



NUCLEODUR® CN / CN-RP NUCLEOSIL® CN / CN-RP

Note: All HPLC columns from MACHERY-NAGEL are supplied with a certificate, which contains specifications and test results of the column. NUCLEODUR® CN and CN-RP columns are quality products based on the high purity and very pressure stable silica NUCLEODUR®, NUCLEOSIL® CN and CN-RP 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) has to be carefully checked by the operator. Chromatographic conditions (mobile phase, flow, temperature etc.) must be adapted to the analytical task. MACHERY-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.

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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 regulations. The use of HPLC columns is only permitted to staff members, who are qualified in their field. Keep HPLC columns away from children. MACHERY-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 spherical silica modified with cyanopropyl by a special procedure. Due to an exhaustive endcapping of the NUCLEODUR® cyano phase, an exceptionally high reproducibility of analysis results is featured.

The normal phase columns NUCLEODUR® CN and NUCLEOSIL® CN are supplied with the eluent *n*-heptane. They can be applied for the separation of compounds (e.g., steroids) 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 in aqueous-organic mobile phases. But then an intermediate flushing is necessary (see eluent).

Eluent in the reversed phase columns NUCLEODUR® CN-RP and NUCLEOSIL® CN-RP is acetonitrile – water. Due to a distinct selectivity for polar organic compounds (e.g., organic acids) as well as for molecules containing π electron systems (e.g., analytes with double bonds, tricyclic antidepressants), this phase clearly shows a different retention behavior compared to C₁₈ or C₈ modified RP phases. A changing to NP conditions is possible, but is not recommended (see eluent).

Application note

Separation of cold medicine ingredients on different RP phases

Columns: A) EC 250/4 NUCLEODUR® 100-5 C₁₈ ec
B) EC 250/4 NUCLEODUR® 100-5 CN-RP

Eluent: acetonitrile – 100 mmol/L sodium citrate, pH 2.5 (15:85, v/v)

Flow rate: 1 mL/min

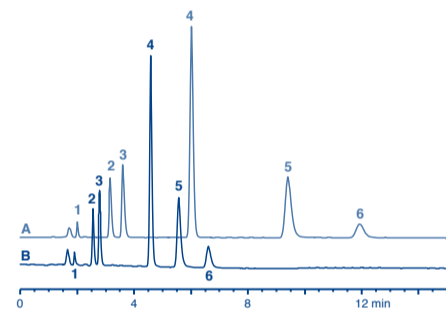
Temp.: 25 °C

Detection: UV, 270 nm

Inj. volume: 10 μ L

Peaks:

1. Maleic acid
2. Norephedrine
3. Ephedrine
4. Acetaminophen
5. Chlorpheniramine
6. Brompheniramine



MN Appl. No. 119340

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 μ m, 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 μ m membrane filter and degassed. For changing to the 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). Typical RP eluents are e.g., acetonitrile or methanol with pure water or phosphate buffer. They should be filtered and degassed. pH stability of NUCLEODUR® CN-RP is between 1 and 8, for NUCLEOSIL® CN-RP between 2 and 8. Strongly acidic or basic conditions, especially for column temperatures higher than 40 °C, 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. An increase of the organic portion can result in precipitation of buffer salts and plugging of the column. Before start of operation with an 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). 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® CN and CN-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.

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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 equilibration with the eluent · contaminated eluent · temperature	longer or better equilibration use freshly prepared solvents and reagents column temperature control
Broad peaks · mixing and/or diffusion before/behind the column · too large sample volume	keep length and ID of capillaries at a minimum smaller injection volume
Peak interference; too fast elution too fast elution and/or insufficient separation by: · improper column temperature or flow rate · elution power of eluent is too high	optimize concerned parameter 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 · precipitation of buffer salts	prepare fresh eluent; prefilter samples and eluent, use in-line filter / rinse LC system, clean the sorbent 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) · dissolution of silica by too high pH value of eluent	use "PEEK Fingertight Fittings", REF 718770 / replace fittings consider pH range of column / replace column

Column regeneration

In some cases the performance 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 using the column for the analysis of samples again.

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 μ m 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
- convert column 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, 100 % tetrahydrofuran with inverse flow direction at 1/5 of original flow rate
- column is converted 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.

After the usage of buffer, directly after finishing a measurement and always before storage RP columns 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.

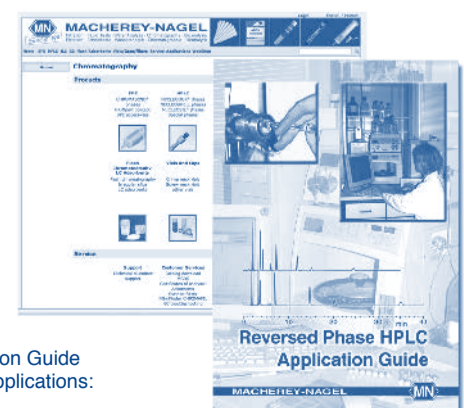
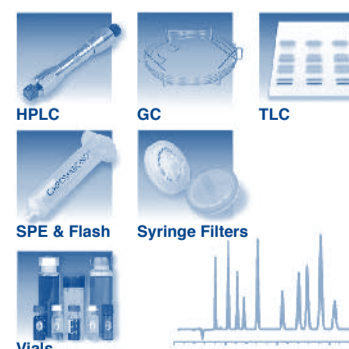
Length [mm]	Inner diameter [mm]	Column volume [mL]			
		2	3	4	4.6
100		0.30	0.70	1.25	1.65
150		0.45	1.05	1.90	2.50
250		0.80	1.75	3.15	4.15

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. An inverse change is not recommended. Eluents should be filtered through a 0.2–0.45 μ m membrane and degassed.
2. Filter samples through a 0.2–0.45 μ m 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.

Please check the full range of MACHERY-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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