NUCLEODUR® NP columns
NUCLEOSIL® NP columns

Note: All HPLC columns from MACHEREY-NAGEL are supplied with a certificate, which contains specifications and test results of the column. NUCLEODUR® NP columns are quality products based on the high purity and very pressure stable silica NUCLEODUR®; NUCLEOSIL® NP columns are 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) must 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., *n*-heptane) 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 normal phase (NP) column NUCLEODUR® SiOH contains unmodified, fully synthetical spherical silica (type B). NUCLEOSIL® NP columns contain unmodified or modified spherical silica (type A).

NP phase	Modification	Property / Stability
NUCLEODUR®		
SiOH	unmodified	polar, ionic, pH 2–8
NUCLEOSIL®		
SiOH	unmodified	polar, ionic, pH 2–8
NO_2	nitrophenyl	polar, hydrophobic, selectivity for aromatic compounds (π-π interactions), pH 2–8
OH	diol	polar (hydrogen bonds), pH 2–8
$N(CH_3)_2$	dimethylamino	polar, hydrophobic, weak ion exchange, pH 2–8

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 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 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

NP columns are supplied with the eluent *n*-heptane. In normal phase mode (NP) *n*-heptane, hexane, dichloromethane or 2-propanol can be used as mobile phase. Eluents should be filtered through a 0.2–0.45 µm membrane and degassed. Polar additives (e.g., water) in the eluent should be avoided for the unmodified NP phases, because they can result in a change of chromatographic separation properties, which is difficult or impossible to reset.

If an application of the modified NP phases should be necessary in reversed phase mode (RP), they must be rinsed with 10 column volumes tetrahydrofuran (THF) before. If a buffer additive is used, the column must be always regenerated after use (see column regeneration). Please consider also the pH stability of the column used. Strong acidic or basic conditions can result in dissolution of the column bed or the organic modification.

Flow rate and pressure

Flow rate (recommended for analytical columns with 2–4.6 mm ID: 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 ID [mm]:	≤ 1	2	3	4	4.6	8	10	16	21	32	40	50
NUCLEODUR® 1.8 µm	900	900	800	600	600	-	-	-	-	-	-	-
NUCLEODUR® 3, 5, 7 and ≥ 10 µm	600	600	600	600	600	400	400	400	400	400	400	400
NUCLEOSIL® 3, 5, 7 und ≥ 10 µm*	400	400	400	400	400	400	400	400	400	400	400	400
* for pore sizes of 300 Å (300 bar), 500 Å (250 bar), 1000 Å and 4000 Å (200 bar) maximum pressure is reduced												

Note, that for a changing to an eluent with higher viscosity than *n*-heptane, the back pressure can increase. Thus, a changing of the eluent composition should be performed at lower flow rate (10–50 % of normal flow rate, in relation to the viscosity). 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, temperature 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).

Column storage

The original eluent (*n*-heptane) is recommended for storage (storage temperature: 15–30 °C). An inert solvent like *n*-hexane or toluene is also possible. Polar solvents (e.g., water or eluents containing water) or solvents with a boiling point only slightly above the storage temperature (e.g., diethyl ether, tetrahydrofuran, dichloromethane) should be avoided. The columns should be stored without buffer additives as well. 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 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 (only for RP mode)	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 with a minimum of 10 column volumes (see table below) at the original flow rate and temperature as follows:
   · 100 % tetrahydrofuran to remove non or medium polar organic compounds
   · if necessary, 100 % tetrahydrofuran with inverse flow direction at 1/5 of original flow rate
   · convert column to storage condition with *n*-heptane
   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.
3. Regeneration (only for modified phases in RP mode): After the usage of buffer in RP mode, 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 for storage gradually increase the organic part in steps of 20 % to acetonitrile – water (70:30, v/v)
   (If the column is to be operated again in NP mode, then intermediate flushing with THF is necessary again!)
4. 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]	**ID [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 eluents nonpolar eluent systems (e.g., *n*-heptane, hexane, dichloromethane or 2-propanol) are recommendable. Eluents should be filtered through a 0.2–0.45 µm membrane and degassed. Please consider intermediate flushing with THF and subsequent regeneration after a possible usage of buffers (RP mode).
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 for analytical columns (ID 2–4.6 mm) 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 after NP application in *n*-heptane, after RP application in acetonitrile – water (70:30, v/v).
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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