

NUCLEOSIL® CHIRAL-1

www.mn-net.com

Note: The column NUCLEOSIL® CHIRAL-1 for enantiomer separation is packed with the established silica NUCLEOSIL® with covalently bonded L-hydroxyproline as chiral selector. Sorbents based on silica are quite 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.

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Description of the column

The NUCLEOSIL® CHIRAL-1 column allows the separation of enantiomers and the control of the optical purity of a substance by means of ligand exchange chromatography. It is based on the formation of mixed complexes between the chiral selector, the optical antipodes of the analyte and a transition metal ion. For the stationary phase NUCLEOSIL® CHIRAL-1, enantiomerically pure L-hydroxyproline is used as covalently bonded ligand. The metal ion copper(II) from the eluent forms the central atom in the complex. Due to the chiral centers of the hydroxyproline, bonding of the optical antipodes of the analytes results in the formation of diastereomeric complexes. The stability differences of these complexes, which are mainly due to sterical effects, cause the chromatographic separation of the enantiomers. Prerequisite for successful separation is the formation of a cyclic complex between metal ion and analyte. Rings with five atoms, like those formed with α -amino acids, *N*-alkylated α -amino acids, and α -hydroxycarboxylic acids, are especially favorable for enantiomer separation.

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.

Precolumn filter and guard columns

A precolumn filter containing 0.5–2.0 μ m 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 NUCLEOSIL® CHIRAL-1 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). Replacement of the guard column 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 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 not exceed 50 μ L to achieve an optimal resolution.

Eluent

Columns are supplied with a solution of 0.5 mM aqueous copper sulfate. As eluents, solutions of aqueous copper sulfate with concentrations between 0.2 and 10 mmol/L should be used. This will prevent loss of copper ions from the stationary phase. Additionally the copper concentration influences the retention times of the analytes due to complexation in the mobile phase. Addition of organic modifiers (e.g., acetonitrile) influences retention times and peak shapes. The amount of organic modifier is limited by the solubility of the used copper salt. The separation is also influenced by the pH value of the eluent (pH stability 2–8). Eluents should be filtered through a 0.2–0.45 μ m membrane and degassed.

Flow rate and pressure

Flow rate (recommended: 0.5–1.0 mL/min) influences the time needed, the resolution and the column lifetime. It is limited by the back pressure, which should not exceed 400 bar. 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

For operation of the columns temperatures up to 60 °C are possible. 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 NUCLEOSIL® CHIRAL-1 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 (see eluent) is recommended for storage. 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.

Application note

Separation of D,L-alanine enantiomers

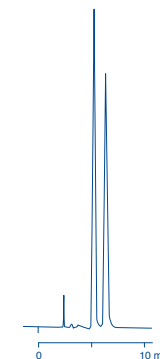
Column: EC 250/4 NUCLEOSIL® CHIRAL-1
REF 720081.40

Eluent: 0.5 mM CuSO₄

Flow rate: 1 mL/min

Temperature: 60 °C

Detection: UV, 250 nm



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NUCLEOSIL[®] CHIRAL-1

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 NUCLEOSIL[®] CHIRAL-1 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 <ul style="list-style-type: none"> insufficient period for equilibration of the eluent contaminated eluent temperature 	longer or better equilibration usage of freshly prepared solvents and buffers column temperature control
Broad peaks <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> improper column temperature or flow rate 	optimize concerned parameter
Increasing back pressure; degradation of the separation performance contamination of sorbent by: <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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) <ul style="list-style-type: none"> faulty fittings (capillaries, ferrules, nuts) compression of column bed by too high flow rate of eluent dissolution of silica by too high pH value of eluent 	use "PEEK Fingertight Fittings", REF 718770 / replace fittings consider maximum flow rate and allowed eluent / replace column 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. It is important, however, to locate the source of contamination before using the column for the analysis of samples again.

- 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.
- Cleaning of sorbent:** To remove contamination ~ 200 mL water are pumped at ambient temperature with a flow of 0.3 mL/min through the inverted column. Then a rinsing with 100 % methanol or a mixture of methanol – tetrahydrofuran (50:50, v/v) should follow. After returning the column to the original flow direction, rinse with methanol – water (60:40, v/v). Then return to the original eluent.
- Regeneration:** For a regeneration of the stationary phase after degradation of the separation or after drying-out, the column can be flushed with ~ 100 mL 0.5 M copper sulfate solution at a flow of 0.3 mL/min.
- Column replacement:** 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.

Abstract

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

- As eluents aqueous solutions of copper sulfate with concentrations between 0.2 and 10 mmol/L are used. Acetonitrile can be added as organic modifier. The eluents should be filtered through a 0.2–0.45 µm membrane and degassed.
- Filter samples through a 0.2–0.45 µm CHROMAFIL[®] Xtra PET syringe filter before injection.
- Use an in-line filter and/or a guard column for protection against impurities.
- The recommended flow rate is 0.5–1.0 mL/min.
- Adjust flow rate to keep column pressure below 400 bar.
- Store the column in 0.5 M solution of copper sulfate
- 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 NUCLEOSIL[®] CHIRAL-1 column. If you have any further questions please contact our service / technical support.