HILICON

iHILIC®-Fusion and iHILIC®-Fusion(+)

HPLC and UHPLC HILIC Columns
Instructions for Use (IFU)

Product Description

iHILIC columns are designed for the separation and purification of polar and hydrophilic compounds using Hydrophilic Interaction Liquid Chromatography (HILIC). The packed stationary phases are charge modulated amide silicas which are covalently bonded with neutral, positively charged, and negatively charged hydrophilic functional groups. Therefore, the separation mechanism with iHILIC columns is based on a combination of hydrophilic interaction, hydrogen bonding, and weak electrostatic interactions. They have unique separation selectivity and high separation efficiency. iHILIC columns are available in two different surface chemistries, providing complementary selectivity as shown in Figure 1.

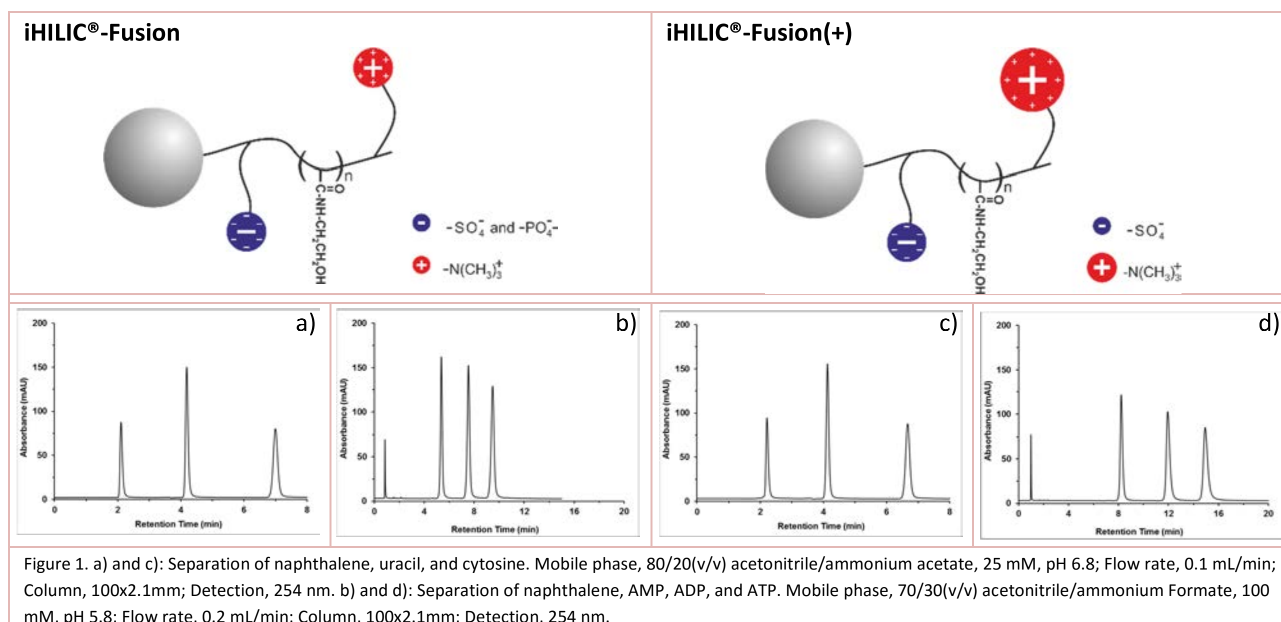

Figure 1. a) and c): Separation of naphthalene, uracil, and cytosine. Mobile phase, 80/20(v/v) acetonitrile/ammonium acetate, 25 mM, pH 6.8; Flow rate, 0.1 mL/min; Column, 100x2.1mm; Detection, 254 nm. b) and d): Separation of naphthalene, AMP, ADP, and ATP. Mobile phase, 70/30(v/v) acetonitrile/ammonium Formate, 100 mM, pH 5.8; Flow rate, 0.2 mL/min; Column, 100x2.1mm; Detection, 254 nm.

Columns

The iHILIC columns are in both poly(ether-ether-ketone) (PEEK) or stainless steel (SS). The column end-fittings have 10-32 UNF threads and a Parker™ port design.

PEEK is a tough engineering plastic and has excellent chemical resistance against a wide range of organic solvents commonly used in HILIC applications, e.g., acetonitrile, formic acid, or alcohols. However, swelling of PEEK material happens in THF, methylene chloride or DMSO, and they should not be used for PEEK columns and HPLC system with PEEK tubing and parts. Furthermore, never use SS ferrules and fittings for PEEK columns, since the metal will cut the PEEK threads and damage the end fittings!

The silica based iHILIC columns can be used between pH 2 to 8, and the maximum operation temperature is 60 °C.

The maximum column operation back pressure is:

- PEEK HPLC columns packed with 3.5 and 5 µm particles: < 350 bar at room temperature (R.T.).
- SS HPLC columns packed with 3.5 µm particles: < 450 bar at R.T.
- SS UHPLC columns packed with 1.8 µm particles: < 650 bar at R.T.

Disposal of iHILIC columns should follow the local authorities and regulations.

Solvents

For HILIC separations, water and buffer solutions are strong eluents and organic solvents are weaker. Acetonitrile is the most favorable solvent. The relative solvent strength for HILIC is: THF < Acetone < Acetonitrile < Isopropanol < Ethanol < Methanol < Water. Contrary to reversed phase chromatography, polar compounds have increased retention when increasing the proportion of organic solvent in mobile phase.

Buffers and Additives

Solutions of 2-50 mM ammonium format and ammonium acetate are the best buffers for HILIC with MS detection. Their pH can be adjusted by adding formic acid, acetic acid, or ammonia. Phosphate buffers should be used with extra caution at lower concentrations due to the lesser solubility of sodium, potassium, and phosphate in HILIC mobile phases, which contain high concentrations of organic solvent. TFA and ion-pair reagents will change the selectivity of HILIC separations and

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often interfere with MS detection. Thus they should be avoided or used consciously to reduce the polarity of amines and the protonation of carboxyl groups for a specific purpose (i.e. isolation of glycopeptides from peptide digests).

Samples

It's highly recommended to do sample preparations with mobile phase or solutions with similar ionic strength and concentration of organic solvent. These would help to get sharp peaks and avoid tailing or split peaks. More organic solvent in the sample solution will sharpen the peak due to peak compression effects. Complex samples such as plasma or urine should be treated with a high proportion of organic solvent to precipitate proteins and salts, and filtered with 0.45 or 0.22 µm syringe filters that are compatible with organic solvents.

Recommended Starting Conditions for Method Development

	Isocratic Elution		**Gradient Elution**	
Mobile phase:	80/20 (v/v) Acetonitrile/5-100 mM ammonium acetate, pH6.8		A: Acetonitrile B: 5-100 mM Ammonium acetate, pH6.8 Gradient profile: 90% to 50% Acetonitrile in 20 minutes (~2%/ min)	
Column equilibration	-		4-10 minutes	
Column I.D.	**1 mm**	**2.1 mm**	**3 mm**	**4.6 mm**
Flow rate (mL/min)	0.05-0.1	0.2-0.5	0.4-1.0	1.0-2.5
Injection (µL)	0.1-1	0.5-10	0.1-20	2-50
Detector cell	Nano, micro	micro, semi-micro	Semi-micro, analytical	Semi-micro, analytical

Optimization of Flow Rate

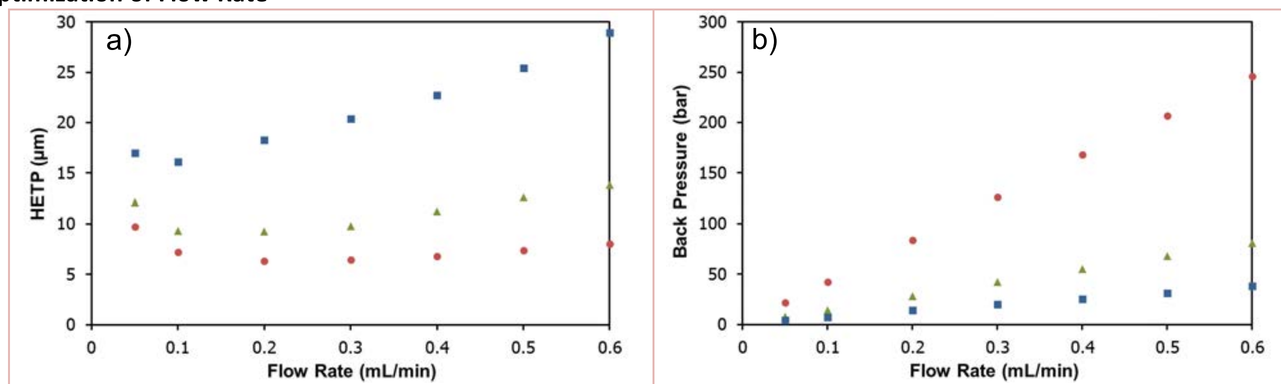

Figure 2. Flow rate versus column HETP a) and back pressure b). Mobile phase, 80/20(v/v) acetonitrile/ammonium Acetate, 25 mM, pH6.8; Sample, cytosine; Detection, 254 nm; iHILIC®-Fusion Columns, 50x2.1 mm with 1.8 µm (●), 3.5 µm(▲), and 5 µm (■) particles.

Column Protection

It's highly recommended to use a guard-column or pre-filter when working with complex or dirty samples such as cell lysates, plasma or urine. They protect the analytical columns from potential clogging by contamination and/or particles and extend column life times.

Column Cleaning or Regeneration

If an increase of column back pressure or a shift of retention time is observed, the following recommended column wash procedure can be helpful. The recommended flow rate for cleaning is 0.1-0.4 and 0.5-2 mL/min for 2.1 and 4.6 mm columns, respectively. However, the column back pressure should never exceed 150 bar in the cleaning process.

1. 60 minutes of deionized water
2. 60 minutes of 0.6 M ammonium acetate
3. 60 minutes of deionized water
4. 30 minutes of mobile phase for the next experiment

If the column is not completely recovered, one last try is to regenerate the column according to the same procedures as above but connect the column in reversed direction. Otherwise, it's time to purchase a new column.

Storage

A new iHILIC column is delivered with 80/20 (v/v) acetonitrile/25 mM ammonium acetate, pH 6.8, which is also the recommended solution for long term storage at a temperature lower than ambient. The column end plugs should be tightened carefully.

Trademarks

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