations of the positively charged benzyltrimethylamine (BTMA, peaks indicated with arrows) and the neutral toluene (void marker), uracil and cytosine on ZIC®-cHILIC (left) and ZIC®-HILIC (right) illustrating differences and similarities in selectivity caused by the different charge orientation of the zwitterionic functional groups (see illustrations). Column dimensions were 100 x 4.6 mm, particles size 3 or 3.5 µm, and pore size 100 Å. Eluent was 80 : 20 acetonitrile/ 25 mM aqueous ammonium acetate pH 6.8 pumped at 0.5 mL/min at 23 °C. Detection by UV absorption at 254 nm.

The selectivity of ZIC®-cHILIC is complementary to ZIC®-HILIC and other types of HILIC columns

HPLC of Nucleotides and Nucleosides

SeQuant® ZIC®-cHILIC can accomplish impressive separation and peak shape of nucleotides thanks to the zwitterionic character of its phosphorylcholine functional groups. The stationary phase provides advantageous weak ionic interactions for enhanced selectivity, without excessive retention or serious peak shape distortion.

On other types of HILIC columns, the negative charges of the nucleotides' phosphate groups can interact strongly with anion exchange sites, resulting in excessive retention or severe peak tailing, making purification and quantification problematic.

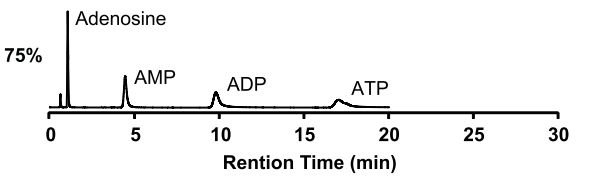


Isocratic separations on SeQuant® ZIC®-cHILIC 100 x 2.1 mm (Ord. No. 1.50657.0001) operated at 23 °C with a flow rate of 0.4 mL/min. Eluent was acetonitrile / 100 mM aqueous ammonium acetate pH 4.5, with acetonitrile ratios of 60 %, 65 %, 70 % and 75 %, respectively (top to bottom). Injection of 5 µL of adenosine, AMP, ADP, ATP diluted in eluent. UV detection at 254 nm.

ZIC®-cHILIC provides enhanced selectivity for nucleotides and many other negatively charged compounds

Comparison to SeQuant® ZIC®-HILIC

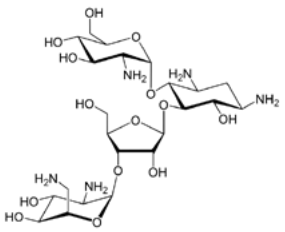
Nucleosides can also be nicely separated on SeQuant® ZIC®-HILIC but tend to be less retained due to slight repulsion from the outermost, more accessible, negative charge on its zwitterionic sulfobetaine functional groups.



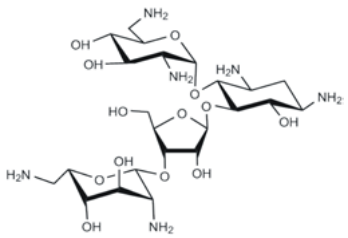
Isocratic separation on SeQuant® ZIC®-HILIC 100 x 2.1 mm (Ord. No. 1.50441.0001) used at identical conditions as above with 75 % acetonitrile in the eluent.

LC-MS of Aminoglycoside Antibiotics

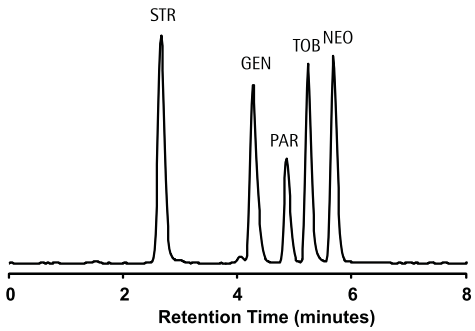
Thanks to the high hydrophilicity and favorable weak repulsion electrostatic interactions with SeQuant® ZIC®-cHILIC columns it is possible to achieve excellent separation of multiple structurally similar aminoglycosides in a minimum amount of time. In an LC-MS set-up, SeQuant® ZIC®-cHILIC columns thus enable quantification of low amounts of aminoglycoside antibiotics in various samples. Aminoglycoside antibiotics contain amino-modified sugar moieties and they are thus very hydrophilic and multiply positively charged at neutral and acidic pH. This makes them very difficult to separate and analyze using reversed-phase and most types of HILIC HPLC columns.



Paromomycin (PAR)



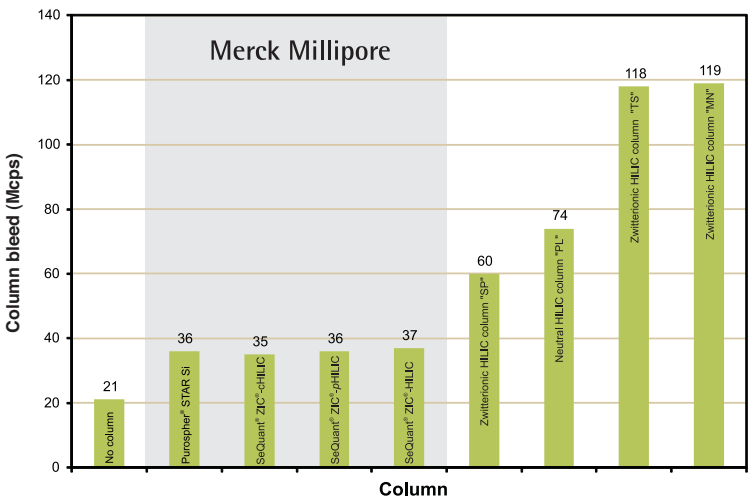
Neomycin (NEO)



Gradient LC-MS on SeQuant® ZIC®-cHILIC 100 x 2.1 mm (Ord. No. 1.50657.0001) operated at 50 °C with a flow rate of 0.4 mL/min. Mobile phases were A: acetonitrile with 1 % (w/w) formic acid and B: 100 mM ammonium acetate with 3% (w/w) formic acid. Gradient was 50 to 95 % B 0-7 min, 95 % B 7-8 min, 95-50 % B 8-16 min (equilibration). Injection of 5 µL 5 µg/mL streptomycin (STR), 25 µg/mL gentamycin (GEN), paromo¬mycin (PAR), tobramycin (TOB), and neomycin (NEO), all diluted in 30 : 70 (v/v) acetonitrile / Milli-Q water. Detection by Shimadzu LCMS 2012EV, detector voltage: 2.0 kV, heat block and CDL temp: 250 °C, SIM in positive mode: m/z 582 (STR), 464 (GEN), 616 (PAR), 468 (TOB), and 615 (NEO).

Maximum LC-MS compatibility

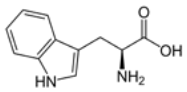
SeQuant® ZIC®-cHILIC HPLC columns have extraordinary low column bleed in LC-MS. Many HILIC columns – including several marketed as "zwitterionic" – tend to display 70–240 % higher bleed than ZIC®-cHILIC. This can cause signal suppression and interfere with quantification while increasing MS instrument wear and service costs.



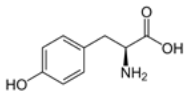
Mass spectrometry intensity data from ESI+ with single quadruple MS measured as total ion current for 20-2000 m/z. Columns were 100 x 2.1 mm operated at 50 °C with a flow rate of 0.1 mL/min. Eluent was 80 : 20 acetonitrile/25 mM aqueous ammonium acetate pH 6.8. Average of 3 measurements, each during 6 minutes. All columns were allowed to equilibrate 1-2 hours until baseline had stabilized before measurement.

HPLC of Amino Acids

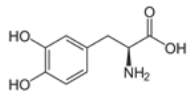
The selectivity of SeQuant® ZIC®-cHILIC is suitable for separating a wide range of amino acids without any pre-column derivatisation reactions or ion pair reagents in the eluent. Amino acids with hydrophilic as well as hydrophobic side chains will retain and can be separated, as can positional isomers.



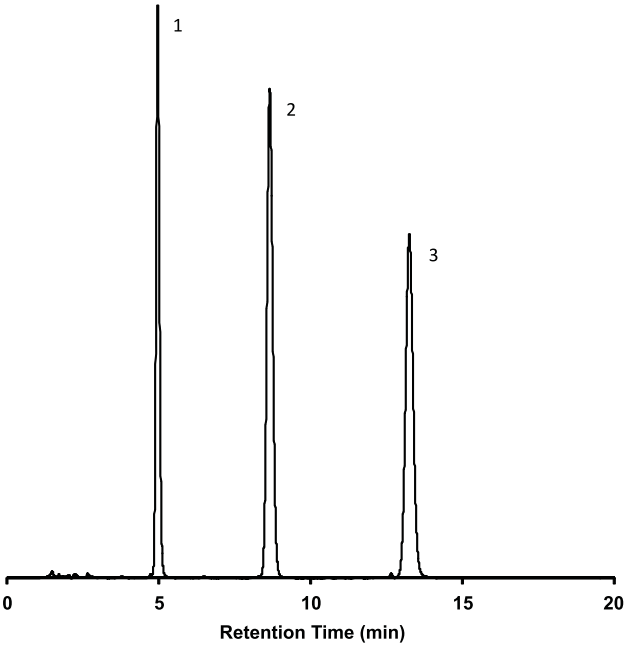
tryptophan (1)



tyrosine (2)



3,4-dihydroxyphenyl-alanine (3)



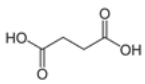
Isocratic separation of amino acids using SeQuant® ZIC®-cHILIC 150x4.6 mm (Ord. No. 1.50661.0001) and a mobile phase containing 80:20 acetonitrile/25mM ammonium acetate pH 6.8, delivered at a flow rate of 1 mL/min, at 23 °C. Detection by UV at 254 nm. Injection of 1 µL standard mixture with tryptophan, tyrosine and 3,4-dihydroxyphenylalanine dissolved in 80:20 acetonitrile / water, acidified with 1 % phosphoric acid.

HPLC of Organic Acids

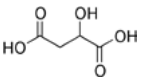
SeQuant® ZIC®-cHILIC is very appropriate for HPLC of small organic acids. Mono-acids, di-acids, and tri-acids can be separated in the same run with high selectivity and resolution. This enables quantification of organic acids in a wide range of samples with a variety of different detection techniques depending on targeted concentration range. This example with UV absorbance detection shows analysis of organic acids in white wine.

1 

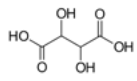
Acetic acid

2 

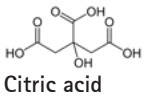
Succinic acid

3 

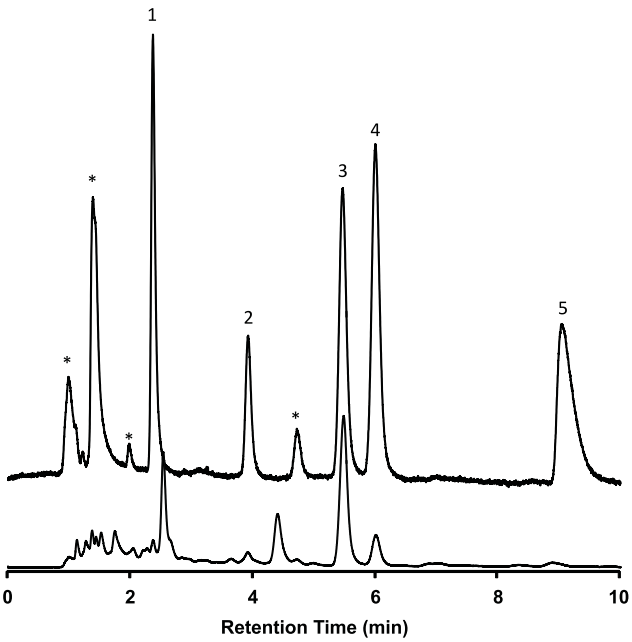
Malic acid

4 

Tartaric acid

5 

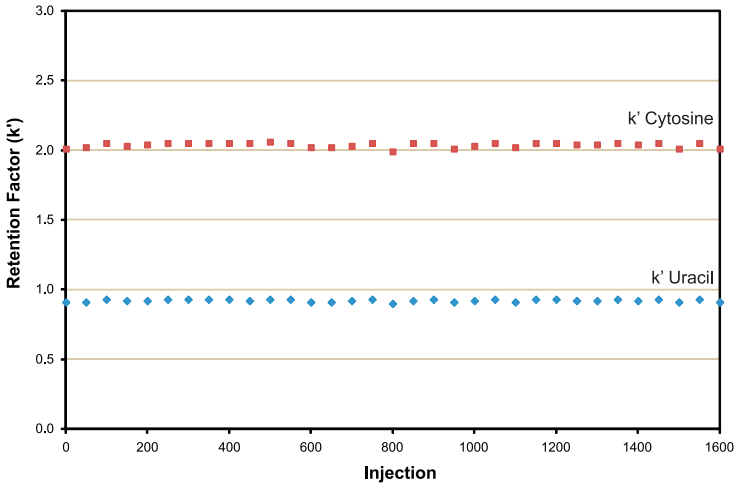
Citric acid



Isocratic HPLC of organic acids on SeQuant® ZIC®-cHILIC 150x2.1 mm (Ord. No. 1.50658.0001). Mobile phase was 75:25 acetonitrile/25mM potassium phosphate buffer pH 6.0, delivered at 0.3 mL/min, at 30 °C. Injection of 5 µL standard mixture containing 10 ppm each of acetic acid (1; 2.4 min), succinic acid (2; 3.9 min), malic acid (3; 5.5 min), tartaric acid (4; 6.0 min) and citric acid (5; 8.9 min) diluted in mobile phase (upper trace), and Riesling wine diluted in water 1:10 and then in acetonitrile 1:10 (bottom trace). Asterisks (*) indicates impurities in standards. Detection by UV at 200 nm using a 2.5 µL semi-micro flow-cell. Total system void time was 1.0 min.

High column stability

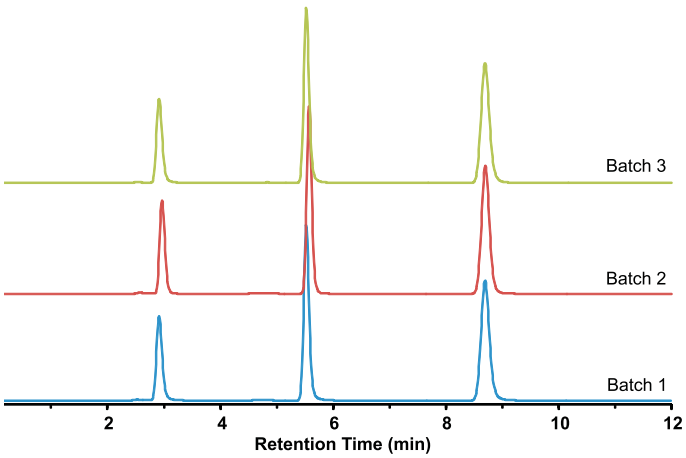
SeQuant® ZIC®-cHILIC shows very high stability and can stand more than 1600 injections during 270 hours of continuous use without showing any signs of change in retention. Its high robustness ensures that you get reproducible chromatography from your column with every injection.



Retention factors of uracil (bottom) and cytosine (top) on SeQuant® ZIC®-cHILIC (Ord. No. 1.50661.0001) 150 x 4.6 mm operated at 23 °C with a flow rate of 0.5 mL/min. Eluent was 80 : 20 acetonitrile/25 mM aqueous ammonium acetate pH 6.8. Injection of 10 µL toluene (void marker), uracil and cytosine in eluent every 12 minutes. Detection by UV absorbance at 254 nm.

Superb batch-to-batch reproducibility

A strictly controlled synthesis procedure gives SeQuant® ZIC®-cHILIC superb batch-to-batch reproducibility so you can rely on your methods delivering consistent results year in and year out.



Isocratic separation on three different sorbent batches of SeQuant® ZIC®-cHILIC (Ord. No. 1.50658.0001) 150 x 2.1 mm operated at 23 °C with a flow rate of 0.1 mL/min. Eluent was 80 : 20 acetonitrile/25 mM aqueous ammonium acetate pH 6.8. Injection of 5 µL toluene (void marker), uracil and cytosine in eluent. Detection by UV absorbance at 254 nm.

Analytical columns

Description	Ordering No.	Particle size	Pore size	Dimension length	Dimension i.d.	Column hard-ware	Contents of one package
SeQuant® ZIC®-cHILIC	1.50656.0001	3 µm	100 Å	50 mm	2.1 mm	PEEK	1 piece
SeQuant® ZIC®-cHILIC	1.50657.0001	3 µm	100 Å	100 mm	2.1 mm	PEEK	1 piece
SeQuant® ZIC®-cHILIC	1.50658.0001	3 µm	100 Å	150 mm	2.1 mm	PEEK	1 piece
SeQuant® ZIC®-cHILIC	1.50659.0001	3 µm	100 Å	50 mm	4.6 mm	PEEK	1 piece
SeQuant® ZIC®-cHILIC	1.50660.0001	3 µm	100 Å	100 mm	4.6 mm	PEEK	1 piece
SeQuant® ZIC®-cHILIC	1.50661.0001	3 µm	100 Å	150 mm	4.6 mm	PEEK	1 piece
SeQuant® ZIC®-cHILIC	1.50662.0001	3 µm	100 Å	250 mm	4.6 mm	PEEK	1 piece
SeQuant® ZIC®-cHILIC Guard Kit*	1.50664.0001	5 µm	100 Å	20 mm	2.1 mm	PEEK	3 pieces

*including column coupler

Capillary columns

Description	Ordering No.	Particle size	Pore size	Dimension length	Dimension i.d.	Column hardware	Contents of one package
SeQuant® ZIC®-cHILIC Capillary	1.50669.0001	3 µm	100 Å	150 mm	300 µm	GL-SS	1 piece
SeQuant® ZIC®-cHILIC Capillary	1.50670.0001	3 µm	100 Å	150 mm	1 mm	GL-SS	1 piece
SeQuant® ZIC®-cHILIC Capillary Guard	1.50665.0001	5 µm	100 Å	5 mm	300 µm	GL-SS	3 pieces
SeQuant® ZIC®-cHILIC Capillary Guard	1.50666.0001	5 µm	100 Å	5 mm	1 mm	GL-SS	3 pieces

PEEK = Poly-ether-ether-ketone column with PEEK frits.
GL-SS = glass-lined stainless steel with stainless steel frits.

Every column (except guards) is individually tested and delivered with a certificate of analysis documenting its performance.

Example references: S. Di Palma et al., Anal. Chem., 83 (2011) 3440-3447. G. Weber et al., J. Sep. Sci., 31 (2008) 1615-1622.

Visit www.sequant.com/scientificpapers for an updated list of research benefiting from ZIC®-cHILIC separation technology.

For more information please visit www.merckmillipore.com/chromatography or www.sequant.com/zicchilic

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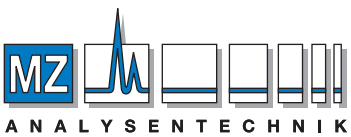

AUTHORIZED DISTRIBUTOR
MZ-Analysentechnik GmbH, Barcelona-Allee 17• D-55129 Mainz
Tel +49 6131 880 96-0, Fax +49 6131 880 96-20
e-mail: info@mz-at.de, www.mz-at.de

For further information on Merck Millipore
and our products contact:
Merck KGaA
64271 Darmstadt, Germany
www.sequant.com
www.merckmillipore.com