NUCLEOSIL® RP columns

Note: All HPLC columns from MACHEREY-NAGEL are supplied with a certificate, which contains specifications and test results of the column. NUCLEOSIL® RP columns are quality products based on the robust silica NUCLEOSIL®. They are specifically 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.) must be adapted to the analytical task. 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.

Table of contents

- Safety indication
- Description of the column
- Installation
- Precolumn filter and guard columns
- Sample
- Eluent
- Flow rate and pressure
- Temperature
- Detection
- Equilibration
- Column storage
- Troubleshooting
- Column regeneration
- Abstract

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 regulations. 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 NUCLEOSIL® RP columns contain a C_{18}, C_8 or special RP phase based on spherical silica (type A), modified with a special method.

NUCLEOSIL® RP phase	Modification	Property / Stability
C_{18} / C_{18} ec	octadecyl, endcapping	hydrophobic, weakly polar, pH 2–8
C_8 ec	octyl, endcapping	weakly hydrophobic, weakly polar, pH 2–8
C_8	octyl, no endcapping	weakly hydrophobic, polar, pH 2–8
C_{18} HD	octadecyl, high density, endcapping	strongly hydrophobic, pH 2–9, LC/MS
C_8 HD	octyl, high density, endcapping	hydrophobic, weakly polar, pH 2–8, LC/MS
C_{18} AB	octadecyl, specially crosslinked, endcapping	strongly hydrophobic, high steric selectivity, pH 1–9, LC/MS
C_{18} Nautilus	octadecyl phase with embedded polar group, endcapping	hydrophobic, polar, stable in 100 % aqueous eluents, pH 2–8
Protect I	RP phase with embedded polar group, endcapping	hydrophobic, polar, stable in 100 % aqueous eluents, pH 2–8

Descriptions of the columns NUCLEOSIL® C_4, C_2, C_6H_5, C_6H_5 ec, C_{18} PPN, C_{18} MPN, C_4 MPN and further RP columns can be found on ***www.mn-net.com***. They can be used and treated in reference to this manual.

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.

Precolumn filter and guard columns

A precolumn filter containing 0.5–2.0 µm porosity stainless steel frits is recommendable between sample injector and column to remove particulates from the eluent stream. For protection and an extension of column lifetime the column should always be used with a guard column. 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 the 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

RP columns are supplied with the eluent acetonitrile – water (depending on the type 80:20, 70:30 or 60:40, v/v; see column certificate for details). As mobile phase typical RP eluents (e.g., acetonitrile or methanol with pure water or buffer) can be used. Eluents should be filtered through a 0.2–0.45 µm membrane filter and degassed. Please consider the pH stability of the used column. Strong acidic or basic conditions can result in dissolution of the column bed or the organic modification. The amount of buffer salts should be kept as low as possible. Note the solubility limit of the buffer in the eluent. The increase of the organic portion can result in precipitation of buffer salts and plugging of the column. Before start of operation with eluent containing a buffer the column should be first preconditioned with a minimum of 10 column volumes acetonitrile – water (25:75, v/v). Always after finishing measurements with buffer-containing eluents the column should be regenerated (see column regeneration). Use of ion pair reagents with phases with embedded polar groups can result in unspecific non-reproducible interactions.

Flow rate and pressure

Flow rate (recommended for analytical columns: 0.2–2.0 mL/min) influences the time required, the resolution and the column lifetime. It is limited by the maximum column back pressure, which should not exceed the limits listed in the table below.

	Maximum pressure [bar]										
Silica Inner diameter [mm]:	2	3	4	4.6	8	10	16	21	32	40	50
NUCLEOSIL® 50 Å, 100 Å, 120 Å	400	400	400	400	400	400	400	400	400	400	400
NUCLEOSIL® 300 Å	300	300	300	300	300	300	300	300	300	300	300
NUCLEOSIL® 500 Å	250	250	250	250	250	250	250	250	250	250	250
NUCLEOSIL® 1000 Å, 4000 Å	200	200	200	200	200	200	200	200	200	200	200

In mixtures of methanol and water viscosity reaches a maximum at about 40 % methanol. For this reason a reduced flow rate is recommended, when changing the eluent composition. We recommend controlling back pressure regularly. 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 up to 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, refractometers and electrochemical detectors can be used with the columns. NUCLEOSIL® C_{18} AB, C_{18} HD or C_8 HD are also suitable for LC/MS detection. 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).

Column storage

The original eluent (see eluent) is recommended for storage. For long-term storage mobile phases containing inorganic salts are not recommended (for a removal of buffer see column regeneration). Methanol is also not recommended for a longer storage, because of a possible impurity with metal ions (e.g., iron(III)). 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 approx. 10 column volumes of 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 their 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 / Repair
Baseline drift	
· insufficient period for equillibration with 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 them 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: A performance loss is not seldom 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 with a minimum of 10 column volumes (see table below) at the original flow rate and temperature as follows:
   - acetonitrile – water or methanol – water (10:90, v/v) for removal of the buffer
   - 100 % methanol to remove polar organic compounds
   - 100 % acetonitrile to remove medium polar organic compounds (possibly T= 40 °C)
   - 100 % tetrahydrofuran to remove nonpolar organic compounds
   - if necessary, 100 % tetrahydrofuran with inverse flow direction at 1/5 of original flow rate
   - convert column to storage condition using acetonitrile – water (80:20, 70:30 or 60:40, v/v) at original flow rate

   An adequate indicator for a clean column is a constant baseline. At constant temperature you should observe less than 2–3 mAU drift during a running time of 5 minutes with an isocratic run.
   After the usage of buffer, directly after finishing a measurement and always before storage of the column rinse with a minimum of 10 column volumes at the original flow rate and temperature as follows:
   - acetonitrile – water or methanol – water (10:90, v/v) for removal of the buffer
   - increase the organic part in steps of 20 % to the conditions of a new measurement run
   - or gradually increase the part of acetonitrile in steps of 20 % to the storage conditions
3. Column replacement: The 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. As RP eluents organic-aqueous eluent systems (e.g., acetonitrile or methanol – water or buffer) are recommendable. Please consider column regeneration after usage of buffers. 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 an in-line filter and / or a guard column for protection against impurities.
4. The recommended flow rate is 0.2–2.0 mL/min.
5. Adjust flow rate to keep column pressure below the maximum value of your column.
6. Store the column in acetonitrile – water (80:20, 70:30 or 60:40, v/v) after removal of buffer salts.
7. Use analytical grade reagents and HPLC grade solvents for all work. Discard any solutions that show evidence of bacterial growth.

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