ZIC®-*p*HILIC HPLC Column

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General Instructions for Care and Use

Introduction
The ZIC®-*p*HILIC column has a zwitterionic stationary phase covalently attached to porous polymer beads. The permanent and hydrophilic zwitterion functionality makes the column suitable for Hydrophilic Interaction Liquid Chromatography (HILIC). Weak electrostatic interactions between charged analytes and the neutral zwitterionic stationary phase results in a unique selectivity, and especially suitable for analytes that are poorly retained on reversed phase columns.

The ZIC®-*p*HILIC column can be used as a tool to change the selectivity or to improve peak resolution for polar and hydrophilic compounds such as carbohydrates, metabolites, acids and bases, organic and inorganic ions, metal complexes, amino acids, peptides and protein digests.

Column Hardware & Chemical Compatibility
Please do not use steel fittings or ferrules!
The column is made from poly(etherether ketone) [PEEK] and has 10-32 UNF female fittings. The frits have a porosity to retain 3-10 µm particles and are made from PEEK. PEEK generally shows excellent chemical resistance to a wide range of organic solvents commonly used in HILIC applications, e.g., acetonitrile, formic acid, and alcohols. Swelling of PEEK material may, however, occur after prolonged exposure to solvents like THF, methylene chloride or DMSO.
The ZIC®-*p*HILIC column can be operated in the pH range 2 to 10, while strongly alkaline solutions and washing with sodium hydroxide should be avoided. The ZIC®-*p*HILIC column can be heated and operated up to 50 °C.

Cleaning and Regeneration
If the backpressure increases or a shift in selectivity is observed, use the following recommended column wash procedure.

30 column volumes of deionised water

30 column volumes of 0.5 M NaCl

30 column volumes of deionised water

An initial washing with deionised water is used to remove organic solvent and polar impurities, followed by a flush with a 0.5 M sodium chloride solution. Finally remove the salt solution with sufficient water and fill the column with 80% (v/v) acetonitrile.

Storage
The column is delivered filled with 80% (v/v) acetonitrile in ammonium acetate buffer (5 mM, pH 6.8) and that is also the recommended solvent for long term storage. Connect the end stop plugs when the column is removed from the system.

Store columns as shipped:
Acetonitrile / NH_4Ac 5 mM, pH 6.8; 80:20 (v/v)

Dispose the column according to local authorities and regulations

Warning
Use of the product in applications not specified, or failure to follow instructions contained in this information insert, may result in improper functioning of the product, personal injury, or damage to property or the product.

Sample Solvent and Solvent Strength
Sample solvents should consist of 60-100% organic solvent, or initial eluent composition. Water should be minimized. Weak HILIC solvents such as acetonitrile are favoured. It is recommended to have about 5% water in the auto sampler wash solution.

The relative solvent strength for HILIC is:
Acetone<Acetonitrile<Isopropanol<Ethanol<Methanol<Water

Mobile Phase Considerations
To obtain reproducible results, maintain at least 3% water in the mobile phase, in order to ensure sufficient hydration of the stationary phase particles

Suitable buffer systems for HILIC separations are formate and acetate, due to their excellent solubility even in very high concentrations of organic solvent. Avoid phosphate, and other low solubility buffers, to prevent precipitation on the column bed. A buffer concentration in the range 5-20 mM is recommended for most analytes, with an upper limit of 200-300 mM, depending on the solubility in the eluent. TFA and other ion pair reagents should be avoided, as they can interfere with the HILIC separation mechanism, and suppress MS signals.

Typical Elution Protocols
Isocratic elution: 80:20 (v/v) acetonitrile / NH_4Ac, (5-20 mM) or other suitable buffer salt.
Gradient elution: 90% to 40% acetonitrile in 20 minutes (~2.5%/ min).

Equilibrating the Column
If not familiar with the column, the recommended initial starting and conditioning procedure is to run a gradient from 90% (v/v) acetonitrile/ 10% (v/v) buffer solution (e.g., 10 mM ammonium acetate), and ending with 40% (v/v) acetonitrile. Initially, use a relatively low flow rate to ensure a suitable linear flow to obtain maximum separation efficiency.

Flow-rate and Injection Volume
The optimal flow-rate and expected backpressure can be seen in the figure and table below. The recommended injection volume is also listed in the table. The low backpressure in HILIC, allows higher flow-rates if sufficient resolution is achieved, but do not exceed the maximum pressure.

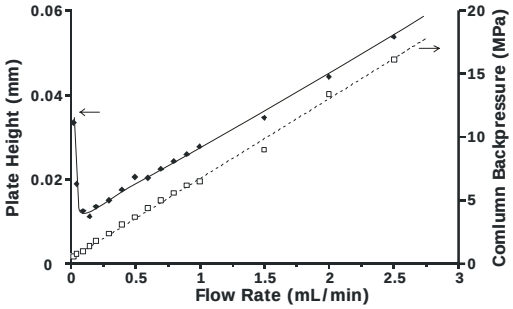

Figure: Column plate height (◆) and backpressure (□) vs. volumetric flow rate. Cytosine injected on a 50 x 4.6 mm ID ZIC®-*p*HILIC column at k' 1.3 using an eluent with 80:20 acetonitrile/buffer

Table: Flow-rate, backpressure and injection volume

Column I.D. (mm)	Injection volume (µL)	Flow-rate (mL/min)	Backpressure Expected (MPa)	Backpressure Max (MPa)
2.1	0.5-5	0.1	2-10	20
4.6	5-50	0.5	2-10	20

Trademarks

References
Visit http://www.mercksequant.com/scientificpapers for an updated list of scientific literature where ZIC®-*p*HILIC has been used.

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