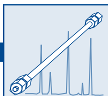
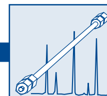




NUCLEODEX β–OH · NUCLEODEX α–PM
NUCLEODEX β–PM · NUCLEODEX γ–PM

Note: NUCLEODEX columns for enantiomer separation are packed with the established silica NUCLEOSIL®. To this, cyclodextrin derivatives are covalently bonded. Such bonded phases are hydrolytically stable. Nevertheless, 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.

Table of contents

Description of the columns
Installation
Precolumn filter and guard columns
Sample
Eluent
Flow rate and pressure
Temperature
Detection
Equilibration
Column storage
Application note
Troubleshooting
Column regeneration
Abstract

Description of the column

NUCLEODEX columns contain bonded cyclodextrins with free (NUCLEODEX β-OH) or permethylated (NUCLEODEX α-PM, β-PM, γ-PM) hydroxy groups. Cyclodextrins are cyclic oligosaccharides, consisting of several glucose molecules. The cyclic structure of the cyclodextrin ring can be described as a truncated cone. α-, β- and γ-cyclodextrins contain an increasing number of glucose units (6, 7 and 8) and, therefore, the diameters of the truncated cones are different. Both open ends of the truncated cone are hydrophilic, due to the primary and secondary hydroxy groups, in contrast to the lipophilic inner surface of the cone. Therefore, non-polar analyte groups of a suitable size (e.g., phenyl or naphthyl substitutents) can penetrate the cyclodextrin ring and form inclusion complexes. Furthermore, analytes can form hydrogen bonds and dipole interactions with the hydroxy groups at the edge of the truncated cone. The chiral glucose units of cyclodextrins allow enantioselective interactions and thus also racemate separations of numerous compounds.

Installation

Columns should be installed in the flow direction indicated on the column label. They are connected with 1/16” capillaries and fittings, typical for HPLC instruments.

Precolumn filter and guard columns

A precolumn filter containing 0.5–2.0 µm porosity stainless steel frits is recommended between sample injector and column to remove particulates from the eluent stream. For protection and an extension of column lifetime the 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 is packed with the same sorbent. Connection of the guard column with the separation column is made using a suitable guard column holder (see ***www.mn-net.com*** or MN chromatography catalog). Cartridge replacement is required when increased column pressure and / or loss of performance is observed.

Sample

Samples, generally dissolved in the starting eluent 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 not exceed 50 µL to achieve an optimal resolution.

Eluent

Columns are supplied with methanol – 0.1 % triethylammonium acetate in water, pH 4. They are normally operated under RP conditions. Both methanol and acetonitrile, in combination with water or buffer solutions are suitable mobile phases. The choice of organic modifiers will influence the selectivity. We recommend phosphate or triethylammonium acetate buffer (TEAA; triethylamine in water, pH value adjusted with acetic acid). When buffering the mobile phase, first establish the solubility of the salt in the mobile phase. The pH value should be between pH 3 and 8. Eluents should be filtered through a 0.2–0.45 µm membrane and degassed.

Flow rate and pressure

The flow rate (recommended: 0.5–1.0 mL/min) obviously defines the total time required for the separation, the resolution, and the shelf life of the column. The maximum permissable flow rate is dictated by the back pressure, which must not exceed 400 bar. In the case of a methanol – water eluent the viscosity and, therefore, the back pressure, depend on the composition of the eluent. With about 40 % methanol maximum viscosity is reached. Thus, changes in the composition of the mobile phases should be carried out at a lower flow rate such as 0.7 mL/min. If a high pressure results from the use of the column at regular flow rates, this usually indicates contamination of the packing material, which must be removed (see troubleshooting).

Temperature

Optimum temperatures for successful separations should be determined empirically, but usually are between 0 and 50 °C. However, they should be at least 30 °C below the boiling temperature of the eluent, in order to ensure proper detection. As the temperature increases, retention times decrease. Generally speaking lower temperatures increase the selectivity.

Detection

Spectrophotometers, refractometers and electrochemical detectors can be used with the NUCLEODEX 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.

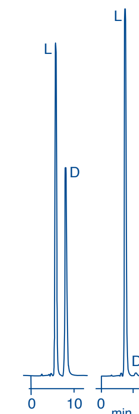
Column storage

The original eluent (s. eluent) is recommended for storage. For long-term storage mobile phases containing inorganic salts are not recommended. 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. In this case, first rinse the column with original eluent at a flow rate of max. 0.2 mL/min.

Application note

Enantiomer separation of dansyl-D,L-leucine

Sample:	left: racemate right: 1 % D- besides 99 % L-isomer
Column:	EC 200/4 NUCLEODEX β-OH REF 720124.40
Eluent:	methanol – 1 % TEAA, pH 4.0 (65:35, v/v)
Flow rate:	0.7 mL/min
Detection:	UV, 254 nm



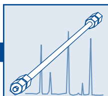
MN Appl. No. 115120

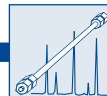
Germany and international:
MACHEREY-NAGEL GmbH & Co. KG
Neumann-Neander-Str. 6–8 · 52355 Düren · Germany
Tel.: +49 24 21 969-0 · Fax: +49 24 21 969-199
info@mn-net.com · ***www.mn-net.com***

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





NUCLEODEX β-OH · NUCLEODEX α-PM
NUCLEODEX β-PM · NUCLEODEX γ-PM

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. Use of a guard column, as well as an appropriate sample pretreatment will help to minimize these risks. All NUCLEODEX 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	longer or better equilibration
· contaminated eluent	usage of freshly prepared solvents and buffers
· 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
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 an in-line filter / rinse LC system, clean the sorbent (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)	use “PEEK Fingertight Fittings”, REF 718770 / replace fittings
· compression of column bed by too high flow rate of eluent	consider maximum flow rate and allowed eluent / replace column
· 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. 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 µm membrane and degassed prior to use.
2. Cleaning of sorbent: To remove contamination at first 50 mL of a mixture of methanol – water (60:40, v/v) is pumped contrary to the conventional flow direction through the column. Back pressure should not exceed 150 bar. Then a rinsing with 100 % methanol or a mixture of methanol – tetrahydrofuran (50:50, v/v) should follow. Subsequently the column is rinsed with 20 mL of a mixture of methanol – water (60:40, v/v) in the regular flow direction. Finally, the column is flushed with the applied mobile phase.
3. Regeneration: After the usage of buffer, directly after finishing a measurement and always before storage of the column rinse with a minimum of 50 mL acetonitrile – water or methanol – water (10:90, v/v) at the original flow rate and temperature. Then increase the organic part in steps of 20 % and adjust to the conditions of a new measurement run or the storage conditions.
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. 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 (phosphate or triethylammonium acetate buffer, pH 3–8) with organic modifiers (methanol, acetonitrile). They 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 an in-line filter and / or a guard column for protection against impurities.
4. The recommended flow rate is 0.5–1.0 mL/min.
5. Adjust flow rate to keep column pressure below 400 bar.
6. Store the column in methanol – 0.1 % triethylammonium acetate in water, pH 4 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.

We wish you success for your work with our NUCLEODEX columns. If you have any further questions please contact our service / technical support.

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

NUCLEODEX 04.11