

SeQuant[®] ZIC[®]-HILIC For all who expect more ...

The better choice for HPLC and LC/MS of all types
of polar and hydrophilic compounds



Poor retention of polar compounds? ZIC[®]-HILIC solves your problem.

From small peptides to ions, from complex carbohydrates to metabolites – all types of hydrophilic compounds can be separated with ZIC[®]-HILIC.

ZIC[®]-HILIC expands your chromatographic toolbox

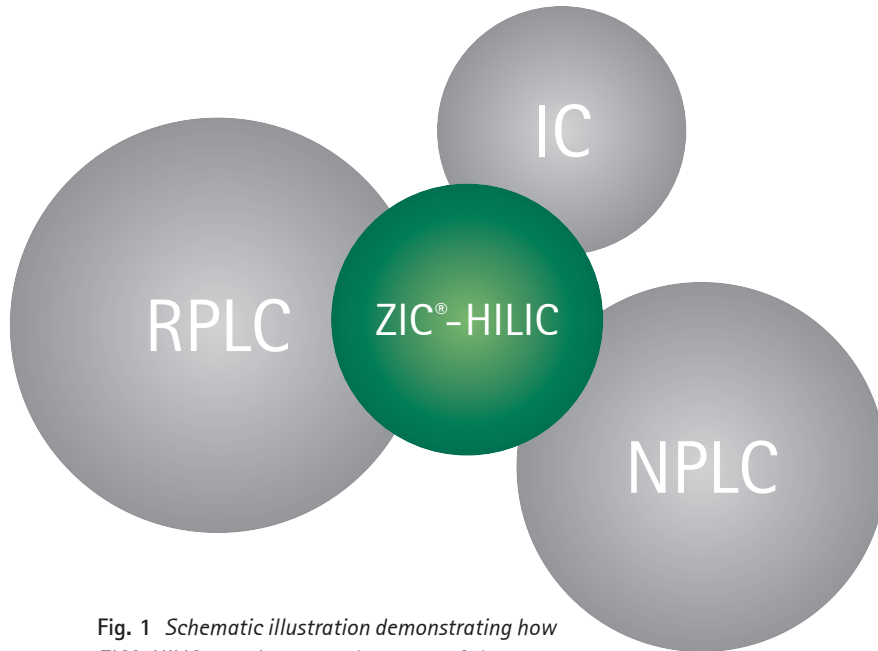


Fig. 1 Schematic illustration demonstrating how ZIC[®]-HILIC complements other areas of chromatography and extends the separation capabilities.

Your benefits

- Low cost per analysis due to robust bonded stationary phase
- Straightforward separation of polar and hydrophilic compounds
- Enhanced MS sensitivity and simpler sample work-up

What is HILIC?

HILIC or Hydrophilic Interaction Liquid Chromatography is a straightforward chromatographic technique for separation of many types of polar and hydrophilic compounds. To put it simple one can say that HILIC is a normal-phase (NPLC) type of separation but uses reversed-phase (RPLC) type eluents.

Thus, in HILIC one has:

- A column with a hydrophilic stationary phase
- An eluent with water, buffer and a high concentration of water-miscible organic solvent.

A typical HILIC application uses an eluent with 50 – 95 % organic solvent in an aqueous buffer that has a high solubility in the solvent, for example acetonitrile in ammonium acetate.

The elution order in HILIC is roughly the opposite of that in RPLC and retention increases with hydrophilicity and charge of the analyte. This enables straightforward separation of compounds that would otherwise elute in the void volume on RPLC columns.

Retention on the ZIC®-HILIC Column increases with hydrophilicity and charge of the analyte.

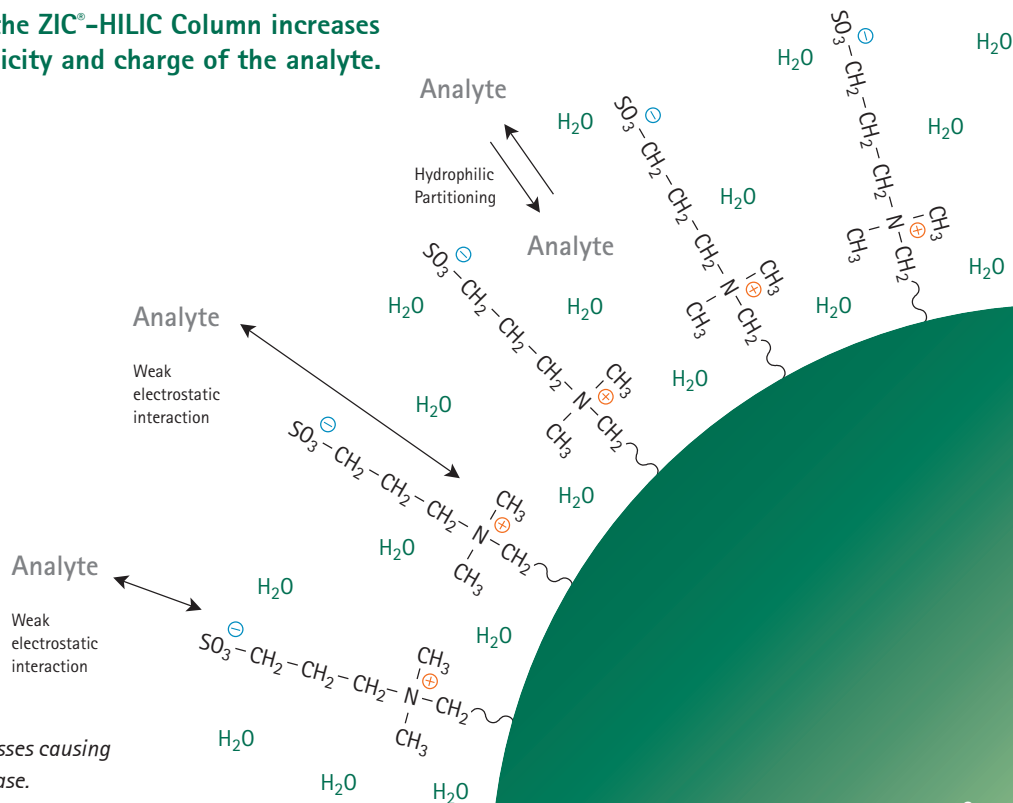


Fig. 2 Schematic illustration of the processes causing retention on the ZIC®-HILIC stationary phase.

SeQuant[®] ZIC[®]-HILIC

High-performance hydrophilic interaction liquid chromatography

- Straightforward separation of polar and hydrophilic compounds
- Orthogonal selectivity compared to reversed-phase
- Permanent zwitterionic functional groups covalently bonded to porous silica
- Low cost per analysis due to robust bonded stationary phase

All classes of polar and hydrophilic compounds

With the ZIC[®]-HILIC Column separation of polar and hydrophilic compounds is straightforward. The selectivity offered by ZIC[®]-HILIC is suitable for a wide variety of molecules containing hydrophilic or ionizable functional groups. This includes compounds such as carbohydrates, metabolites, acids and bases, organic and inorganic ions, metal complexes, amino acids, peptides, protein digests, plant and cell extracts, plus much more. These compounds are normally characterized by a small or negative LogP value* and have poor retention on reversed-phase columns. The ZIC[®]-HILIC Column is designed to retain and separate these types of polar and hydrophilic molecules with a selectivity that is orthogonal to reversed-phase.

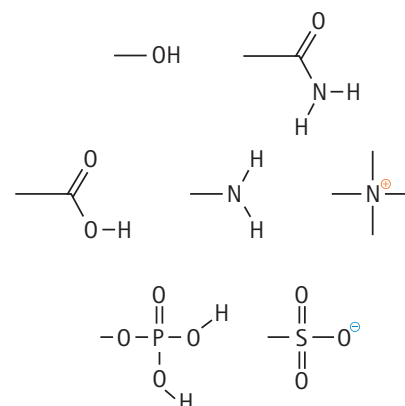


Fig. 3 Examples of hydrophilic functional groups.

ZIC[®]-HILIC range

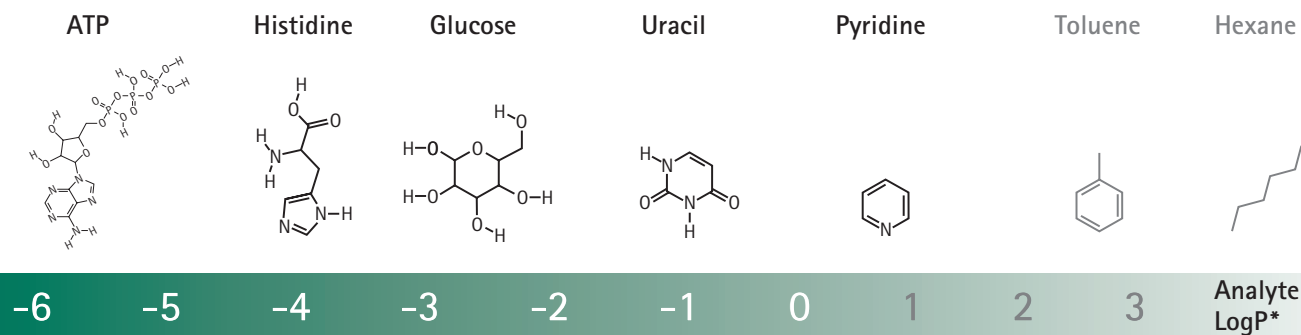


Fig. 4 Examples of polar and hydrophilic compounds with different LogP values* that can be separated with ZIC[®]-HILIC. In contrast, two compounds that are too hydrophobic to be retained (toluene and hexane) are also displayed.

*octanol-water partition coefficient





Robust zwitterionic sorbent

The ZIC®-HILIC Column 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) is affected during eluent optimization.

The robust bonded hydrophilic phase on ZIC®-HILIC ensures a stable environment for the HILIC partitioning process during analyte retention. The compatibility of ZIC®-HILIC with a range of different buffers, organic solvents and temperatures makes it a straightforward task to develop robust isocratic and gradient methods for MS, ELSD, UV and other detection techniques. The ZIC®-HILIC robustness and excellent batch-to-batch reproducibility also ensures method scalability all the way from nano and capillary columns to semi-preparative size.

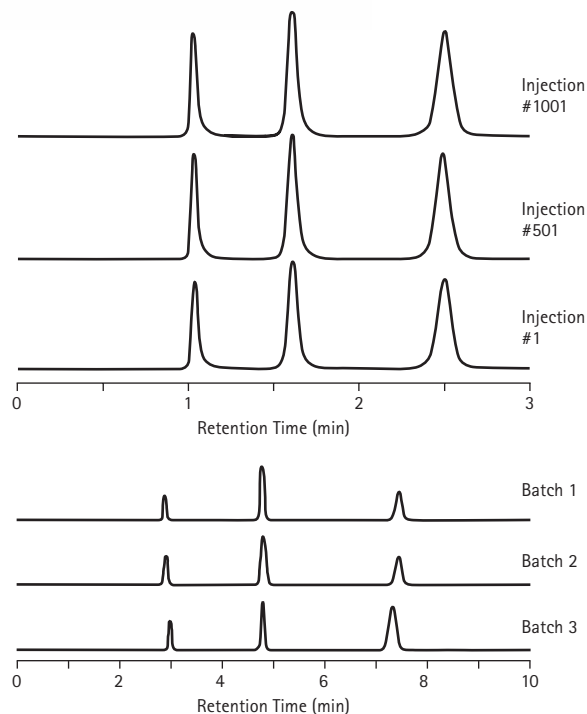


Fig. 5 Excellent column robustness and batch-to-batch reproducibility of ZIC®-HILIC. Analytes: Toluene (void volume marker), uracil and cytosine. Column: ZIC®-HILIC 50 x 4.6 mm, 5 μ m (top) or 150 x 4.6 mm, 5 μ m (bottom). Flow-rate: 0.5 mL/min. Eluent (v/v): 80 % Acetonitrile 20 %, Ammonium Acetate 25 mM, pH 6.8.

Application examples

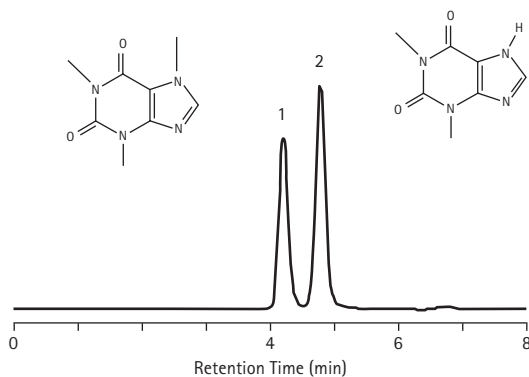


Fig. 6 Isocratic separation of Caffeine and Theophylline on a ZIC®-HILIC PEEK Column 150 x 2.1 mm, 3.5 μ m, 200 \AA , (Ord. No. 1.50448.0001). Eluent (v/v): 90 % Acetonitrile and 10 % Ammonium Acetate 100 mM; pH 4.50 (total ionic strength 10 mM). Flow-rate: 0.1 mL/min. Injection: 5 μ L (25 μ g/mL) of each compound in mobile phase. Detection: UV at 254 nm.

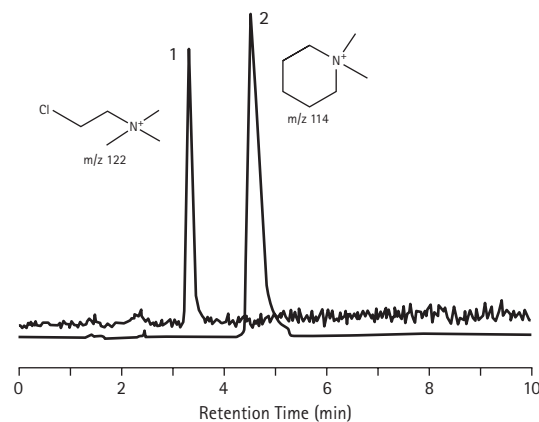


Fig. 7 Isocratic separation of Chlormequat and Mepiquat on a ZIC®-HILIC PEEK Column 100 x 2.1 mm, 3.5 μ m (Ord. No. 1.50447.0001). Eluent (v/v): 80 % Acetonitrile and 20 % Ammonium Acetate 25 mM. Flow-rate: 0.2 mL/min. Injection: 20 μ L in mobile phase. Detection: Electrospray-MS in positive mode (Esi+), Single ion monitoring (SIM) at m/z 114 and 122. By courtesy of: Dr.-Ing. Ludmila Havlik, Chemisches Labor Dr. Wirts + Partner, Hannover, Germany, www.wirts.de

Ordering information for ZIC®-HILIC Columns see page 8 and 9

SeQuant[®] ZIC[®]-pHILIC

Polymer phase for advanced hydrophilic interaction liquid chromatography

- Solves challenging separations of polar and hydrophilic compounds
- Permanent zwitterionic functional groups covalently bonded to porous polymer beads
- Enhanced selectivity adjustments due to broad pH application range

Challenging separations of polar and hydrophilic compounds

When you need to solve the most demanding separations of hydrophilic compounds, it is an advantage to be able to explore a wide variety of different eluent conditions. The polymer-based ZIC[®]-pHILIC Column offers an extended pH stability range (pH 2-10) to increase the choice of buffer systems for the eluent. This introduces a unique opportunity to change the selectivity by altering the ionization of functional groups on the analyte. This feature also enhances sensitivity in negative ESI MS detection.

The ZIC[®]-pHILIC stationary phase has the same highly polar, bonded, permanent zwitterion, functional group as the silica based ZIC[®]-HILIC Column. The user can therefore expect the same selectivity, however, with a trade-off in flow-rate range, and separation efficiency, common with polymeric materials. The more durable support allows for use in an extended pH range, which can be beneficial for certain applications.

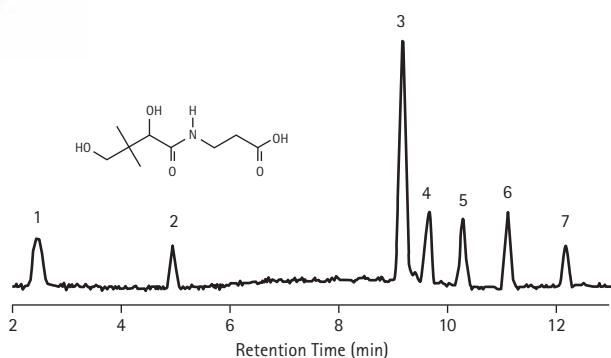


Fig. 8 Gradient separation of Pantothenate (2), Acetyl-CoA (3), AMP (4), CoA (5), ADP (6) and ATP (7) on ZIC[®]-pHILIC PEEK Column 150 x 2.1 mm, 5 μ m (Ord. No. 1.50460.0001).
Eluent (v/v): A: Acetonitrile, B: 10 mM (NH₄)₂CO₃ + 0.2% NH₄OH.
Gradient 20 – 60% B in 15 min, 5 min at 60% B, Equilibration 15 min at 20% B.
By courtesy of T. Pluskal, K. Nagao and M. Yanagida, GO Cell Unit, Initial Research Project, Okinawa Institute of Science and Technology, Okinawa 904-2234, Japan.



Example of enhanced selectivity at high pH

The application example below illustrates how the selectivity of the ZIC®-pHILIC material can be enhanced by performing the separation at elevated pH. The chromatograms show isocratic separations of gentisic acid, protocatechuic acid and isophthalic acid on a ZIC®-pHILIC Column. The pH-increase also results in higher retention and improved peak shape for these analytes.

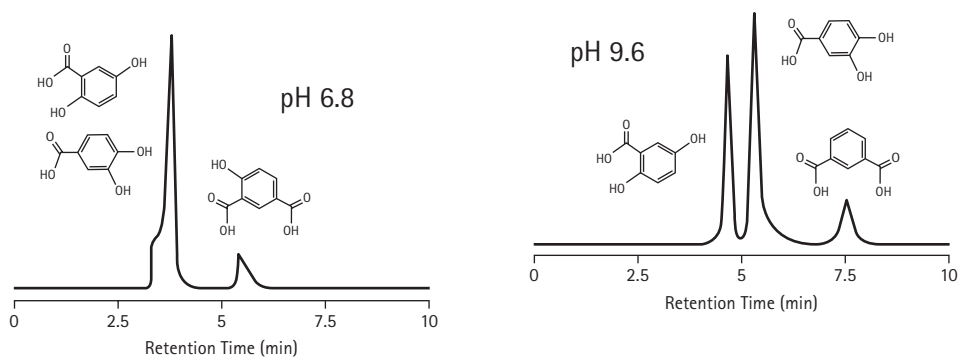
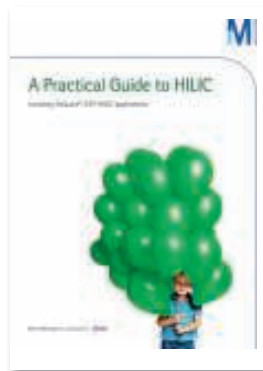


Fig. 9 Separation of gentisic acid, protocatechuic acid, and isophthalic acid on a ZIC®-pHILIC Column. Eluent: 75 : 25 Acetonitrile / aqueous buffer pumped at 0.5 mL/min. Buffer salt: ammonium acetate (17 mM, pH 6.8) or ammonium carbonate (17 mM, pH 9.6).

For the most up-to-date information, products and applications, please visit www.sequant.com and ask for your free copy of our booklet **A Practical Guide to HILIC**.



Ordering information ZIC®-HILIC

ZIC®-HILIC Columns are available in a broad range of column formats, from analytical columns made of PEEK with PEEK frits, to capillaries and microbore columns made of glass-lined stainless steel.

Semi-preparative columns are available in stainless steel.

ZIC®-HILIC analytical columns

Description	Particle Size	Porosity	ID	Length	Pieces	Ord. No.
ZIC®-HILIC PEEK Fitting Guard Column	5 µm	200 Å	1 mm	14 mm	5	150434.0001
ZIC®-HILIC Guard Column	5 µm	200 Å	2.1 mm	20 mm	1	150435.0001
ZIC®-HILIC Guard Column Kit*	5 µm	200 Å	2.1 mm	20 mm	3	150436.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	100 Å	2.1 mm	20 mm	1	150439.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	100 Å	2.1 mm	50 mm	1	150440.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	100 Å	2.1 mm	100 mm	1	150441.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	100 Å	2.1 mm	150 mm	1	150442.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	100 Å	2.1 mm	250 mm	1	150443.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	100 Å	4.6 mm	150 mm	1	150444.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	200 Å	2.1 mm	50 mm	1	150445.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	200 Å	4.6 mm	50 mm	1	150446.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	200 Å	2.1 mm	100 mm	1	150447.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	200 Å	2.1 mm	150 mm	1	150448.0001
ZIC®-HILIC PEEK HPLC Column	3.5 µm	200 Å	4.6 mm	150 mm	1	150449.0001
ZIC®-HILIC PEEK HPLC Column	5 µm	200 Å	2.1 mm	50 mm	1	150450.0001
ZIC®-HILIC PEEK HPLC Column	5 µm	200 Å	4.6 mm	50 mm	1	150451.0001
ZIC®-HILIC PEEK HPLC Column	5 µm	200 Å	2.1 mm	100 mm	1	150452.0001
ZIC®-HILIC PEEK HPLC Column	5 µm	200 Å	4.6 mm	100 mm	1	150453.0001
ZIC®-HILIC PEEK HPLC Column	5 µm	200 Å	2.1 mm	150 mm	1	150454.0001
ZIC®-HILIC PEEK HPLC Column	5 µm	200 Å	4.6 mm	150 mm	1	150455.0001
ZIC®-HILIC PEEK HPLC Column	5 µm	200 Å	2.1 mm	250 mm	1	150457.0001
ZIC®-HILIC PEEK HPLC Column	5 µm	200 Å	4.6 mm	250 mm	1	150458.0001

*includig column coupler

All products are not intended for use as in-vitro diagnostics in terms of European Directive 98/79/EC. They are for research purposes only, for investigating in-vitro samples without any medical objective.



ZIC®-HILIC nano, capillary & microbore columns

Description	Particle Size	Porosity	ID	Length	Pieces	Ord. No.
ZIC®-HILIC Guard Column	5 µm	200 Å	1 mm	5 mm	1	150483.0001
ZIC®-HILIC Guard Column	5 µm	200 Å	1 mm	5 mm	5	150490.0001
ZIC®-HILIC Guard Column	5 µm	200 Å	300 µm	5 mm	1	150484.0001
ZIC®-HILIC Guard Column	5 µm	200 Å	300 µm	5 mm	5	150492.0001
ZIC®-HILIC Nano Column	5 µm	200 Å	75 µm	150 mm	1	150465.0001
ZIC®-HILIC Nano Column	3.5 µm	200 Å	100 µm	100 mm	1	150466.0001
ZIC®-HILIC Microbore Column	3.5 µm	100 Å	1 mm	150 mm	1	150487.0001
ZIC®-HILIC Capillary Column	3.5 µm	200 Å	300 µm	30 mm	1	150489.0001
ZIC®-HILIC Microbore Column	3.5 µm	200 Å	1 mm	30 mm	1	150478.0001
ZIC®-HILIC Capillary Column	3.5 µm	200 Å	300 µm	150 mm	1	150479.0001
ZIC®-HILIC Microbore Column	3.5 µm	200 Å	1 mm	150 mm	1	150480.0001
ZIC®-HILIC Capillary Column	5 µm	200 Å	300 µm	30 mm	1	150491.0001
ZIC®-HILIC Capillary Column	5 µm	200 Å	300 µm	150 mm	1	150481.0001
ZIC®-HILIC Microbore Column	5 µm	200 Å	1 mm	150 mm	1	150482.0001

Semi-preparative columns

Description	Particle Size	Porosity	ID	Length	Pieces	Ord. No.
ZIC®-HILIC PEEK HPLC Column	5 µm	200 Å	7.5 mm	150 mm	1	150456.0001
ZIC®-HILIC Stainless Steel Column	5 µm	200 Å	10 mm	50 mm	1	150495.0001
ZIC®-HILIC Stainless Steel Column	5 µm	200 Å	10 mm	150 mm	1	150493.0001
ZIC®-HILIC Stainless Steel Column	5 µm	200 Å	10 mm	250 mm	1	150494.0001
ZIC®-HILIC Stainless Steel Column	5 µm	200 Å	21.2 mm	50 mm	1	150496.0001
ZIC®-HILIC Stainless Steel Column	5 µm	200 Å	21.2 mm	150 mm	1	150497.0001
ZIC®-HILIC Stainless Steel Column	5 µm	200 Å	21.2 mm	250 mm	1	150671.0001

Ordering information ZIC®-pHILIC

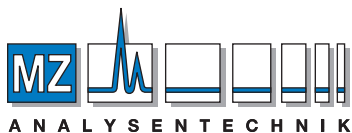
ZIC®-pHILIC Columns are available as PEEK Columns with PEEK frits.

Description	Particle Size	ID	Length	Pieces	Ord. No.
ZIC®-pHILIC Guard Column	5 µm	2.1 mm	20 mm	1	150437.0001
ZIC®-pHILIC Guard Column Kit*	5 µm	2.1 mm	20 mm	3	150438.0001
ZIC®-pHILIC PEEK HPLC Column	5 µm	2.1 mm	50 mm	1	150459.0001
ZIC®-pHILIC PEEK HPLC Column	5 µm	2.1 mm	100 mm	1	150462.0001
ZIC®-pHILIC PEEK HPLC Column	5 µm	2.1 mm	150 mm	1	150460.0001
ZIC®-pHILIC PEEK HPLC Column	5 µm	4.6 mm	50 mm	1	150463.0001
ZIC®-pHILIC PEEK HPLC Column	5 µm	4.6 mm	100 mm	1	150464.0001
ZIC®-pHILIC PEEK HPLC Column	5 µm	4.6 mm	150 mm	1	150461.0001

*including column coupler



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