Chromolith® CapRod® RP Monolithic Capillary column

General information and guidelines for care and use

Introduction

Chromolith® CapRod® capillary column is a monolithic porous silica rod inside fused glass tubing. The rod is made of a defined silica skeleton with macropores and mesopores providing a very high porosity. Consequently, Chromolith® columns can be operated at higher flow rates with no loss of performance or limitations due to the column length or back pressure. CapRod® capillaries are developed for efficient separation of small molecules, peptides and proteins, and are especially suited for micro and nano/capillary-LC combined with UV or MS detection. Every analytical column is supplied with a Certificate of Analysis indicating test results and operating parameters.

General Considerations

Chromatographic performance depends on the entire system, not just on the column. Most variations are due to extra-column effects created by the design of the whole system (tubing, flow cell, injector, operator technique etc.). If you have any questions regarding your test results or the column quality please contact your local Merck KGaA, Darmstadt, Germany or MilliporeSigma subsidiary.

Contents of Package

1 Chromolith® CapRod® Capillary Column
1 Certificate of Analysis (not for Trap columns)
1 Care and Use Instructions
2 PEEK fittings 1/16” and sleeves (not with Trap columns)

Mobile Phase Selection

Generally, choice of buffer, concentration and operating temperature influence column lifetime.

- Use only HPLC or MS grade solvents and water (e.g. LiChrosolv® Hypergrade solvents).
- Use only highest purity chemicals & reagents.
- Degas all mobile phases prior to use.
- Use only mobile phases that are compatible with the column packing material.
- Switch only between mutually miscible mobile phases.
- The pH should be between 2.0 - 7.5.

Silica-based columns are pH sensitive. pH lower than 2 will hydrolyse the bonded phase and pH higher than 7.5 will solubilize the silica.

Column Installation

The tubing & fittings contribute to system dead volumes. Therefore, dead volumes should be minimized.
Chromolith® CapRod® column packages are equipped with PEEK 1/16” fittings and sleeves. The fittings and sleeves fit with any 360 µm O.D. fused glass tubing.

1. Connection to injector
Always use the column in the flow direction indicated by the arrow on the column label.
Connect the 360 µm O.D. capillary with the PEEK 1/16” fittings and the sleeves to tighten the tubing.
Make sure that the tubing is seated all the way down into the fittings. Make sure that the tubing enters all the way to the bottom of the injector port.
Keep the tubing as short as possible to avoid dead volumes.
Be careful in bending the capillary. Angle of bend should not exceed 45°.

2. Connection to the detector
Chromolith® CapRod® columns can be directly connected to any nano/capillary-HPLC UV detector (e.g. equipped with a nano/capillary flow cell) or a mass spectrometer.
Connections are made with either a fingertight fitting or with PTFE tubing.

Equilibrating the column

Reversed phase Chromolith® CapRod® columns are shipped in acetonitrile/water (70:30). As the column can dry out during storage and shipping, equilibrate by passing 10 column volumes of mobile phase at normal flow rate until you achieve a stable base line. (Note: flow rates should not exceed values indicated below).

- Install your column as described above. Make sure that no air bubbles are in the system.
- Verify that your mobile phase is miscible with the shipping solvent.
- It may be advantageous to run a blank gradient.

Validating Column Performance (does not apply to Trap columns)

If you want to validate the performance of a Chromolith® CapRod® column by measuring the efficiency on your own system, run the column using the test conditions stated on the Certificate of Analysis. Repeat this procedure periodically to check the column over time.
Please note: variations may be obtained on different HPLC systems. Mass spectrometry background noise may be caused by the chemicals and solvents used.

Sample Considerations

Sample quality greatly affects the column lifetime and performance. Whenever possible, use samples free of any debris (use of a pre-column might be advantageous). Filtration of samples through a 0.2 µm filter is highly recommended.

Running the Column

- Keep back pressure below certain limitations. Please find the recommended backpressures for the Chromolith® Cap Rod® Capillary products in the table below.
- Flow rates: to maximize column performance, flow rates should be adjusted to the inner column diameter and the nature of the sample. For recommended flow rates please check the table below.

Column Removal

- Stop the flow and wait until decompression is completed. This usually takes 5 minutes.
- Never remove the column when the column inlet pressure is > 5 bar (~70 psi). Otherwise the column can be damaged.
- Avoid unnecessary column removal; keep it installed whenever possible.
  Caution: Do not remove the capillary before decompression is fully completed.

Column Storage

- For prolonged storage, flush the column with a mobile phase of 60 to 80% acetonitrile or methanol in water.
- If the column has been used with buffers, flush the column with several column volumes of 60 - 80% acetonitrile or methanol.
- Never store columns for a long time with buffer or acid containing solvents or pure water.
- When not in use, store the column in the protective shipping box.

Cleaning and Regeneration

A shift in retention or resolution or unspecific background noise may indicate contamination of the column.

- Use 95% acetonitrile/0.1% formic acid for cleaning.
- Make sure that your in-column solvent or mobile phase is miscible with the cleaning solvent.
- Flush the column with 2 - 4 column volumes of 95% acetonitrile/ 0.1% formic acid.
- Equllibrate as described above.

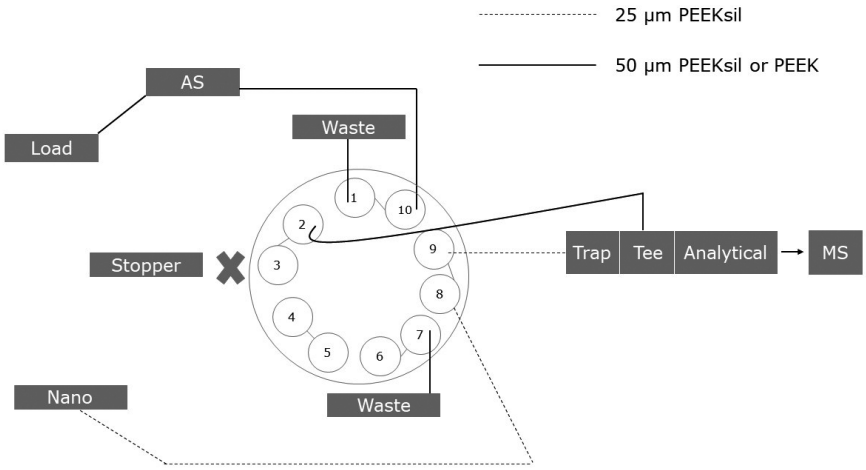
Various Connections to nano/capillary LC systems

Fittings, sleeves and dead volume connections from Upchurch Scientific, USA: Items #: F-230; F-130x; P-742; P-770.
Fitting and adaptors from VICI AG International, Switzerland: Items #: FS1.4PK-5; EZU1C.

Recommended use and flow rate ranges

Recommended use	RP-18e 150 x 0.05 mm	RP-8e 150 x 0.1 mm	RP-8e 50 x 0.1 mm Trap	RP-18e 150 x 0.1 mm	RP-18e 300 x 0.1 mm	RP-18e 150 x 0.1 mm HR	RP-18e 50 x 0.2 mm Trap	RP-18e 150 x 0.2 mm	RP-18e 150 x 0.2 mm HR
Separation of small molecules	✓		✓	✓	✓	✓	✓	✓	✓
– of peptides	✓	✓	✓	✓	✓	✓	✓	✓	✓
– of proteins		✓							
Micro ESI		✓		✓	✓	✓		✓	✓
Nano ESI	✓	✓		✓	✓	✓			✓
High Resolution						✓			
Flow rates (µl/min)	**0.2 - 0.8**	**0.4 - 3**	**1 - 10**	**0.4 - 3**	**0.2 - 1.5**	**0.1 - 0.4**	**10 - 50**	**5 - 20**	**0.5 - 2**
Max backpressure (bar)	**200**	**200**	**200**	**200**	**200**	**218**	**218**	**218**	**218**

Suggested installation of a trapping column



Ordering information

Product name and description	I.D.	Length	Content	Ordering Number
Chromolith® Cap Rod® RP-18e	0.05 mm	150 mm	1 analytical column	1.50403.0001
Chromolith® Cap Rod® RP-8e	0.1 mm	150 mm	1 analytical column	1.50400.0001
Chromolith® Cap Rod® RP-18e Trap	0.1 mm	50 mm	1 trapping column	1.50426.0001
Chromolith® Cap Rod® RP-18e	0.1 mm	150 mm	1 analytical column	1.50402.0001
Chromolith® Cap Rod® RP-18e	0.1 mm	300 mm	1 analytical column	1.50424.0001
Chromolith® Cap Rod® RP-18e HR	0.1 mm	150 mm	1 analytical column	1.50404.0001
Chromolith® Cap Rod® RP-18e Trap	0.2 mm	50 mm	1 trapping column	1.50409.0001
Chromolith® Cap Rod® RP-18e	0.2 mm	150 mm	1 analytical column	1.50405.0001
Chromolith® Cap Rod® RP-18e HR	0.2 mm	150 mm	1 analytical column	1.50407.0001

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Made in Germany

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