Supelco®

SeQuant®

ZIC®-*p*HILIC HPLC Column

Analytical PEEK coated Columns

General Instructions for Care and Use

Contents of Package

1 ZIC®-*p*HILIC HPLC Column
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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 and Chemical Compatibility

The column is made from poly(etherether ketone) [PEEK] coated stainless steel and has 10 - 32 UNF female fittings. The frits have a porosity to retain 3 - 10 µm particles and are made from Titan. 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 equilibrate the column with 80 % (v/v) acetonitrile. On-line cleaning of the column can be accomplished by increasing the aqueous portion of the eluent close to 100 % while keeping a sufficient ion strength. This procedure can be included at the end of a gradient run and is especially useful for routine applications with samples containing appreciable amounts of salts, such as urine and plasma.

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.

Sample Solvent and Solvent Strength

Sample solvents should consist of 60 - 100 % organic solvent, orinitial 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. THF is normally a poor solvent for HILIC separations due to its strong hydrogen bonding ability.

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. Avoid very steep eluent gradients combined with high flow-rates as this can cause pressure shocks irreversibly damaging the polymeric column bed.

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

Equilibrate the column with 7 - 8 column volumes of initial mobile phase composition. Note that equilibration can be performed at higher flow rates to save time.

Flow-rate and Injection Volume

Never exceed the maximum backpressure listed in the table below.The low viscosity of acetonitrile can enable very high flow-rates before max pressure is reached. A suitable injection volume is about 1 % of the total column volume.

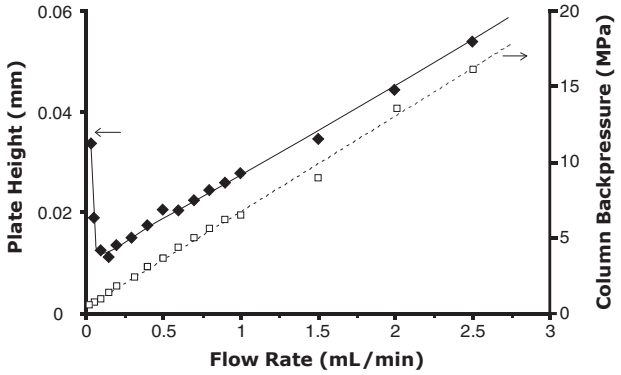

Figure: Column plate height (◆) and backpressure (□) vs. volumetric flow rate. Cytosine injected on a 50 x 4.6 mm ID ZIC®-*p*HILIC column at k' 1.3 using an eluent with 80 : 20 acetonitrile/buffer

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	0.05 - 0.5	2 - 10	20
4.6	5 - 50	0.25 - 2.5	2 - 10	20

Trademarks

ZIC® is a registered trademark of Merck KGaA, Darmstadt, Germany.

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.

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Made in Germany

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