Supelco®

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1.25251.0001

Chromolith® Prep Si 100-25 mm

1.25252.0001

Chromolith® Prep RP18e 100-25 mm

Instruction manual
Chromolith® Prep Si and RP18e - Columns

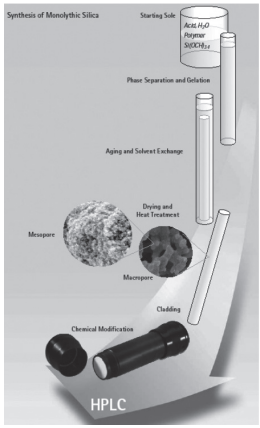
2024/10

MERCK

Content

1 General Instructions for the Care and Use of Chromolith® Prep Columns

1.1 General Information



Contrary to particulate HPLC columns the Chromolith® Prep is a monolithic type of column.

The Chromolith® Prep columns were developed based on a new sol gel process for the preparation of monolithic porous silica rods using highly pure metal free alkoxysilanes.

The Chromolith® Prep column possesses a defined bimodal pore structure typically consisting of macropores and mesopores in the skeleton providing a significantly higher porosity in comparison to particulate columns (Fig. 1).

Consequently, Chromolith® Prep columns can be operated at high er flow rates without loss of performance up to the maximum column backpressure of 100 bar thus significantly increasing productivlty.

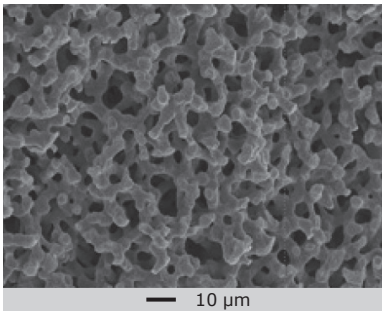
The column backpressure is limited to 100 bar (1450 psi). Every manufacturing step is tightly controlled and each column is individually tested. Certificates of analysis and column efficiency are provided with each column. Test the column immediately to verify performance and quality.

This Chromolith® Prep column has been extensively tested and inspected to ensure the highest quality.

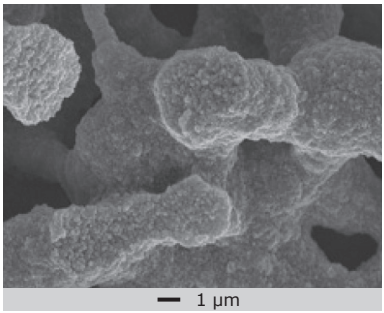
Please check the column you received fot possible physical damage possibly caused during transit. If damage has occurred, immediately notify both your local Merck KGaA, Darmstadt, Germany representative and the delivery carrier.

All Chromolith® Prep columns are shipped in testing solvent.

Fig. 1 SEM of a cross section of a Chromolith® prep



macropores, 2 µm



mesopores 130 Å (13 nm)

The macropores can be described as through-pores which determine the permeability of the column. The mesopores are located on the silica skeleton providing the necessary surface area needed for the chromatographic separation process.

Due to the presence of macropores, the Chromolith® Prep possesses a much higher porosity than conventional particulate HPLC columns.

1.2 Column Information

The label attached to the column indicates article number, column dimensions, flow direction, max backpressure and column serial number. Keep this important information with the column at all times. If a problem occurs, the column serial number allows us to trace the manufacturing history of your column.

1.3 Installation of the Column

Connect your Chromolith® Prep column to the injector corresponding to the direction of the flow label on the column. All connections must be made with compatible fittings of Merck KGaA, Darmstadt, Germany in order to avoid loss of efficiency due to increased dead volume.

Before connecting the column outlet to the detector, flush the column with mobile phase (take care of air bubbles). Reverse the flow periodically to prevent particles and non eluting sample components from accumulating on the column. When reversing the flow, flush the column before connecting it to the detector. Equilibrate the column with the starting solvent of your separation.

1.4 Validating the Performance

Before running the column, validate its performance by measuring the efficiency on your own system using the test conditions and the test sample shown on the certificate. Repeat this procedure periodically to check the column over time. Slight variations may be obtained on different preparative HPLC systems due to system electronics, plumbing, operating environment, reagent quality, and operator technique.

1.5 Equilibrating the Column

The Chromolith® Prep Si columns are shipped in heptane/dioxane (95/5, v/v). Verify thaI your mobile phase is miscible with the shipping solvent before equilibrating your column for use with the new mobile phase. It is possible that the column can dry out during storage and shipping, but this does not harm the column. Simply resolvate and activate the column by equilibrating for 5 minutes with heptane/dioxane (95/5, v/v) at a flow rate of 40 mL/min. Continue conditioning the column with your mobile phase until a stable baseline is achieved.

The Chromolith® Prep RP18e columns are shipped in acetonitrile. Verify that your mobile phase is miscible with the shipping solvent before equilibrating your column for use with the new mobile phase. It is possible that the column can dry out during storage and shipping, but this does not harm the column.

Simply flush the column for 5 minutes with acetonitrile (100%) at a flow rate of 40 mL/min. Continue conditioning the column with your mobile phase until a stable baseline is achieved.

2 Column Care

For optimum column performance and maximum life, the following conditions are recommended:

2.1 Column Hardware

Chromolith® Prep columns are clad with a PEEK-polymer. PEEK polymer is inert and stable to almost all solvents used in preparative chromatography. Nevertheless, a few solvents should not be used in pure form or at higher temperatures. Those solvents are listed in the data sheet (see solvent stability of Chromolith® Prep).

Merck KGaA, Darmstadt, Germany can not be hold responsible for any defects, damage, or nonconformity resulting from abuse, neglect lack of reasonable care, or the attachment of improper devices to the column.

2.2 pH

2.2.1 Chromolith® Prep Si column

In general, Chromolith® Prep Si columns should be used in the pH range of 2.0 to 7.5. Higher pH's will dissolve the silica, creating voids in the column. These defects will cause changes in retention times and loss of resolution.

2.2.2 Chromolith® Prep RP18e column

In general, Chromolith® Prep RP18e columns should be used in the pH range of 2.0 to 7.5. Higher pH's will dissolve the silica, and lower pH's can cleave the bonded phase, but does so very slowly. Experience has shown that mobile phases containing up to 1% of a strong acid, (TFA, up to 0.2%), which are often used for protein and peptide separations, do not affect the column selectivity or efficiency over long periods of use (up to 4–6 months). If using buffers in the near basic range (7.5 to 8.0), great care should be taken as these mobile phases might have an apparent pH that is higher once they are added to a miscible organic solvent. This increase in apparent pH can be as much as 1 pH unit, hence this effect can potentially damage all silica based resins.

2.3 Mobile Phase

2.3.1 Chromolith® Prep Si column

Chromolith® Prep Si column can be used with all common HPLC grade organic solvents except 100% dimethylsulfoxide and dichloromethane. These can swell the PEEK® tubing used to clad the monolith. Chromolith® Prep Si is stable at room temperature (23°C) in 100% tetrahyrofuran. Higher temperature should be avoided (see appendix).

Verify that solvents are miscible when changing mobile phases.

Avoid to pump water through this silica column so that the hydroxyl groups of Chromolith® surface will be deactivated and the column loses separation efficiency.

For the best performance of the Chromolith® Prep Si, we recommend to use mixtures of heptane/ethyl acetate as mobile phase.

2.3.2 Chromolith® Prep RP18e column

Chromolith® Prep RP18e column can be used with all common HPLC grade organic solvents except 100% tetrahydrofuran, dimethylsulfoxide (DMSO) and dichloromethane. It is possible to use these solvents in combination with water to lesson their harmful effect on the PEEK tubing (see appendix).

Verify that solvents are miscible when changing mobile phases to avoid buffer precipitation on the top of the Chromolith® Prep RP18e.

For the best performance of Chromolith® Prep RP18e, mobile phases of acetonitrile/water in varying ratios are recommended.

2.4 Strong Acids

Do NOT use strong acids (i.e. hydrochloric, nitric, and sulfuric) in the column. If such an acid needs to be used in a mobile phase, the column should be dedicated to that application.

2.5 Column Life

Column life is highly dependent on the sample and conditions, and cannot be generalized. For samples with large quantities of contaminants, we recommend to apply one or more sample preparation methods prior to separation (e.g. solid phase extraction, filtration, centrifugation, etc.). Make sure that your samples and the mobile phases are clean and particulate free by using HPLC grade solvents and reagents.

If buffers or other salts are used, a final filtration of the mobile phase should be done with a membrane filter.

2.6 Pressure

Chromolith® Prep columns are made to operate at pressures up to 100 bar (1450 psi), measured at the column head as a result of the column and the connected detection system.

2.7 Temperature

Chromolith® Prep columns have been used successfully at temperatures up to 45°C. It is not recommended to use the column at temperatures above 45°C.

2.8 Column Storage

2.8.1 Chromolith® Prep Si column

When storing the Chromolith® Prep Si column for several days or longer, store the column in heptane/dioxane (95/5, v/v).

2.8.2 Chromolith® Prep RP18e column

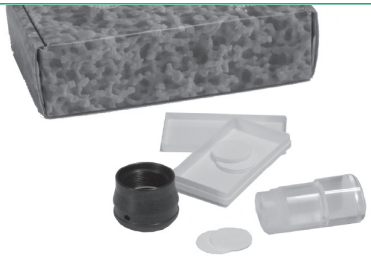
When storing the Chromolith® Prep RP18e column for several days or longer, store the column in 100% acetonitrile. If the last mobile phase contained a buffer salt, flush the column with 3–5 volumes of water before changing over to acetonitrile.

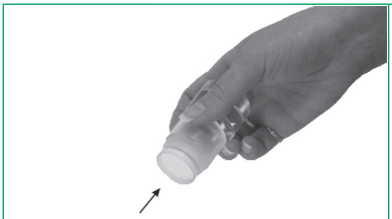
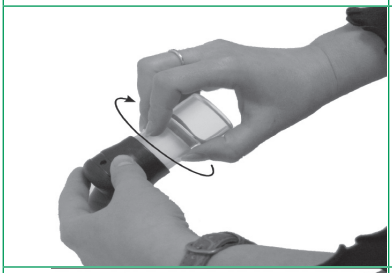
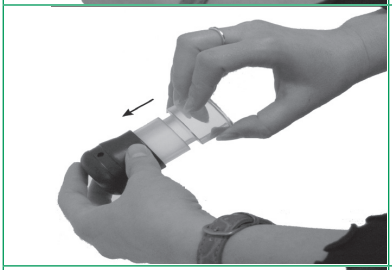
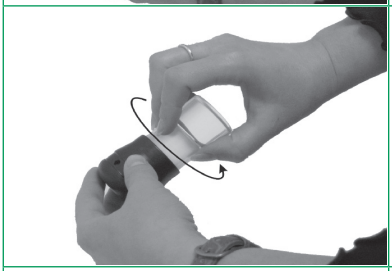
3 Exchanging the Frit

The Chromolith® Prep column has been developed to operate at high flow rates without loss of performance. The column has one frit on the inlet side. The frit increase column lifetime and optimize the distribution of sample and mobile phases on the column. The frit should be replaced with a new one from time to time as necessary. The need for change can be indicated by an increase of column back pressure or a decrease in the separation performance over time.

3.1 Instructions for Changing the frit

For easy change of the frit use the Chromolith® Prep tool set, Ordering No. 1.25255.0001

	1) Remove the end Gap from the Chromolith by using the mounting tool and the hook wrench from the Chromolith® Prep tool set (Ordering No. 1.25255.0001). Remove the frit by levering off with a spatula or tweezers. Take a new frit from the spare part Chromolith® Prep frit set (Ordering No. 1.25257.0001).
	2) Pull back the plunger of the filter mounting tool about 5 cm.

	3) Insert frit, into the hollow space now created.
	4) Screw the filter mounting tool clockwise into the inlet until some resistance is felt.
	5) Press the plunger downwards. The frit will now be properly located in the inlet. Note: It is also possible to place the inlet cap on the table to press the plunger down.
	6) Unscrew the filter mounting tool anticlockwise to remove it trom the inlet cap. Note: Sometimes a small part of the border from the frit are cut of. It is important to remove this with a small spatula.
	7) The frit is now inserted into the inlet cap. The inlet cap can now be mounted onto the inlet of the Chromolith® Prep Si or RP18e.

4 General Information: Performance and Selectivity

4.1 Performance and Selectivity of the Chromolith® Prep Columns

4.1.1 Chromolith® Prep Si column

The selectivity of a Chromolith® Prep Si column is comparable to common straight phase columns. It provides you with an excellent tool to solve your separation problems regarding non-polar basic until middle polar compounds, region isomers, stereo isomers and natural products.

In most cases your existing methods from using particulate columns or Chromolith® performance Si 100-4.6 can easily be transferred to Chromolith® Prep Si. However for same applications it is worth optimizing the method to make use of the full potential of this enhanced technology.

4.1.2 Chromolith® Prep RP18e column

The selectivity of a Chromolith® Prep RP18e column is comparable to common RP18e reversed phase columns. It provides you with an excellent tool to salve your separation problems regarding non-polar basic and acidic compounds as well as peptides.

In most cases your existing methods from using particulate columns can easily be transferred to Chromolith® prep. However for some applications it is worth optimizing the method to make use of the full potential of this enhanced technology.

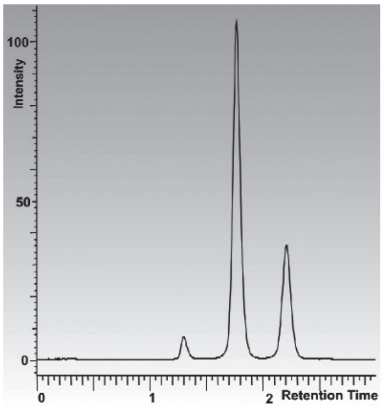
4.1.3 Chromolith® Prep 100-25 opens the door to high speed separation in preparative Chromatography

Monolithic silica rod technology puls an end to the back pressure problem immediately! Compared to modern particulate columns filled with particles of a size of 3.6 µm the Chromolith® Prep RP18e shows a significant reduction of back pressure at flow rates of 40 mL/min and even higher!

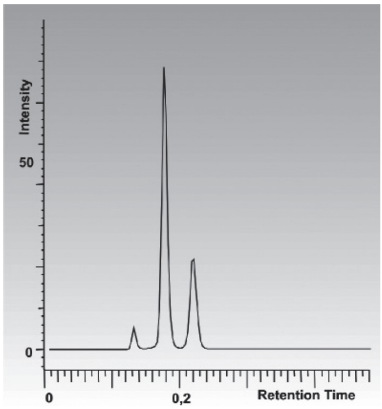
5 Applications: Chromolith® Prep Si 100-25

5.1 Separation of phthalate-mixture

Flow rate 40 mL/min



Flow rate 390 mL/min

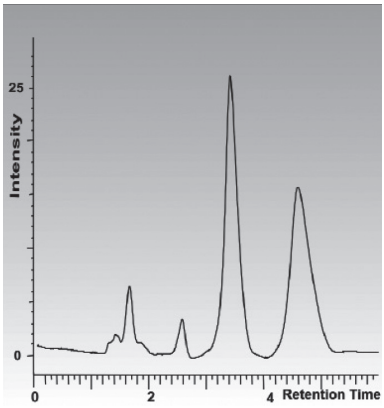


Column: Chromolith® Prep Si 100-25
Mobile phase: n-heptane/dioxane (90/10,v/v)
Sample: toluene, dimethyl-, dibutylphthalate

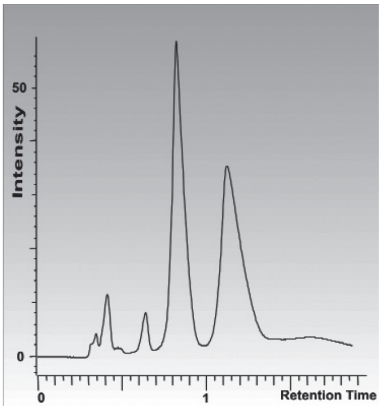
5.2 Separation of γ- and δ-tocopherol from sunflower oil at different flow rates

By using Chromolith® Prep Si the separation could be reduced to only 2 minutes, still resulting in pure target product.

Flow rate 40 mL/min



Flow rate 160 mL/min

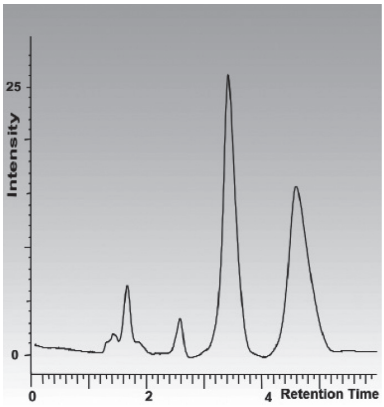


Column:	Chromolith® Prep Si 100-25
Mobile phase:	n-heptane / ethyl acetate (90/10, v/v)
Flow rate:	40 and 160 mL/min
Detection:	UV 280 nm
Sample:	130 mg tocopherol mixture

Monolithic silica rod technology makes it possible to speed-up your separation significantly! Higher flow rates up to 300 mL/min are not a problem at all only limited by the maximum back pressure tolerance of 100 bar.

6 Applications: Chromolith® Prep RP18e 100-25

6.1 Separation of Hirudin (filtrate of crude extract)



Sample: 23 mg Hirudin
(filtrate of crude extract) in
5 mL solution injected

Flow rate: 60 mL/min

UV detection: 254 nm
Chromolith® Prep RP18e 100-25

Eluent A: water + 0.1% formic acid

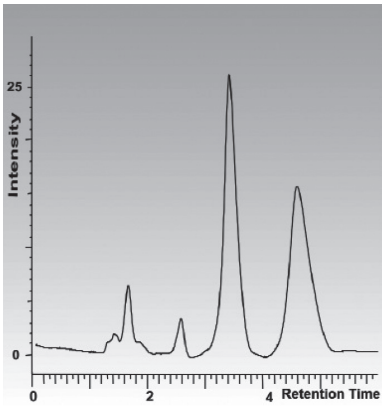
Eluent B: acetonitrile (100%)

Gradient:

Time [min]	% A	% B
0	90	10
8	70	30
10.1	90	10

By using Chromolith® Prep RP18e, the separation could be reduced to only 5 minutes, still resulting in pure target product.

6.2 Separation of Dishydropyridines



Sample: 90 mg mixture

1	Nifedipin
2	Nimodipin
3	Nisoldipinin

(1/1/1,v/v/v) in 450 µL DMSO

Flow rate: 100 mL/min

UV detection: 220 nm
Chromolith® Prep RP18e 100-25

Eluent A: water

Eluent B: acetonitrile (100%)

Gradient:

Time [min]	% A	% B
0	20	80
8	80	20
8.1	20	80

7 Disclaimer

We advise our customers on technological matters to the best of our knowledge under given circumstances. Our information and recommendations are without obligation. Existing laws and regulations are to be observed in all cases. This also applies in respect to any rights of third parties. Our suggestions do not relieve our customers of the responsibility of checking the suitability of our products for the envisaged purpose.

8 Important Safety Information

Retain and follow all product safety and operating instructions. Observe all warnings on the product and in the operating instructions.

9 Ordering Information

9.1 Chromolith® Prep Si 100-25 and RP-18e 100-25

Designation	Ordering No.	Dimension [mm]	Content
Chromolith® Prep Si 100-25	1.25251.0001	100-25	1 piece
Chromolith® Prep RP-18e 100-25	1.25252.0001	100-25	1 piece

9.2 Chromolith® Prep Accessories

Designation	Ordering No.	Dimension [mm]	Content
Chromolith® Prep sealing set	1.25254.0001	25	2 pieces
Chromolith® Prep Prep tool set	1.25255.0001		1 mounting tool filter 1 mounting tool 1 hook wrench
Chromolith® Prep end cap set	1.25256.0001	25	1 inlet cap 1 outlet cap
Chromolith® Prep frit set	1.25257.0001	25	10 pieces

If you need additional info or assistance please do not hesitate to contact us.

Status: 2024-10-23
Made in Germany

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