Chromolith® Widepore 300 HPLC columns

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

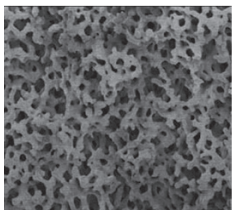
The label attached to the column indicates catalogue number, packing type, column dimensions and column number. Keep this important information with the column. If you have a problem, the column number allows us to trace the manufacturing history of your column.

Monolithic silica

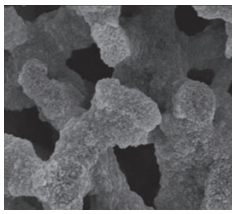
Chromolith® Widepore columns are made from a single piece of high-purity polymeric silica gel and are not packed with small silica particles. This new technology achieves a very high separation performance along with a large reduction in operating pressure.

Chromolith® Widepore 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.



a)



b)

Figure 1

Electron-microscope photographs a) Macropores; b) Mesopores

Specifications

Table 1

Component	Description
Silica type	High purity (Type B)
Particle size	Monolithic
Macropore size	2 µm
Mesopore size	30 nm (300 Å)
Pore volume	1 mL
Total porosity	>80%
Surface area	~ 120 m^2/g
Pressure limit	200 bar
pH stability	1.5 - 7.5
Storage temperature	15 - 25 °C (2 - 8 °C for Chromolith WP Protein A)
Operating temperature	max. 60 °C

Connection of Chromolith® columns to HPLC systems

Chromolith® columns are cladded 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.

The end-fittings of Chromolith® columns are connected with standard 1/16" fittings suitable for all standard HPLC, UHPLC and UPLC® systems. Short capillary tubing is recommended to minimize extra-column volumes. Install the column in the correct direction as shown on the column label.

We strongly recommend using adjustable plastic ferrules in order to avoid a possible damage to the plastic 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.

Mobile phase

Chromolith® Widepore 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% Tetrahydrofurane (THF), 5% Chlorinated solvent (eg. Dichloromethane) or 5% Dimethylsulfoxide (DMSO). However pure DMSO can be used as solvent 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 1.5 to 7.5** with Chromolith® Widepore columns. Higher pH values will dissolve the silica, creating voids in the column. Lower pH values can eventually strip away some of the bonded phase. These defects will cause changes in retention times and loss of resolution.

Equilibrating the column

Chromolith® Widepore RP-18, RP-8, RP-4 columns are shipped in 100% acetonitrile. 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 which is between 0.7 mL/min and 5 mL/min. Then, continue conditioning the column with your mobile phase until you get a stable baseline. Check beforehand that your mobile phase is miscible.

However, regardless from the used buffer system, it is necessary to use high purity reagents and salts, and filter those (0.22 or 0.45 µm) prior to use.

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: 1.0 µL

Cleaning and regeneration procedure

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, connect the Chromolith® Widepore column in the reverse flow direction. The simplest procedure is to pump 100% methanol or acetonitrile for 5 min at about 2 mL/min. 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 about 5 mL/min: water, acetonitrile, 2-propanol, heptane, 2-propanol, acetonitrile, water, mobile phase.

Storing the column

It is recommended to store the columns in at least 50% acetonitrile. The preferred storage medium for Chromolith® Widepore columns is 100% acetonitrile, especially when storing columns for several days or longer.

The columns should not be stored in highly aqueous mobile phases with less than 50% organic mobile phase. The shorter the chains of the surface bonding are, the more organic mobile phase should be used for storing the column.

Maximum operating temperature and pressure

The maximum operating temperature for Chromolith® Widepore columns is 60 °C. Please note that any temperature above ambient will have a negative impact on column lifetime, also depending on pH and buffer conditions used.

Important Tip - for good peak symmetry, the mobile phase must be preheated 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® Widepore columns is 200 bar (3000 psi).

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.

Chromolith® WP columns were burdened with 15.000 column volumes (CV) of water and acetonitrile containing 0.1% TFA. The chromatographic change in separation behavior is shown in Figure 2.

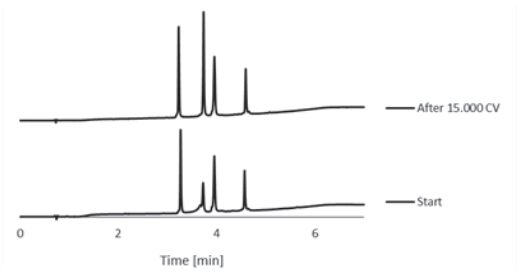


Figure 2

Separation of four proteins on a Chromolith® WP RP-8, 100-4.6 mm

Mobile phase:
A 0.1% TFA in water
B 0.1% TFA in acetonitrile

Detection:
220 nm

Gradient:
1 min 4% B; 10 min 4 - 60% B; 5 min 60% B

Peak Identification:
1) Ribonuclease
2) Cytochrome C
3) Holo-Transferrin
4) Apomyoglobin

Guard columns

It is generally good practice to protect the analytical column with a pre-column (guard column) in order to ensure maximum column lifetime. A Chromolith® Widepore guard column could also be used as a trapping column. For use in bioanalysis, it is recommended to use the bio-inert Chromolith® guard cartridge holder. Using guard columns can lead to slightly different peak shapes and separation efficiencies.

Low column back pressure

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

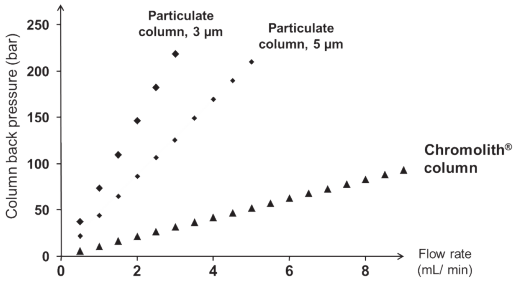


Figure 3

Column back pressure at different flow rates
Comparison of a Chromolith® Widepore column vs. equivalent fully porous particle-packed HPLC columns

A mixture of five peptides demonstrates the extreme time savings and high separation efficiency made possible with Chromolith® Widepore columns. Due to excellent mass transfer properties of the monolithic skeleton, high-efficiency separation is possible even at high flow rate.

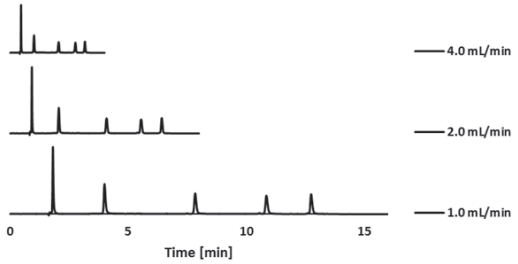


Figure 4

Separation of five peptides on a Chromolith® WP RP-18, 100-4.6 mm at various flow rates

Mobile phase:
A 0.1% TFA in water
B 0.1% TFA in acetonitrile

Detection:
220 nm

Gradient:
1 min (0.5/0.25 min) 10% B; 10 min (5/2.5 min) 10 - 20% B; 5 min (2.5/1.25 min) 20% B

Peak Identification:
1) Gly-Tyr
2) Val-Tyr-Val
3) Met enkephalin
4) Leu enkephalin
5) Angiotensin II

Applications

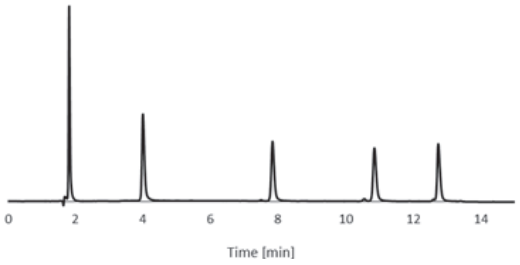


Figure 5

Separation of five peptides on a Chromolith® WP RP-18, 100-4.6 mm

Mobile phase:
A 0.1% TFA in water
B 0.1% TFA in acetonitrile

Flow rate: 1.0 ml/min

Detection: 220 nm

Gradient:
1 min 10% B; 10 min 10 - 20% B; 5 min 20% B

Peak Identification:
1) Gly-Tyr
2) Val-Tyr-Val
3) Met enkephalin
4) Leu enkephalin
5) Angiotensin II

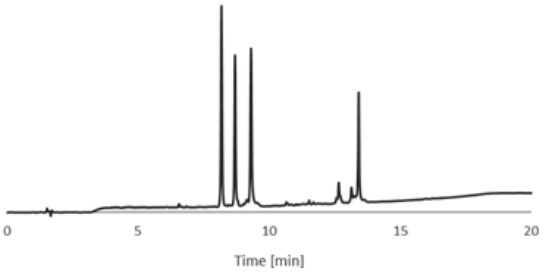


Figure 6

Separation of peptide/protein mixture on a Chromolith® WP RP-8, 100-4.6 mm

Mobile phase:
A 0.1% TFA in water
B 0.1% TFA in acetonitrile

Flow rate: 1.0 ml/min

Detection: 220 nm

Gradient:
1 min 4% B; 15 min 4 - 60% B; 5 min 60% B

Peak Identification:
1) Angiotensin II
2) Neurotensin
3) Ribonuclease
4) Myoglobin

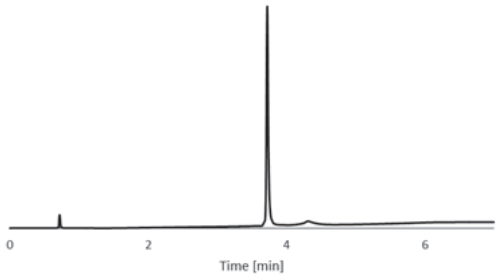


Figure 7

Analysis of Cetuximab® on a Chromolith® WP RP-4, 100-4.6 mm

Mobile phase:
A 0.1% TFA in water
B 0.1% TFA in acetonitrile

Flow rate:
2.2 mL/min

Gradient:
0.1 min 4% B; 4.9 min 4 - 60% B; 2 min 60% B

Detection:
220 nm

Sample:
5 mg/mL Cetuximab®

Ordering information for Chromolith® WP 300 products

Column dimension							
Length (mm)		ID (mm)	RP-18	RP-8	RP-4	Protein A	Epoxy
Chromolith® WP 300 HPLC Column [1 unit]							
25	x	4.6				1.52258.0001	1.52252.0001
25	x	2				1.52358.0001	1.52352.0001
50	x	4.6	1.52271.0001	1.52266.0001	1.52261.0001		1.52251.0001
50	x	2	1.52371.0001		1.52361.0001		1.52351.0001
100	x	4.6	1.52270.0001	1.52265.0001	1.52260.0001		1.52250.0001
100	x	2	1.52370.0001		1.52360.0001		1.52350.0001
Chromolith® Guard cartridges [3 units]							
5	x	4.6	1.52273.0001	1.52268.0001	1.52263.0001		1.52254.0001
5	x	2	1.52372.0001		1.52362.0001		1.52353.0001
10	x	4.6	1.52272.0001	1.52267.0001	1.52262.0001		1.52253.0001
Chromolith® Guard cartidge Holder							
5	x	4.6	1.52032.0001				
10	x	4.6	1.52033.0001				
Chromolith® Guard cartridge Holder							
for dimension			**Material**	**Item No.**			
5	x	2	Bioinert	1.52355.0001			
5	x	4.6	Bioinert	1.52255.0001			
10	x	4.6	Bioinert	1.52256.0001			

Status: 2024-10-08
Made in Germany

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