NUCLEOGEL® GPC columns

Note: All HPLC columns from MACHEREY-NAGEL are supplied with a certificate, which contains specifications and test results of the column. NUCLEOGEL® GPC columns are quality products based on a mechanically stable polymer. They are specifically developed for chromatographic high performance analysis. If carefully and properly used excellent chromatographic results and long column lifetime can be achieved. These products can be used for exclusion chromatography (gel permeation chromatography, GPC) of many water-insoluble substances. All GPC separation columns must exclusively be used in accordance with universally accepted laboratory regulations and working methods of high performance liquid chromatography, especially of gel permeation chromatography. 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 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.

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Safety indication

Follow the general safety instructions for handling of the mobile phases used (e.g., tetrahydrofuran, toluene) 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 GPC columns must follow international, national and local environmental protection regulations. The use of GPC columns is only permitted to staff members, who are qualified in their field. Keep GPC 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 NUCLEOGEL® GPC columns contain a highly crosslinked macroporous, spherical polystyrene-divinylbenzene polymer matrix (PS/DVB). Due to an optimized polymer cross-linking the macroporous, spherical particles of this matrix show nearly no shrinking when changing from polar to nonpolar solvents. However, the procedures for changing an eluent should be followed (see eluent).

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. Capillaries should be as short as possible to avoid dead volume. Several columns can be coupled in series using a capillary union (short capillary with nuts and ferrules, see ***www.mn-net.com***) for complex separations. For this purpose, connections in order of decreasing pore size have proved to be advantageous.

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 capillary union. A replacement of guard column is required when increased column pressure and / or loss of performance is observed.

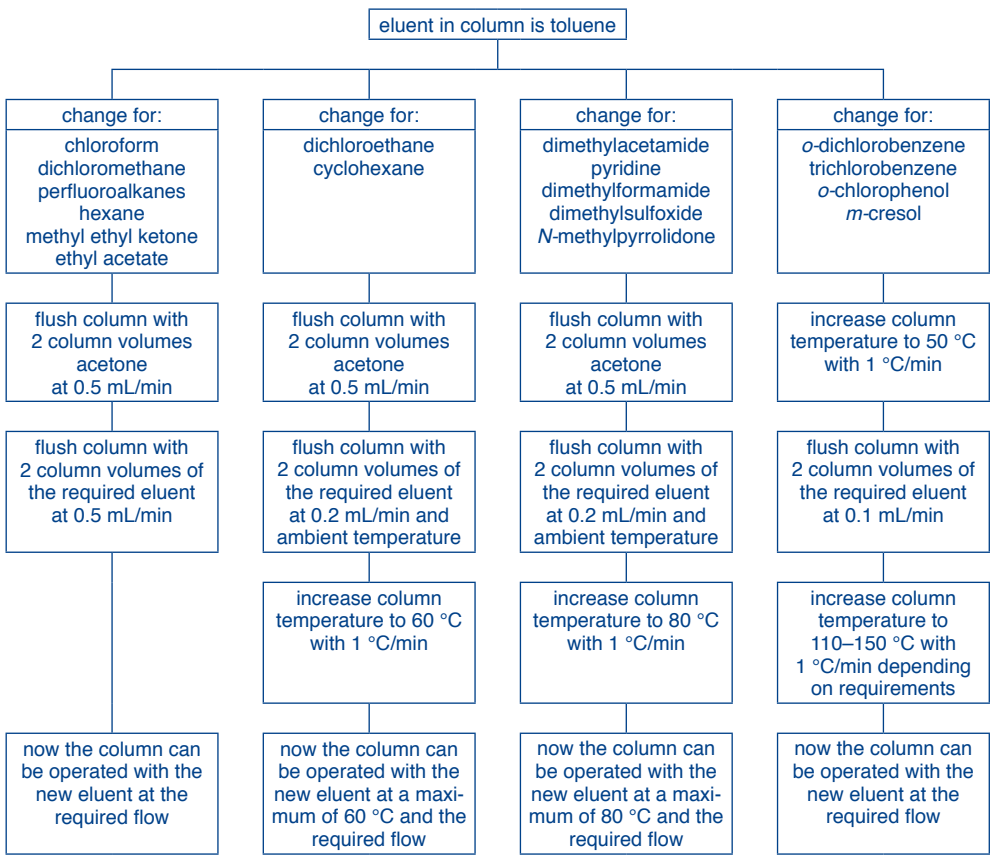
Sample

Generally, the sample is dissolved in the eluent and 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. Optimal sample volumes and concentrations depend on the individual application and should be determined empirically. Samples with a broad distribution of molecular weight can be generally injected at a higher concentration than samples with narrow distribution. Furthermore, loadability can be increased by several columns coupled in series.

Eluent

GPC columns are supplied with the eluent toluene. As mobile phases toluene, tetrahydrofuran, chloroform or *o*-dichlorobenzene are most commonly used. The usage of unstabilized tetrahydrofuran (e.g., HPLC grade) is not recommended, becasue the formation of peroxides can alter the surface characteristics of the polymer, resulting in peak tailing and adsorption of polar analytes. According to requirements further eluents are possible (see figure). Changing from toluene to tetrahydrofuran can be accomplished without flushing step at a reduced flow rate (0.5 mL/min for the first 2 column volumes). For changing to an eluent with another solvent follow the procedures below:

Procedures for changing the eluent



1 column volume (300 mm length x 7.7 mm ID column) ≙ 14 mL

If the column was operated at elevated temperature reduce the flow rate to 0.1 mL/min and reduce the temperature at 1 °C/min until ambient temperature is reached.
Eluents should be filtered through a 0.2–0.45 µm membrane and degassed.

Flow rate and pressure

As optimum flow rate for analytical columns with 7.7 mm ID, 1 mL/min is recommended. For low viscosity eluents (< 0,6 cP), the maximum flow rate is 3 mL/min for columns with 10 µm and 2 mL/min for columns with 5 µm particle size. Higher viscosity eluents should be used at lower flow rates and / or at elevated temperatures. Flow rates should be changed in small steps and pressure pulses should be avoided. In all instances the maximum column pressure should not exceed 150 bar. 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 150 °C are possible. Optimum temperatures depend on application and eluent. The temperature should be at least 30 °C below the boiling temperature of the eluent, in order to ensure proper detection. The temperature increase to operation temperature or cooling down to ambient temperature should be made in steps of approx. 1 °C/min.

Detection

In GPC refractometric detectors are preferentially used. However, the columns can be also used with spectrophotometers, mass spectrometers and electrochemical detectors. 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 (toluene) is recommended for storage (storage temperature: 15–30 °C). Other eluents except unstabilized tetrahydrofuran are also possible. Columns used with dimethylformamide or solvents with similar polarity are best stored in these eluents. 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.

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. Polymer columns 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 (see column regeneration)
Insufficient separation; degradation of the separation with regular column pressure	
contamination with:	
· 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
· compression of column bed by too high flow rates and inappropriate eluent changing	consider maximum flow rate and allowed eluent / expand the polymer bed (see column regeneration)

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 at 0.1 mL/min and 60 °C as follows (if necessary, inverse flow direction):
   - 100 % tetrahydrofuran to remove non or medium polar organic compounds
   - 10 % methanol in tetrahydrofuran to remove polar organic compounds
   - convert column to storage condition with toluene

   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. Decompression of polymer bed: The polymer consist of compressible spherical particles. The particles are deformed by a back pressure above 150 bar. Thus, a compression of the column bed and a further increase of pressure results. To decompress the column bed, shut off the pump and allow the polymer to “relax” for about 30 min. Invert the column and pump the eluent through the column with 0.1 mL/min overnight (viscous eluent at 60 ˚C). Then return the column to normal operating conditions.
4. Column replacement: The above procedures will not restore performance only in certain cases. Some organic contaminants are particularly refractory and may not respond to treatment. Under these circumstances, column replacement is necessary. It is highly advisable to locate the cause of the problem before installing a new column.

Abstract

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

1. As eluents, e.g., toluene, tetrahydrofuran or dimethylsulfoxide are used. (Pay attention to procedures for changing the eluent!) 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 7.7 mm) is 1.0 mL/min.
5. Adjust flow rate to keep column pressure below the maximum value of 150 bar.
6. Preferentially store the column in toluene after application with nonpolar eluents and in the eluent after application with polar eluents (e.g., dimethylsulfoxide).
7. Use analytical grade solvents and stabilized tetrahydrofuran for all work.

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

NUCLEOGEL GPC A052181 / 1050.2