NUCLEODUR® HILIC
NUCLEOSHELL HILIC

Note: All HPLC columns from MACHEREY-NAGEL are supplied with a certificate, which contains specifications and test results of the column. NUCLEODUR® HILIC columns are quality products based on the high purity and very pressure stable silica NUCLEODUR®; NUCLEOSHELL HILIC is based on highly efficient core-shell silica. They are especially developed for HPLC analysis. If carefully and properly used excellent chromatographic results and long column lifetime can be achieved. HPLC columns are designed for qualitative and quantitative analysis of mixtures of substances and single components. They must exclusively be used in accordance with universally accepted laboratory regulations and HPLC working methods. Before running the column the entire analytical system (column and equipment) has to be carefully checked by the operator. Chromatographic conditions (mobile phase, flow, temperature etc.) have to be adapted to the analytical problem. MACHEREY-NAGEL does not give any warranty and is not liable for the success of a separation or application. If you have any questions after reading this manual, please call our service / technical support.

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Safety indication

Follow the general safety instructions for handling of HPLC solvents used as mobile phases (e.g., acetonitrile, methanol) and take precautions against any kind of injuries or damage to health (e.g., skin and eye protection in case of broken capillaries). Disposal of used HPLC columns must follow international, national and local environmental protection laws. The use of HPLC columns is only permitted to staff members, who are qualified in their field. Keep HPLC columns away from children. MACHEREY-NAGEL disclaims and excludes all warranties of any kind or nature whatsoever and MN shall not be liable for any damages (whether direct, indirect, foreseeable, incidental, compensatory, consequential or special), whether based upon warranty, contract, tort or strict liability, if damages and / or losses occur caused by improper use, maintenance, neglect or improper treatment (especially opening of the column and exposure of the column bed).

Description of the column

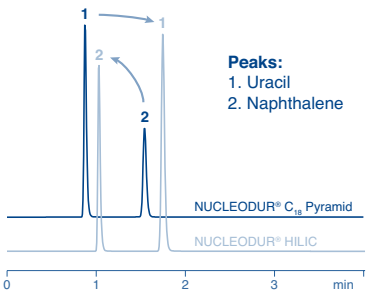
As stationary phase NUCLEODUR® HILIC columns contain fully synthetical spherical silica (type B) modified with ammonium – sulfonic acid ligands; NUCLEOSHELL HILIC columns contain accordingly modified core-shell silica. HILIC columns are especially suited for the separation of hydrophilic, polar and ionic analytes under reversed phase conditions. These analytes are only poorly retained on reversed phase columns. The behavior is truly orthogonal on NUCLEODUR® HILIC (see figure below) which makes it an ideal tool to enhance chromatographic separations. The stationary phase is modified with a strong cationic and anionic ion exchanger. The zwitterionic functionality is responsible for the hydrophilic character. Due to a special modification step NUCLEODUR® / NUCLEOSHELL HILIC provide a total charge balance. The hydrophilic character and the ability to exhibit only weak electrostatic interactions are properties which are vital for a broad applicability in "**H**ydrophilic **I**nteraction **Li**quid **C**hromatography".

Separation of uracil and naphthalene

Orthogonal retention between HILIC and RP column

Columns: EC 125/4 NUCLEODUR® HILIC, 3 µm, REF 760531.40
EC 125/4 NUCLEODUR® C_{18} Pyramid, 3 µm, REF 760260.40
Eluent: acetonitrile – water (90:10, v/v)
Flow rate: 1.0 mL/min
Temperature: 25 °C
Detection: UV, 254 nm

MN Appl. Nos. 122911 / 122912



Installation

The column should be installed in the flow direction indicated on the column label. It is connected with 1/16" capillaries and fittings, typical for HPLC instruments.

Guard columns

For protection and an extension of column lifetime the column should always be used with guard columns. The filter elements and the adsorbent in the guard column retain contaminants from the sample or the eluent. Connection of the guard column with the separation column is made by a suitable guard column holder (see ***www.mn-net.com*** or MN chromatography catalog). Cartridge replacement is required when increased column pressure and / or loss of performance is observed.

Sample

Sample solutions should be passed through a syringe filter (e.g., CHROMAFIL® Xtra PET, 0.45 µm, 25 mm, REF 729220) before entering the column. If injected sample solutions are still turbid even after filtration, the lifetime of the column may be significantly reduced. The sample volume should be as small as possible to achieve an optimal resolution.

Eluent

The retention behavior for HILIC is inverse to RP chromatography. Therefore, the relative solvent strength for HILIC is also inverse to RP chromatography:

water > methanol > ethanol > 2-propanol > acetonitrile > tetrahydrofuran > acetone

The columns are supplied with the eluent acetonitrile – water (80:20, v/v). A suitable mobile phase for HILIC includes acetonitrile (40–98 %) and water (60–2 %). Other solvents miscible with water, like acetone, THF, 2-propanol or ethanol can also be used for HILIC columns. In order to establish a water layer on the surface of the stationary phase it is vital that at least 2 % water remains in the mobile phase. Volatile buffers, such as ammonium acetate and ammonium formate are commonly used to control the ionic strength and the pH of the mobile phase (pH range: 2–8.5). A buffer concentration of 0–25 mM is recommended for midpolar and polar compounds, like uracil, melamine, adrenaline, creatine or mepiquat. For extremely polar compounds, such as adenosine triphosphate, citric acid or aminoglycosides, higher concentrations of buffer (100-200 mM) are required. Ion pair reagents like TFA should be avoided since they suppress MS signals. Never use phosphate buffers as mobile phase component nor as solvent for your samples! Eluents should be filtered through a 0.2–0.45 µm membrane and degassed.

Flow rate and pressure

Flow rate influences the time required, the resolution and the column lifetime. Typical flow rates used for HILIC columns are:

Column ID [mm]	Optimum flow rate [mL/min]		Maximum back pressure [bar]	
	NUCLEODUR	NUCLEOSHELL	NUCLEODUR	NUCLEOSHELL
2	0.15	0.15	900	600
3	0.34	0.34	800	600
4	0.60	0.60	600	600
4.6	0.80	0.80	600	600

Considerably higher flow rates can be employed if sufficient resolution is achieved.
If a high pressure results from the use of the column at nominal flow rates, this usually indicates that some contaminants have become deposited on the packing material, which must be removed (see troubleshooting).

Temperature

Column temperatures between 0 and 60 °C are possible; for a long lifetime 30–40 °C is recommended. However, they should be at least 30 °C below the boiling temperature of the eluent, in order to ensure proper detection. Variation of the temperature influences retention times and especially the peak shape. Optimum temperatures for successful separations should be determined empirically.

Detection

Spectrophotometers, mass spectrometers, refractometers and electrochemical detectors can be used with the columns. If electrochemical detectors are used, please note that high temperatures may be incompatible with some working electrodes. If a higher sensitivity is required, post-column derivatizations with an appropriate detector for the reaction product can be used.

Equilibration

Prior to measurement of samples the column must be rinsed with the eluent at the same flow rate and temperature as the method to be applied. Column equilibration is finished, when the baseline of the detector no longer shows a drift (generally after 10 column volumes).

Method development

If no application note is available the following procedure is recommended for method development:

Isocratic elution

a) acetonitrile – 0 to 25 mM ammonium acetate (75:25, v/v) for midpolar and polar compounds, like uracil, melamine, adrenaline, creatine or mepiquat
b) acetonitrile – 100 to 200 mM ammonium acetate (70:30, v/v) for extremely polar compounds, such as adenosine triphosphate, citric acid or aminoglycosides

Gradient elution

90 to 50 % acetonitrile in 20 min (2 %/min); the buffer salt concentration depends on the nature of the compounds analyzed as described for the isocratic elution

retention time to short	→	increase the amount of acetonitrile stepwise by 5 to 10 %
retention time to long	→	increase the amount of buffer stepwise by 5 to 10 %
MS sensitivity to small	→	decrease the buffer concentration gradually

Column storage

The column should be stored at room temperature with acetonitrile – water (80:20, v/v) or with acetonitrile – 5 mM NH_4Ac, pH 5.3 (80:20, v/v). For column storage be sure the end fittings are tightly sealed using column end plugs, because storage without these seals can result in drying of the packing material. Under these circumstances rinse the column with the eluent of storage at a flow rate of max. 0.2 mL/min.

Troubleshooting

The following outline describes the symptoms of performance loss and its cause. All columns are subject to the strict regulation and control of our quality assurance system. Columns based on silica are robust and hold their separation efficiency for long periods by correct maintenance and treatment. According to experience, column failures are mostly a result of injection of contaminants to the sorbent bed. The usage of a guard column, as well as an appropriate sample pretreatment will help to minimize these risks.
Use the outline below to help determine the cause of a possible performance loss:

Symptom / Error / Cause	Prevention / Remedy
Baseline drift	
· insufficient period for equillibration of the eluent	longer or better equilibration
· contaminated eluent	use freshly prepared solvents and reagents
· temperature	column temperature control
Broad peaks	
· mixing and / or diffusion before / behind the column	keep length and ID of capillaries at a minimum
· too large sample volume	smaller injection volume
Peak interference; too fast elution	
too fast elution and / or insufficient separation by:	
· improper column temperature or flow rate	optimize concerned parameter
· elution power of eluent is too high	optimize eluent system
Increasing back pressure; degradation of the separation performance	
contamination of sorbent by:	
· particulate accumulation on frit or sorbent bed from sample, eluent or system	prepare fresh eluent; prefilter samples and eluent, use in-line filter / rinse LC system, clean the sorbent
· precipitation of buffer salts	check solubility of buffer salts before / remove it by rinsing (see column regeneration)
Insufficient separation; degradation of the separation with regular column pressure	
contamination with:	
· fats, oils, lipids from sample (coating of sorbent surface) and other organic substances from improperly prepared eluent or matrices	remove organic substances by sample preparation / clean the sorbent (see column regeneration)
Double peaks (dead volume)	
· faulty fittings (capillaries, ferrules, nuts)	use "PEEK Fingertight Fittings", REF 718770 / replace fittings
· dissolution of silica by too high pH value of eluent	consider pH range of column / replace column

Column regeneration

In some cases the perfomance of the column can be restored by removing contaminants from the sorbent bed or by regeneration of the phase. It is important, however, to locate the source of contamination before again using the column for the analysis of samples.

1. Prepare fresh eluent: In some cases the performance loss is traced to eluent contamination. Therefore, prepare fresh eluent and flush all liquid lines before using the column again. The eluent should be filtered through a 0.2–0.45 µm membrane and degassed prior to use.
2. Cleaning of sorbent: To remove contamination rinse the column by using the following cleaning procedures.
   Polar impurities:
   · 20 column volumes of deionized water
   · 30 column volumes 0.5 M ammonium acetate, pH 6
   · 30 column volumes of deionized water
   Non polar impurities:
   · 20 column volumes of acetonitrile – water (50:50, v/v)
   · 20 column volumes acetonitrile
   · 20 column volumes of acetonitrile – water (50:50, v/v)
   Any other solvent miscible with water (acetone, tetrahydrofuran, methanol etc.) can also be used.
3. Column replacement: Above procedures will restore performance only in certain cases. Some organic contaminants are particularly refractory and may not respond to treatment. Also dead volume, due to column compression can generally not be repaired. Under these circumstances, column replacement is necessary. It is highly advisable to locate the cause of the problem before installing a new column.

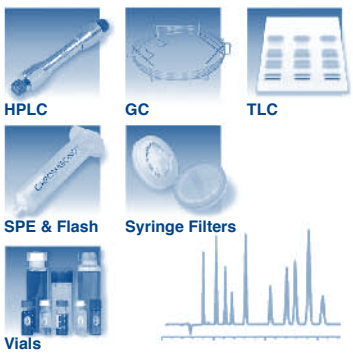
		Column volume [mL]			
Length [mm]	Inner diameter [mm]:	2	3	4	4.6
100		0.30	0.70	1.25	1.65
150		0.45	1.05	1.90	2.50
250		0.80	1.75	3.15	4.15

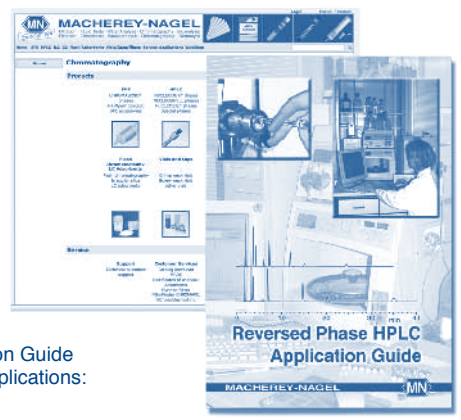
Abstract

To extend column lifetime, please keep in mind the following:

1. Suitable eluents for HILIC include acetonitrile (40–98 %) and water (60–2 %). Other solvents miscible with water (e.g., acetone, THF, 2-propanol, ethanol), as well as volatile buffers (ammonium acetate, ammonium formate) can be used. The eluents should be filtered through a 0.2–0.45 µm membrane and degassed.
2. Filter samples through a 0.2–0.45 µm CHROMAFIL® Xtra PET syringe filter before injection.
3. Use a guard column for contaminated samples.
4. The recommended flow rate is 0.15–0.80 mL/min.
5. Adjust flow rate to keep column pressure below the maximum value of your column.
6. Store the column in acetonitrile – water or acetonitrile – 5 mM NH_4Ac, pH 5.3, (80:20, v/v).
7. Use analytical grade reagents and HPLC grade solvents for all work. Discard any solutions that show evidence of bacterial growth.

Please check the full range of MACHEREY-NAGEL chromatography products!





… for applicative support please ask for our HPLC Application Guide or visit our website with more than 3000 chromatography applications:
www.mn-net.com/apps

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