NUCLEODUR® NH_2 / NH_2–RP
NUCLEOSIL® NH_2 / NH_2–RP

Note: All HPLC columns from MACHEREY-NAGEL are supplied with a certificate, which contains specifications and test results of the column. NUCLEODUR® NH_2 and NH_2-RP columns are quality products based on the high purity and very pressure stable silica NUCLEODUR®; NUCLEOSIL® NH_2 and NH_2-RP are based on the robust silica NUCLEOSIL®. They are specifically developed for HPLC analysis. Due to the chemical nature of amino phases, the lifetime of the column highly depends on the measurement and the treatment after measurement. Consequently, prior to column installation, you should familiarize yourself with the contents of this instruction leaflet. 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 leaflet, please call our service / technical support.

Table of contents

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).

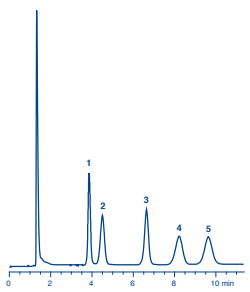
Description of the column

As stationary phases the columns contain amino phases, based on spherical silica and modified with a special procedure. The normal phase columns NUCLEODUR® NH_2 and NUCLEOSIL® NH_2 are supplied with the eluent *n*-heptane. They can be applied for the separation of compounds like amines, esters and chlorinated pesticides in normal phase chromatography (NP) with nonpolar mobile phases. As so-called multi-mode columns, they can also be used for reversed phase (RP) applications like the determination of sugars in aqueous-organic mobile phases. But then a intermediate flushing is necessary (see eluent).
Eluent in the reversed phase columns NUCLEODUR® NH_2-RP and NUCLEOSIL® NH_2-RP is acetonitrile – water. Thus, they can be used for RP applications without prior intermediate flushing.

Application note

Reversed phase separation of sugars

Column: EC 250/4 NUCLEODUR® 100-5 NH_2-RP
REF 760732.40
Eluent: acetonitrile – water (79:21, v/v)
Flow rate: 2 mL/min
Detection: RI
Peaks:
1. Fructose
2. Glucose
3. Saccharose
4. Maltose
5. Lactose



MN Appl. No. 122160

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 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

NP columns: Eluent in the column is *n*-heptane. As mobile phases in normal phase mode (NP) *n*-heptane, hexane, dichloromethane or 2-propanol are used. Eluents should be filtered through a 0.2–0.45 µm membrane filter and degassed. For a changing to reversed phase mode (RP), columns must be rinsed with 10 column volumes tetrahydrofuran (THF).
RP columns: They 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). For the choice of an RP eluent (e.g., acetonitrile or methanol with pure water or phosphate buffer; filtered and degassed) please keep in mind the following. A pH value below 2 and above 9 should be always avoided. 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). Furthermore, depending on the pH value of the eluent, the NH_2 group can be protonated. Therefore, it acts as a weak anion exchanger, which alters the retention behavior. Especially bulky buffer salts like phosphate can develop ionic interactions, thereby effectively shielding the polar NH_2 group. These phenomena lead to a retention time shift. Always after finishing measurements with buffer containing eluents, or if problems with a retention shift happen after this application, the column should be regenerated (see column regeneration). A changing to NP mode is not recommended. If necessary, it should only be made with an intermediate flushing step with THF.

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 back pressure, which should not exceed the maximum of 600 bar (NUCLEODUR®) / 400 bar (NUCLEOSIL®). 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. NUCLEODUR® NH_2 and NH_2-RP 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 (see 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 / Remedy
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 beforehand / 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)
protonation of NH_2 group by:	
· usage of acidic buffers as eluent	deprotonation by rinsing with weakly basic solutions (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:
   NP columns:
   · 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
   · column is converted to storage condition with *n*-heptane at original flow rate
   RP columns:
   · 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 non polar organic compounds
   · if necessary, with 100 % tetrahydrofuran with inverse flow direction at 1/5 of original flow rate
   · convert column to storage condition with 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.
3. Regeneration (only for RP columns): After finishing measurement and for a possible retention shift, after the usage of buffer, rinse as follows:
   · acetonitrile – 20 mM ammonium acetate or formate, pH 8–8.5 (95:5, v/v)
   · acetonitrile – water (10:90, v/v)
   · gradually increase the part of acetonitrile in steps of 20 % to the storage conditions
   The necessary time frame for each step depends on the measurement conditions like buffer concentration, pH value, flow rate, column length and diameter. As a rule of thumb, each step should at least take 30–50 minutes for a 250 x 4 mm column with a flow of 1 mL/min.
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]	**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 NP eluents nonpolar organic solvents (e.g., *n*-heptane, dichloromethane, 2-propanol) and as RP eluents organic-aqueous eluent systems (e.g., acetonitrile – water or buffer) are recommendable. For a change from NP to RP mode the column must be always rinsed with THF between the steps. 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 for analytical columns (ID 2–4.6 mm) is 0.2–2.0 mL/min.
5. Adjust flow rate to keep column pressure below 600 / 400 bar.
6. Store the NP column in *n*-heptane and the RP column 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.

Germany and international:
MACHEREY-NAGEL GmbH & Co. KG
Neumann-Neander-Str. 6–8 · 52355 Düren · Germany
Tel.: +49 24 21 969-0 · Fax: +49 24 21 969-199
info@mn-net.com · ***www.mn-net.com***

Switzerland:
MACHEREY-NAGEL AG
Hirsackerstr. 7 · 4702 Oensingen · Switzerland
Tel.: 062 388 55 00 · Fax: 062 388 55 05
sales-ch@mn-net.com

France:
MACHEREY-NAGEL EURL
1, rue Gutenberg · 67722 Hoerdt · France
Tel.: 03 88 68 22 68 · Fax: 03 88 51 76 88
sales-fr@mn-net.com

USA:
MACHEREY-NAGEL Inc.
2850 Emrick Boulevard · Bethlehem, PA 18020 · USA
Tel.: 484 821 0984 · Fax: 484 821 1272
sales-us@mn-net.com

NUCLEODUR / NUCLEOSIL NH_2 2/0/10.12 PD · A041166