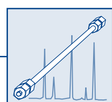

NUCLEOGEL® SUGAR 810 Ca · SUGAR Ca SUGAR Pb · SUGAR Na

Note: All HPLC columns from MACHEREY-NAGEL are supplied with a certificate, which contains specifications and test results of the column.
NUCLEOGEL® SUGAR columns are quality products based on a polymer that requires special care. Introduction of organic solvents into the column except as described below will cause the polymer to swell resulting in column overpressure. Consequently, prior to column installation, you should familiarize yourself with the contents of this manual. The columns are specifically developed for chromatographic high performance analysis. If carefully and properly used excellent chromatographic results and long column lifetime can be achieved. Depending on modification these products can be used for separation and for a quantitative determination of mono-, di-, and oligosaccharides as well as sugar alcohols from food, biological samples and starch hydrolyzates. All HPLC separation columns must exclusively be used in accordance with universally accepted laboratory regulations and working methods of high performance liquid 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 eluents used for column regeneration (e.g., acetonitrile) 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 NUCLEOGEL® SUGAR columns contains a sulfonated, spherical polystyrene/divinylbenzene polymer matrix (PS/DVB) in different forms (Ca^{2+}, Pb^{2+}, Na^{+}). Depending on the ionic form the columns show different selectivities. Thus, they can be specifically used for sugar analysis. NUCLEOGEL® SUGAR 810 Ca is particularly suitable for separation of mono-, di- and oligosaccharides as well as for sugar alcohols and alcohols, NUCLEOGEL® SUGAR Ca for mono- and oligosaccharides as well as sugar alcohols, NUCLEOGEL® SUGAR Pb for mono- and disaccharides from food and biological samples and NUCLEOGEL® SUGAR Na for oligosaccharides from starch hydrolyzates and food. The SUGAR 810 Ca phase differs from SUGAR Ca by a different selectivity. Thus, the range of application is considerably enlarged. Separation mechanisms include hydrophobic interaction and steric exclusion from the polystyrene matrix, ligand exchange and partition effects, the ligand exchange being mostly the predominant force, since the hydrated metal ions form strong interactions with the hydroxyl groups of the sample molecules. Size exclusion phenomens are more important for higher oligomeres.

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. They should be as short as possible to avoid dead volume.

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

The usually aqueous sample 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. Avoid introduction of fats, oils, proteinaceous materials and particulates into the column. These substances will ultimately cause an increase in operating pressure and may be difficult or impossible to remove. Furthermore, certain samples found in the food industry may contain organic matter that is soluble in the food sample, but not in the eluent. Build-up of these compounds can lead to a plugging and a column overpressure. Only in a few cases a regeneration (see column regeneration) can recreate the primary column efficiency. Impurities like salts and proteins change the retention behavior of the column. Hence, they must be removed from the samples before injection by an adequate sample preparation (e.g., protein precipitation). Numerous methods of sample purification can be found in the literature. Also a sample preparation by solid phase extraction with the SPE columns CHROMABOND® for example is usable for a removal of interfering compounds and matrix components.
The maximum injection volume, which can be applied on the column, must be empirically determined for a particular sample. Generally smaller sample volumes cause higher separation efficiencies. If the injection volume is too large, peaks can broaden or merge with nearby peaks. Thus, we recommend sample volumes in the 10 to 50 µL range.

Eluent

Bacteria-free, demineralized water is used as eluent, filtered with a 0.2–0.45 µm membrane and degassed prior to use. In order to avoid a loss of metal ions by ion exchange, you may add up to 10 mmol/L of the relevant metal salt (e.g., for SUGAR 810 Ca / SUGAR Ca: calcium chloride, SUGAR Pb: lead nitrate, SUGAR Na: sodium chloride). Other salts as well as acids, bases and organic modifiers, especially methanol and tetrahydrofuran are to be avoided. The eluents should always be prepared with demineralized water and analytical grade reagents, free of external metal ions.

Flow rate and pressure

It is good practice to limit flow rates such that the pressure does not exceed 70 bar. Typical flow rates are between 0.1–0.7 mL/min. Changes of the flow rate should be performed in small steps. 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

Optimum temperatures for successful separations of sugars should be determined empirically. The temperature range is mostly between 50 and 90 °C. Thus, a column heating device is necessary. In general, higher column temperatures result in reduced sample retention, higher separation efficiency, and lower column pressure. Temperatures below 50 °C can be useful for some applications. In this case the flow rate should be adjusted such that the pressure does not exceed 70 bar.

Detection

Refractometric detectors are preferentially used in sugar analysis. But spectrophotometers, mass spectrometers and electrochemical detectors can be also 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 column is supplied with demineralized water. This is also the recommended eluent for storage. When the column is stored, 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. If the column should be dried up, it results an increased back pressure under the general working conditions. Under these circumstances rinse the column with demineralised water at 90 °C with a flow rate of 0.3 mL/min. Gradually increase flow rate to 0.5 mL/min and observe that the maximum pressure of 70 bar will be not exceeded.

Application notes

Diverse application notes can be found under ***www.mn-net.com/apps***. Select here your NUCLEOGEL® SUGAR phase with "Search criteria: Phase" in the pull-down menu of "Search word".

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. Polymer columns 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 or usage of wrong, improper solvents. The usage of a guard column, as well as an appropriate sample pretreatment and application of the column 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
· presence of external cations in the eluent (e.g., K^+ or H^+)	use eluent free of external cations
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 / rinse LC system, clean the sorbent (see column regeneration)
· accumulation of proteinaceous material from microbial growth in sample or in eluent	keep sample cool, prepare fresh eluent / clean the sorbent (see column regeneration)
Insufficient separation; degradation of the separation with regular column pressure contamination with:	
· metal ion from LC system or sample	PEEK capillaries, remove metal ion from sample / regenerate column (see column regeneration)
· fats, oils, lipids from sample (coating of polymer 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
· compression of column bed by too high flow rates and by usage of an improper organic modifier	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 decompression of the polymer bed. 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: Set column temperature at 65 °C and pump a solution of water with max. 30 % acetonitrile with 0.1 mL/min through (inverted) column overnight. The pressure must not exceed 70 bar. Under circumstances a dark-colored eluate coming from the column can be observed at the column end. The next day, replace the mixture acetonitrile – water by water and continue pumping with 0.1 mL/min to determine if pressure has subsided. If pressure is lower, return column temperature to 90 °C and gradually increase eluent flow rate to 0.4 mL/min. Test the column under normal operating conditions. If the pressure has returned to normal status, but the performance remains inadequate, attempt to regenerate the column.
3. Column regeneration: Prepare the following solutions in reference to the column type:
   - SUGAR 810 Ca: saturated calcium chloride solution
   - SUGAR Ca: saturated calcium chloride solution
   - SUGAR Pb: 1 % aqueous solution of lead nitrate with 0.25 % EDTA
   - SUGAR Na: saturated sodium chloride solution

   Pump these through the (inverted) column at 90 °C with 0.1 mL/min. The pressure must not exceed 70 bar. Subsequently rinse with 10 column volumes water. Then operate the column in normal fashion. An initial minimal baseline drift should be rapidly stabilized by the equilibration step. If excessive drift is observed, operate column at 85 °C with deionized and degassed water until the baseline stabilizes.
4. Decompression of polymer bed: The polymer consists of compressible spherical particles. The particles are deformed by a back pressure above 70 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 at 90 °C overnight. Then return the column to normal operating conditions.
5. Column replacement: The above procedures will restore performance only in certain cases. Under circumstances, column replacement is necessary. It is highly advisable to locate the cause of the problem before installing a new column.

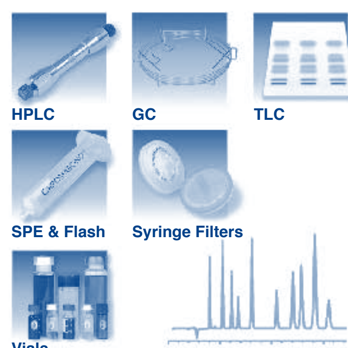
1 column volume (300 mm length x 7.8 mm ID column) ≙ 14 mL

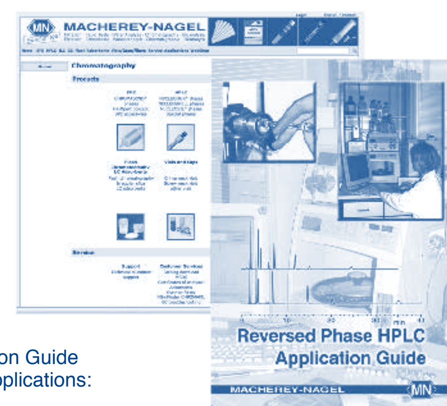
Abstract

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

1. The recommended eluent is demineralized water. 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 is 0.1–0.7 mL/min.
5. Adjust flow rate to keep column pressure below the maximum value of 70 bar.
6. When the column is not to be used for extended periods, store equilibrated in demineralized water.
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 MACHEREY-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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