

Chromolith® HPLC columns (2 mm, 3 mm, 4.6 and 10 mm internal diameter)

General information and guidelines for care and use

All Chromolith® Widepore columns have been extensively tested and inspected to ensure highest quality. Please examine your column for any possible damage caused in transit. If damage has occurred, immediately notify your local Merck KGaA, Darmstadt, Germany or MilliporeSigma representative and the delivery carrier.

Column information

The label attached to the column indicates catalogue number, packing type, column dimensions and column number. Keep this important information with the column. If you have a problem, the column number allows us to trace the manufacturing history of your column.

Column description

Chromolith® columns are made from a single piece of high-purity polymeric silica gel and are not packed with small silica particles. This novel technology achieves a very high separation performance along with a large reduction in operating pressure.

Specifications

Component	Description
Silica type	High purity
Structure	Monolithic
Macropore size	2 µm for 3, 4.6 and 10 mm i.d. columns 1.5 µm for 2 mm i.d. columns) 1.1 µm for HR products
Mesopore size	15 nm (150 Å) for HR products 13 nm (130 Å) all Chromolith® columns except HR
Pore volume	~ 1 mL/g
Total porosity	>80%
Surface area	300 m ² /g all Chromolith® columns except HR 250 m ² /g HR products
pH stability	pH 2.0 - 7.5
Temperature stability	max 50 °C
Pressure stability	max. 200 bar (~3000 psi) for 2, 3 and 4.6 mm i.d. columns max. 150 bar (~2250 psi) for 10 mm i.d. columns

Monolithic silica

Chromolith® HPLC columns are made from highly porous monolithic rods of silica with a revolutionary bimodal pore structure providing a unique combination of macropores and mesopores. The **Macropores** allow a rapid flow of the mobile phase at low pressure.

The **Mesopores** form the fine porous structure and create the large uniform surface area on which adsorption takes place, thereby enabling high performance chromatographic separations.

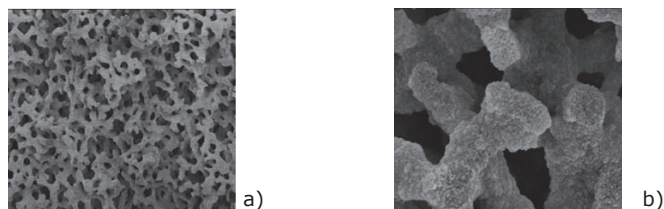


Figure 1
Electron-microscope photographs a) Macropores; b) Mesopores

Connection of Chromolith® columns to HPLC systems

The end-fittings of Chromolith® columns are connected with standard 1/16" fittings to all standard HPLC, U-HPLC and UPLC® systems. Chromolith® columns have a standard Parker connection port. The connection capillary from ferrule till the end must not be longer than 2 mm (0.09 in.).

Short capillary tubing is recommended to minimize extra-column volumes.

We strongly recommend using adjustable plastic ferrules in order to avoid a possible damage to the PEEK end-fitting of the Chromolith® column. The use of stainless steel ferrules is not recommended because they can damage the column end-fitting. Before connecting the column outlet to the detector, flush the column with mobile phase to remove any air.

Equilibrating the column

Chromolith® RP-18 and RP-8 endcapped columns are shipped in acetonitrile/water (60/40, v/v). As the column can dry out during stocking and shipping, equilibrate the column before use for 5 minutes with 100 % acetonitrile or methanol at optimum flow rate (see Figure 3). Then continue conditioning the column with your mobile phase until you get a stable baseline. Check beforehand that your mobile phase is miscible.

Chromolith® NH₂ columns are shipped in acetonitrile/water (90/10, v/v). We recommend to start the equilibration of the column in this solvent, followed by your mobile phase.

Chromolith® Si columns are shipped in heptane/dioxane (95/5 v/v). We recommend equilibrating the column with dioxane, followed by your mobile phase.

Fast chromatograms require fast instrument settings:

1) Detector response time

Most HPLC detectors have a variable response time or time constant. If the response time is too slow, peaks may appear broad and show tailing. Chromolith® 2 mm columns typically produce fast narrow peaks, particularly when run at flow rates higher than 0.3 mL/min.

Important Tip – fast peaks on Chromolith® 2 mm columns require a fast detector time constant, such as 0.05 seconds.

Please note – by reducing the time constant from 2 to 0.1 sec the plate count for Chromolith® columns may improve greatly.

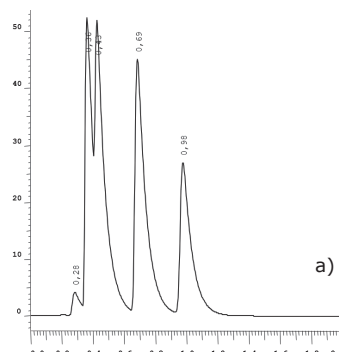


Figure 2

a) Detector response time
2.0 seconds

a) Detector response time
0.1 seconds

Column: Chromolith® Performance
RP-18e 100-4.6 mm
Mobile phase: acetonitrile/water
40/60 v/v

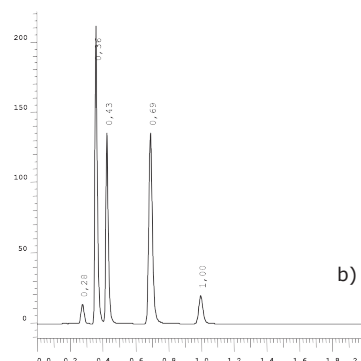
Flow rate: 5.0 mL/min

Detection: UV 254 nm, standard
cell,

Injection: 10.0 µL

Peak identification:

1. Uracil
2. Pyridine
3. Aniline
4. 4-Ethylaniline
5. Benzene



2) Data system settings

Fast chromatographic peaks can be just a few seconds wide. For good integration of the peak area and good optical presentation of the chromatogram, the data system settings must enable approximately 20 data samples to be acquired during the peak width time. We recommend to check the data acquisition rate of the data system.

Important Tip – fast peaks on Chromolith® 2 mm columns require a fast data acquisition rate, such as 20 Hz (ie. 20 data points per second, which means 1 data measurement in 50 milliseconds).

Column hardware

Chromolith® columns are clad with a mechanically stable and chemically robust polymer (PEEK - Poly Ether Ether Ketone). The end fittings are made of the same material. Do not remove the end fittings from the column.

Mobile Phase – Chromolith® columns can be used with all commonly used HPLC grade organic solvents, with the following restrictions. The mobile phase should NOT contain more than 50 % Tetrahydrofuran (THF), 5 % Chlorinated solvent (eg. Dichloromethane) or 5 % Dimethylsulfoxide (DMSO). However pure DMSO can be used as sol-vent for samples.

Buffers, organic modifiers and ion pair reagents present no problems as long as the appropriate pH range is not exceeded. Ion pair reagents are often difficult to completely flush from the column. Therefore columns used with these reagents should be dedicated to the particular analysis involved.

Do not exceed the **pH range from 2.0 to 7.5** with Chromolith® columns. Higher pH's will dissolve the silica, creating voids in the column. Lower pH's can eventually strip away some of the bonded phase. These defects will cause changes in retention times and loss of resolution.

Verify that solvents are miscible when changing mobile phases and that no buffer precipitation will occur.

Chromolith® Si (silica) columns are generally used with solvents such as n-heptane and dioxane, which are typical solvents for adsorption chromatography.

Optimal flow rates

Optimal Chromolith® HPLC column flow rates depends on the column inner diameter. Flow rate values for different inner diameter columns can be found in figure 3 below:

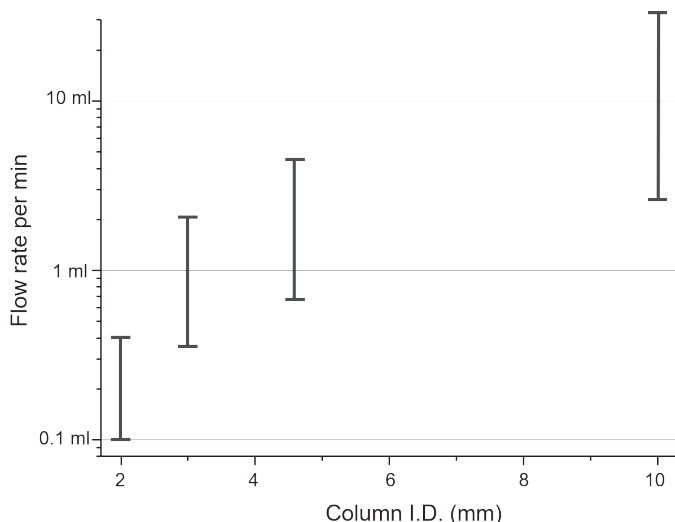


Figure 3
Optimal Flowrates

Maximum operating temperature and pressure

The maximum operating temperature for Chromolith® columns is 50 °C.

Important Tip – for good peak symmetry, the mobile phase must be pre-heated to enter the column already at the column temperature. If cold solvent enters the hot column, peaks may become unsymmetrical.

The maximum operating pressure for Chromolith® columns with 2, 3 and 4.6 mm i.d. is 200 bar (3000 psi). The maximum operating pressure for Chromolith® columns with 10 mm i.d. is 150 bar (2250 psi).

Use with mass spectrometers – procedure to maintain low column bleeding

Chromolith® columns are optimized for LC/MS by a surface modification process minimizing column bleed. **Important Tip** – before connecting a RP-18 or RP-8 column to LC/MS instrument, we strongly recommend pumping iso-propanol plus 0.1% formic acid at middle optimal flow rate (see Figure 3) through the column for one hour. This cleans any trace organic material out of the column. Plain Si columns should be washed with dioxane at middle optimal flow rate (see Figure 3) for one hour.

Column bleed will be low when the maximum solvent strength of the mobile phase used is equivalent to methanol or acetonitrile. If stronger solvents are used in the mobile phase, eg. Tetrahydrofuran or DMSO, then we recommend first to pump approximately 10 mL of this stronger mobile phase plus 0.1% formic acid through the column before connecting to the detector.

Column lifetime

Column lifetime 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.

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.

Cleaning and regeneration procedure for Chromolith® columns

To extend the lifetime of the column, "wash" the column after use and before storage to remove trace of samples and buffers from the column. For cleaning and regeneration of non-polar phases (RP-18, RP-8, Diol, CN, NH₂-if used in RP mode), connect the Chromolith® column.

in the reverse flow direction. The simplest procedure is to pump 100% methanol or acetonitrile for 5 min at middle optimal flow rate (see Figure 3). If buffers have been used, first pump 100 % water and then methanol. If the column is strongly contaminated, then pump the following solvents one after the other through the column for 5 minutes at the upper limit of the corresponding column optimal flow-rate range (see Figure 3): water, acetonitrile, 2-propanol, heptane, 2-propanol, ace-tonitrile, water, mobile phase.

For cleaning and regeneration of polar phases (Si, Diol, CN, NH₂) connect the Chromolith® column in the reverse flow direction, then pump the following solvents one after the other through the column for 5 minutes at the upper limit of the corresponding column optimal flow-rate range (see Figure 3): heptane, chloroform, ethanol or 2-propanol, chloroform, heptane, mobile phase.

Storing the column

When storing the column for several days or longer, store non-polar phase columns in 100 % acetonitrile. If the mobile phase contained a buffer salt, flush the column with pure water for 5 minutes at optimal flow rate (see Figure 3) before changing over to 100 % acetonitrile. Polar phase columns stored in heptane/dioxane (80/20).

Validating the column performance

Check the performance of the column by measuring the efficiency on your own system using test conditions and a test sample similar to that shown on the certificate. Repeat this procedure periodically to check the column over time. (Please note that it is not unusual for the results measured to differ from those on the certificate of analysis; this is caused by differences in injection volume, dead-volume of connectors and capillary tubing, detector cell volume, detector response time, data system settings etc.). Sample volume for column performance test:

2 mm	0.1 µL
3 mm	1.0 µL
4.6 mm	1.0 µL
10 mm	10 µL

Important Tip – for highest column efficiency, we recommend 0.1 µL injection volume, connecting tubing with 0.12 or 0.13 mm internal diameter and a detector with a micro flow cell. Larger sample volumes and detector cells can be used, but peak widths will be wider.

Low column back pressure

Owing to the very high porosity of the Chromolith® column, very high flow rates can be applied with very low pressures. The following diagrams show data for a 4.6 mm internal diameter column.

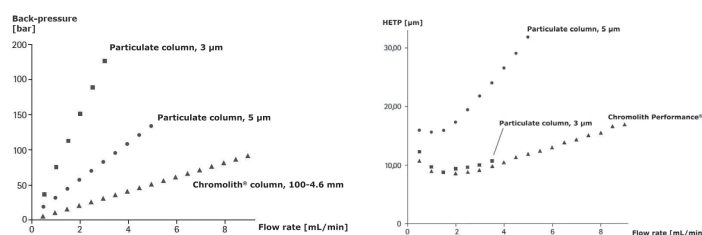


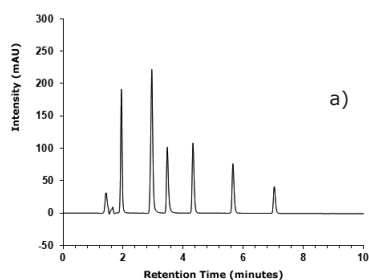
Figure 4
Column back pressure at different flow rates
Comparison of a Chromolith® Performance column vs. equivalent classical particulate HPLC columns.

Figure 5
A van Deemter plot of the height equivalent to a theoretical plate (HETP) vs. flow rate for a Chromolith® Performance column and equivalent classical particulate HPLC columns.

The van Deemter plot of the Chromolith® column demonstrates clearly that separation efficiency does not decrease significantly when the flow rate is increased, as is the case with particulate columns. It is therefore possible to operate monolithic columns at high flow rates with minimal loss of peak resolution (Figure 5).

Comparison of selectivity and column backpressure for monolithic and particulate HPLC columns

Chromolith® columns with C18 surface modification and end-capping are comparable in selectivity to particulate reversed-phase columns. However, the column back-pressure of monolithic columns is significant lower in comparison to fully porous silica particles (FPP) and Superficially porous particles (SPP) with similar chromatographic performance especially in comparison to FPP.



a)

Figure 6

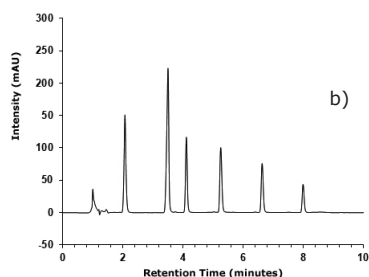
- a) Chromolith® HighResolution RP-18e 100-4.6 mm
 b) Fully porous silica particle (FPP) C18, 3 µm 100-4.6 mm
 c) Superficially porous silica particle (SPP) C18, 2.7 µm 100-4.6mm

Eluent: A: 100% acetonitrile
 B: 20 mM Phosphate buffer pH 4.5

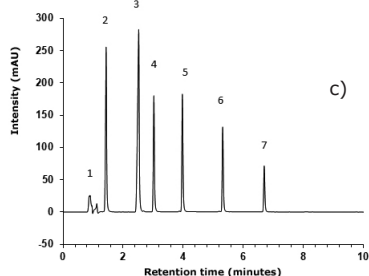
Gradient: Time/min %A %B
 0 20 80
 12.0 80 20

Flow rate: 1.0 mL/min
 Pressure: 36/146/170 bar
 Detection: UV 230 nm
 Detection cell: Standard 11 µL
 Temperature: 22 °C
 Injection volume: 2.0 µL

- Sample: 1. Ascorbic acid
 2. 4-Hydroxybenzoic acid
 3. Benzoic acid
 4. Sorbic acid
 5. Methyl 4-hydroxy benzoic acid
 6. Ethyl 4-hydroxy benzoic acid
 7. Propyl 4-hydroxy benzoic acid



b)



c)

Monolithic columns for faster analysis and higher sample throughput

A mixture of five beta-blocking drugs demonstrates the extreme time savings and high separation efficiency made possible with Chromolith® columns. Due to excellent mass transfer properties of the monolithic skeleton, high-speed separation is possible even at high flow rate. The beta-blockers were well separated with excellent peak symmetry. At 9 ml/min with a 4.6 mm internal diameter column, the analysis time is less than 1 minute and the column back-pressure is only 153 bar.

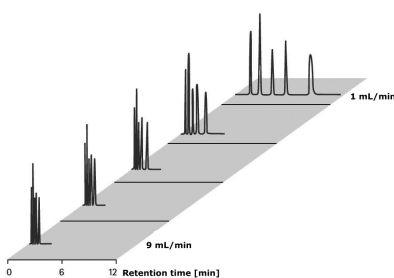
Figure 7

Separation of five beta-blocking drugs on a Chromolith® Performance RP-18 endcapped, 100-4.6 mm at various flow rates

Mobile phase: acetonitrile/0,1% TFA in water (20/80, v/v);
 Detection: UV 254 nm

Peak identification:

1. Atenolol
 2. Pindolol
 3. Metoprolol
 4. Celiprolol
 5. Bisoprolol



Longer columns for highest separation performance

For complex separations it is still necessary to use long columns in order to provide the separation efficiency required for resolution of all compounds of interest. Chromolith® HPLC columns can be connected in series to produce a column with higher plate count at relatively low back-pressure. (We recommend column connector –part number 151467.0001).

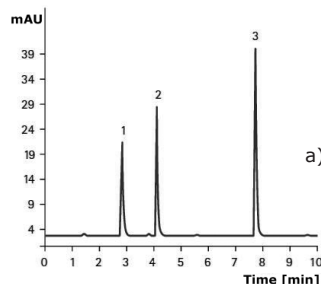
No. of Chromolith® Performance Columns (1021290001)	Length [mm]	Back pressure (bar) at 3 mL/min	Plate Number per column (Anthracene)
Chromolith® Performance 1x	100	30	10.000
Chromolith® Performance 2x	200	60	19.000
Chromolith® Performance 3x	300	90	27.000
Chromolith® Performance 4x	400	120	35.000
Chromolith® Performance 5x	500	150	41.000

Guard columns

It is generally good practice to protect the analytical column with a pre-column (guard column) in order to ensure maximum column life-time. Use of a pre-column might result in a slight shift of the chromatographic parameters. A Chromolith® guard column also could be used as a trapping column.

Scale-up from analytical to semi-prep

The loadability of the Chromolith® SemiPrep 100-10 mm RP-18 endcapped column will vary from sample to sample and also on the solubility in the mobile phase. Figure 8 shows a typical chromatogram where the column is not overloaded.



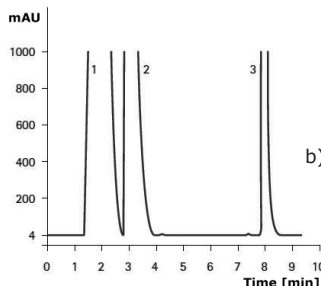
a)

Figure 8

Scale-up from analytical to semi-prep column Column: Chromolith® RP-18e

Gradient:
 a) acetonitrile with 0,1% TFA
 b) water with 0,1% TFA

0 –10 min 15 – 80% A
 Detection: UV 270 nm



b)

Performance 100-4.6 mm

Flow rate: 1 mL/min
 Injection: 2 µL

Sample: 1. Nadolol 1 mg/mL
 2. Metoprolol 1 mg/mL
 3. Propranolol 0,5 mg/mL

SemiPrep 100-10 mm

Flow rate: 4.7 mL/min
 Injection: 100 µL

Sample: 1. Nadolol 100 mg/mL
 2. Metoprolol 100 mg/mL
 3. Propranolol 50 mg/mL

Ordering information for Chromolith® products

Column dimension											
Length (mm)	ID (mm)	RP-18e	HR RP-18e	RP-8e	HR RP-8e	Phenyl	CN	Diol	NH ₂	Si	
Chromolith® HPLC Column [1 unit]											
25	x	4.6	1.51463.0001	1.52020.0001			1.52056.0001	1.52046.0001	1.53170.0001	1.52026.0001	
25	x	3	1.52003.0001								
25	x	2	1.52014.0001	1.52320.0001							
50	x	4.6	1.51450.0001	1.52021.0001			1.52057.0001	1.52047.0001	1.53171.0001	1.52027.0001	
50	x	3	1.52002.0001								
50	x	2	1.52007.0001	1.52321.0001							
100	x	4.6	1.02129.0001	1.52022.0001	1.51468.0001	1.52064.0001	1.52058.0001	1.52048.0001	1.53172.0001	1.52028.0001	1.51465.0001
100	x	3	1.52001.0001								
100	x	2	1.52006.0001	1.52322.0001							
150	x	4.6		1.52023.0001							
100	x	10	1.52016.0001								1.52015.0001
100	x	25	1.25252.0001								1.25251.0001
Validation Kits [3 Chromolith® HPLC cartridges from 3 different sorbent batches]											
50	x	2	1.52062.0001								
100	x	4.6	1.51466.0001	1.52019.0001							
100	x	3	1.52063.0001								
10	x	10	1.52036.0001								1.52035.0001
Chromolith® Guard cartridges [3 units]											
5	x	4.6	1.51451.0001	1.52025.0001	1.52013.0001		1.52059.0001	1.52050.0001	1.53175.0001	1.52030.0001	1.52011.0001
10	x	4.6	1.51452.0001								
5	x	3	1.52005.0001								
5	x	2	1.52009.0001	1.52325.0001							
Chromolith® Guard cartridge Set [1 starter kit with holder and 3 guard cartridges]											
5	x	2	1.52008.0001								
5	x	3	1.52004.0001								
Chromolith® Guard cartridge Holder											
for dimension		Type	Material	Item No.							
5	x	2 and 3	a	PEEK	1.52004.0001						
5	x	2	b	Bioinert	1.52355.0001						
5	x	4.6	b	Bioinert	1.52255.0001						
10	x	4.6	b	Bioinert	1.52256.0001						
5	x	4.6	c	SST	1.52032.0001						
10	x	4.6	c	SST	1.52033.0001						
10	x	10	d	PEEK/SST	1.52037.0001						
Chromolith® Column coupler											
for analytical columns			1.51467.0001								
for 25 mm ID columns			1.25259.0001								

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Made in Germany

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Merck Life Science KGaA, 64271 Darmstadt, Germany,
Tel. +49(0)6151 72-2440

www.sigmaaldrich.com/HPLC

