NUCLEOGEL® RP columns

Note: NUCLEOGEL® RP columns are packed with a polymeric material that requires special care. Application of organic solvents onto the column except as described below will cause the polymer to swell resulting in overpressure. Consequently, prior to column installation, you should familiarize yourself with the contents of this manual. Improper use will invalidate the warranty. If you have any questions after reading this manual, please call our service / technical support.

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Description of the column

NUCLEOGEL® RP columns contain a strongly crosslinked polystyrene-divinylbenzene copolymer. Different pore sizes allow the use of these columns for the separation of organic molecules and biological macromolecules.

Precolumn filter and guard columns

A precolumn filter containing 0.5–2.0 µm porosity stainless steel frits is recommendable between sample injector and column to remove particulates from the eluent stream. For protection and an extension of column lifetime NUCLEOGEL® RP columns should always be used with guard columns. The filter elements and the adsorbent in the guard column retain contaminants from the sample or the eluent.The corresponding guard column (REF 719542) is packed with the same polymer. Connection of the guard column with the separation column is made with guard column holder B (REF 719539). 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. When using isocratic systems the sample should be dissolved in the eluent. For gradient systems the sample should be dissolved in the buffer component with the lowest eluent strength, to receive maximum solute interaction.

Eluent

Due to the chemical and physical stability of this column packings, buffered mobile phases within the pH range 1 to 13 and of salt concentrations < 0.5 M can be used without detrimental effect to the column. Mobile phases with organic modifier contents between 1 and 100 % can be used. The common reversed-phase organic modifiers, e.g., acetonitrile and tetrahydrofuran are all suitable for use.
If an eluent of methanol – water is necessary, then 10 % THF or acetonitrile may be added to improve peak symmetry and efficiency.

Flow rate and pressure

The maximum operating pressure for the NUCLEOGEL® RP columns is 180 bar. Low viscosity mobile phases will allow flow rates up to 4.0 mL/min for a 4.6 mm ID column. However, optimum resolution and lifetime is normally achieved with flow rates between 1.0 and 2.0 mL/min. An increase in the operating temperature to a maximum of 80 °C is permissible to reduce the eluent viscosity or to control the solute retention. 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 trouble shooting).

Temperature

Optimum temperatures for successful separations should be determined empirically, but usually are between 20 and 80 °C. Please take care, that the pressure does not exceed 180 bar.

Detection

Spectrophotometers, refractometers and electrochemical detectors can be used with the NUCLEOGEL® RP 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.

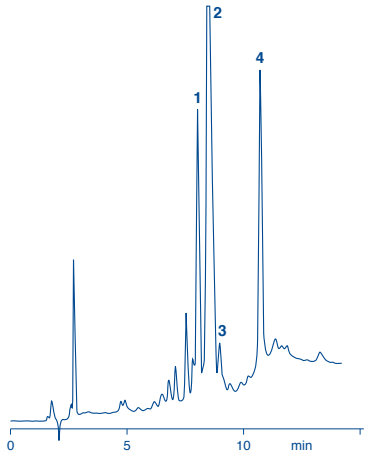
Column storage

NUCLEOGEL® RP columns are supplied with acetonitrile – water (9:1, v/v). This is also the recommended eluent for storage. For long-term storage mobile phases containing inorganic salts are not recommended. 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. If the column is dried up, this results in an increased back pressure under general working conditions. Under these circumstances rinse the column with acetonitrile – water (9:1, v/v) at 80 °C and a flow rate of 0.3 mL/min. Gradually increase the flow rate to 0.5 mL/min and make sure that the maximum pressure of 180 bar will not be exceeded.

Application note

Analysis of the synthetic acyl carrier protein ACP(65–74)

Column: VA 150/4.6 NUCLEOGEL® RP 100-8 REF 719456
Eluent A: 0.1 % TFA in acetonitrile – water (1:99, v/v)
Eluent B: 0.1 % TFA in acetonitrile – water (99:1, v/v)
Flow rate: 1 mL/min
Detection: UV, 220 nm
Peaks:
1. ACP(66–74)(H-Gln)
2. ACP(65–74)
3. ACP(66–74)(Glp)
4. Thioanisole



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Trouble shooting
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 polymer bed. The usage of a guard column, as well as the appropriate sample pretreatment will help to minimize these risks. All NUCLEOGEL® columns are thoroughly tested prior to shipment and are supplied with a sample chromatogram illustrating performance of that particular column.
Use the outline below to help determine the cause of a possible performance loss:

Symptom / Error / Cause	Prevention / Repair
Baseline drift · insufficient period for equillibration of the eluent · contaminated eluent	 longer or better equilibration usage of freshly prepared solvents and buffers
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
Broad peaks; too fast elution too fast elution and/or insufficient separation by: · improper column temperature or flow rate	 optimize concerned parameter
Increasing back pressure; degradation of the separation performance contamination of polymer by: · particulate accumulation on frit or polymer bed from sample, eluent or system	 prefiltration of samples and eluent, usage of in-line filter / rinse LC system, clean the polymer (see column regeneration) keep sample cool, prepare fresh eluent / clean the polymer (see column regeneration)
Insufficient separation; degradation of the separation with regular column pressure contamination with: · 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 polymer (see column regeneration)
Double peaks compression of column bed (dead volume): · by too high flow rate of eluent · by usage of not recommended 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 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. Decompression of polymer bed: The polymer consist of compressible spherical particles. The particles are deformed by a back pressure above 180 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 80 °C overnight. Return the column to normal operating conditions.
3. Cleaning of polymer: To remove hydrophobic contamination a high strength eluent is pumped at 80 °C with 0.1 mL/min through (inverted) column overnight (e.g., 100 % of the organic modifier which is used in the mobile phase). Next day, replace the usual working conditions. Where the contamination may be due to ionizable species an acid or alkali wash may be advantageous. Peptide and protein contamination may be removed by using 0.1 % TFA in an acetonitrile gradient. Between each wash cycle a high organic wash is recommended.
4. Column replacement: Above procedures will restore performance only in certain cases. Some organic contaminants are particulary 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. Recommended eluents are mixtures of deionized water or aqueous buffers (up to 0.5 M, pH 1–13) with organic modifiers (acetonitrile, methanol, THF etc.). They should be filtered through a 0.2–0.45 µm CHROMAFIL® syringe filter and degassed.
2. Filter samples through a 0.2–0.45 µm syringe filter before injection.
3. The recommended flow rate is 0.2–0.6 mL/min.
4. Use an in-line filter and a guard column.
5. Adjust flow rate to keep column pressure below 180 bar.
6. Store the column in acetonitrile – water (9:1, v/v) after removal of buffer salts.
7. Use analytical grade reagents and HPLC grade solvents for all work. Discard any solutions that show evidence of bacterial growth.

Application notes for this and many further products under *www.mn-net.com/apps*