NUCLEOSIL® CHIRAL-2 / CHIRAL-3

Note: NUCLEOSIL® CHIRAL-2 and NUCLEOSIL® CHIRAL-3 columns for enantiomer separation are packed with the established silica NUCLEOSIL® with covalently bonded D- or L-conformation of *N*-(3,5-dinitrobenzoyl)-phenylglycine 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.

Table of contents

Description of the columns

The columns NUCLEOSIL® CHIRAL-2 and CHIRAL-3 have been developed for control of optical purity of compounds under normal phase conditions. They are recommended for the analysis of stereoisomers (enantiomers and diastereomers), control of optical purity (e.g, plant protectives, pharmaceuticals) and for product control in chiral organic synthesis. As chiral selector *N*-(3,5-dinitrobenzoyl)-phenylglycine is covalently bonded to NUCLEOSIL® silica. Both adsorbents differ only in the configuration of the chiral selector. CHIRAL-2 and CHIRAL-3 thus feature, for any pair of enantiomers, equal selectivity and chromatographic resolution, but a reversed elution sequence of the optical antipodes. For control of optical purity the user now has the option to select conditions such that the minor component, which is present as an impurity, is eluted before the main peak. Thus, overlapping peaks are avoided.

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 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). 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 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 achive an optimal resolution.

Eluent

Columns are used in Normal Phase mode. Therefore they are supplied with an eluent of *n*-heptane – 2-propanol – trifluoroacetic acid (100:0.05:0.05, v/v/v). For separation of enantiomers nonpolar organic eluents (*n*-heptane, *i*-octane) are recommended. Retention time can be influenced by adding polar organic compounds (e.g., tetrahydrofuran, alcohols, chlorinated hydrocarbons). Small amounts of a strong acid like trifluoroacetic acid in the eluent (up to 0.1 vol%) can considerably improve separation of enantiomers. Pay attention to the pH stability of 2–8 and avoid any traces of water. Eluents should be filtered through a 0.2–0.45 µm 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-2 and CHIRAL-3 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.

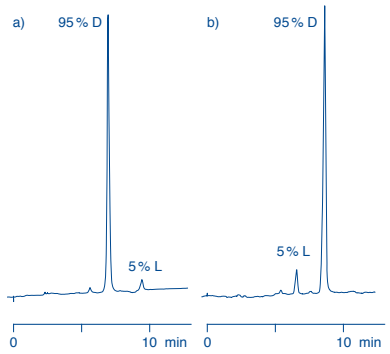
Column storage

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

Control of optical purity of mecoprop methyl

Sample:	mecoprop methyl 90 % ee
Columns:	a) EC 250/4 NUCLEOSIL® CHIRAL-2 REF 720088.40
	b) EC 250/4 NUCLEOSIL® CHIRAL-3 REF 720350.40
Eluent:	*n*-heptane – 2-propanol – TFA (100:0.05:0.05, v/v/v)
Flow rate:	1 mL/min
Temperature:	ambient
Detection:	UV, 230 nm
Injection:	1 µL



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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-2 and NUCLEOSIL® CHIRAL-3 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	prefilter of 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 50 mL 2-propanol are pumped with a flow of 0.1 mL/min through the inverted column. Then return to the original eluent in the original flow direction.
3. Regeneration: By rinsing with *n*-heptane – 2-propanol – trifluoroacetic acid (100:0.05:0.05, v/v/v) at a flow rate of 0.2 mL/min the phase can be regenerated.
4. Column replacement: 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 nonpolar organic eluents (*n*-heptane, *i*-octane). Polar solvents (e.g., tetrahydrofuran, alcohols, chlorinated hydrocarbons) and small amounts of acids (e.g, trifluoroacetic acid) can be added. 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 *n*-heptane – 2-propanol – trifluoroacetic acid (100:0.05:0.05, v/v/v).
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 NUCLEOSIL® CHIRAL-2 or CHIRAL-3 column. 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*