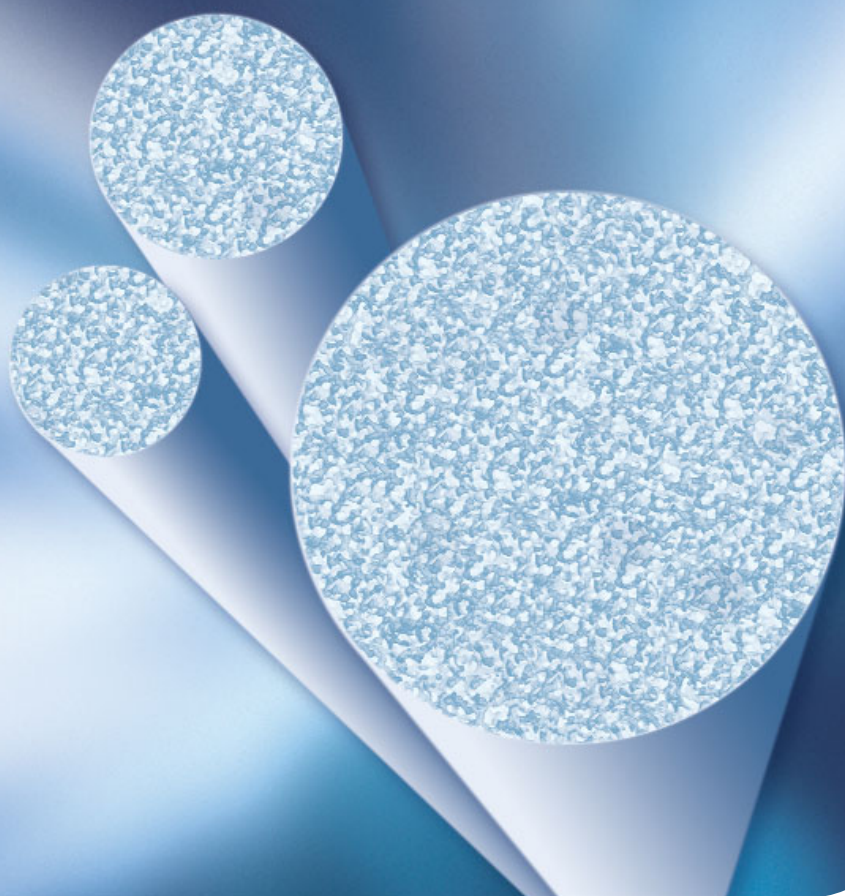


Speed and Performance in Monolithic Form. Chromolith® HPLC Columns

Long lifetime and high reproducibility –
Capillary, analytical and semi-prep HPLC columns



Chromolith® HPLC columns – a revolutionary technology wins worldwide acclaim

Scientists throughout the world have rapidly realized the revolutionary benefits made possible by the Chromolith® HPLC column technology. Over one hundred scientific papers have been published.

Revolutionary – monolithic silica replaces particles

Chromolith® HPLC columns are not filled with minute silica particles like conventional packed HPLC columns, but consist of a single rod of high purity monolithic silica.

Revolutionary – bimodal pore structure

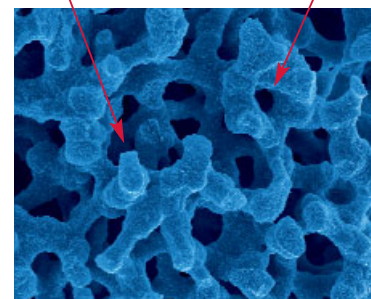
Chromolith® silica has a porosity exceeding 80% and a unique bimodal pore structure, which gives greatly improved chromatographic performance in terms of separation performance and column back-pressure.

Macropores dramatically reduce the column back-pressure and allow the use of faster flow rates, thereby considerably reducing the analysis time. Mesopores form the fine porous structure and provide the very large active surface area for high efficiency separations.

SEM picture of a cross section from a silica monolith

Mesopores: 13 nm

Macropores: 2 μm



Total porosity > 80%

Revolutionary – good peak resolution, low column back-pressure

When compared with 5 μm particulate columns of the same length, the column back-pressure with Chromolith HPLC columns is typically 4 times lower. The peak resolution however remains virtually unchanged.

Revolutionary – the long lifetime and robustness

Monolithic Chromolith® HPLC columns demonstrate very high mechanical stability and long operative lifetimes, in most cases far exceeding column lifetimes for particulate columns.

Benefits of Chromolith® HPLC Columns at a glance

1. Speed of Analysis

- Separations two times faster at half the column back-pressure compared to 5 µm columns
- Higher sample throughput – separations up to 9 times faster if required
- Fast column re-equilibration between analyses

2. Improved HPLC system security

- Significantly increased column lifetime
- Reduced maintenance on HPLC pump and injector seals
- Reduced need for sample preparation as columns are very resistant to blocking (even with biological samples)

3. Column length no longer pressure limited

- Very high peak resolution by column coupling

4. Standard HPLC instruments are ideally suited for use with Chromolith® HPLC columns

- Chromolith® columns clad in PEEK are very easy-to-use and handle

5. Cost Savings

- Cost savings due to faster sample throughput (in USA typically \$400 per day if analysis time is halved) can repay revalidation expenses (in USA typically \$12,000) in about 6 weeks
- Cost saving due to significantly longer column lifetime

The speed and performance of particulate HPLC columns are limited by column pressure

Particulate HPLC columns are filled with particles, typically silica with 3 or 5 micron particle size. Very small particle size brings one advantage – high separation performance. But small particle size also brings three limitations:

- high column back-pressure limits the speed of analysis and the column length
- particulate columns can easily block, thereby reducing lifetime
- lifetime of HPLC instrument and injector seals is reduced at high pressure

Acquity UPLC™ columns use even smaller particles (1.7 µm) requiring ultra-high pressures. Here the user requires a special new instrument, in order to operate at these high pressures.

(Acquity UPLC™ is a trademark of Waters Corporation)

Chromolith® Performance

100-4.6 mm columns -

Faster separations at lower back-pressure

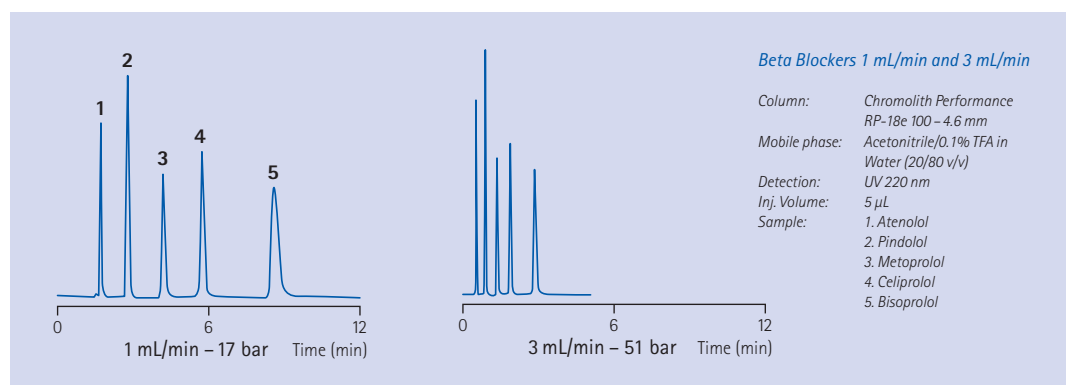
Chromolith® Performance is the ideal alternative to a 5 µm particulate column

Chromolith®
Performance
100-4.6 mm

At 1 mL/min flow rate a chromatogram run on a Chromolith® Performance column looks almost identical to the same chromatogram run on the corresponding particulate column. The striking difference is that the column back-pressure is typically 4 times lower compared to a 5 µm column.

Three times faster analysis at lower column pressure

Simply by increasing the flow rate to 3 mL/min, the analysis is 3 times faster, the quality of the separation is unchanged and the column back-pressure remains lower than with the 5 µm column. If even faster analysis is required, simply increase the flow rate further – up to 9 times faster.



Easy method transfer from particulate to Chromolith® HPLC columns

Method transfer from particulate to monolithic Chromolith® columns is made easy as the chromatographic selectivity of Chromolith® columns is very similar to that of many modern particulate HPLC columns (eg. Purospher®). Chromolith® columns are included in databases of USP compatible columns in the categories L1, L3 and L7. The USP has proposed the inclusion of Chromolith® columns in the L1 category in USP 29.

Choice of selectivity

- RP-18 endcapped and RP-8 endcapped for reversed-phase HPLC
- Unmodified silica for normal phase HPLC

Validation Kit

For correct method validation, it is essential to assess all possible sources of variations. To assist the validation process, the Chromolith® Validation Kit includes three columns from three different production batches.



Chromolith® column coupler

Very high efficiency separations at moderate pressure

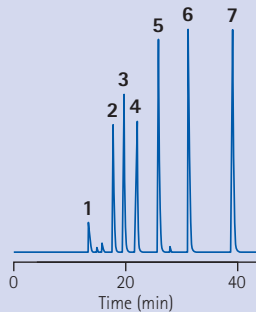
Longer columns give greatly improved peak resolution

Column
Coupler

Using the Chromolith® column coupler, longer columns are readily available, giving high separation performance at a fraction of the back-pressure that 5 µm particles generate.



Connecting ten 100 mm columns can give column efficiency exceeding 80,000 theoretical plates. Connecting four 100 mm columns will double the peak resolution.



81,000 plates at 85 bar pressure

Column: 10 columns of Chromolith Performance RP-18e, 100–4.6 mm

Mobile phase: 80/20 Acetonitrile/Water

Flow rate: 1 ml/min

Detection: UV 254 nm

Temp.: ambient

Inj. Volume: 10 µL

Sample: 1. Thiourea

2. Benzene

3. Toluene

4. Ethylbenzene

5. Propylbenzene

6. Butylbenzene

7. Phenylbenzene



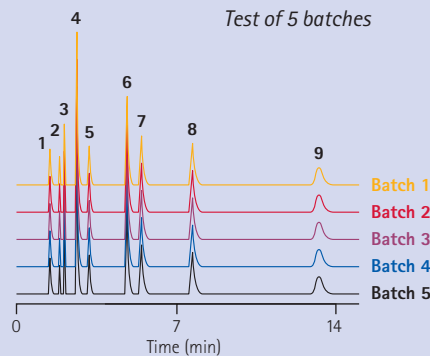
Column length (mm)	Back pressure (bar)	Plate Number N (anthracene)
200	22	19000
300	33	27000
400	44	35000
500	54	41000

Chromolith® column length and plate count at 3 mL/min.

Excellent batch-to-batch reproducibility

The batch-to-batch reproducibility of Chromolith® HPLC columns is tightly controlled and fulfils the requirements for QA/QC laboratories.

Test of 5 batches



Chromatographic Conditions

Column: Chromolith Performance RP-18e, 100–4.6 mm

Mobile phase: Methanol/Water 55/45 (v/v)

Flow rate: 1 ml/min

Detection: UV 254 nm

Temp.: ambient

Sample: 1. Thiourea

2. Aniline

3. Phenol

4. 2,3-Dihydroxynaphthalene

5. 4-Ethylaniline

6. Diethylphthalate

7. N,N-Dimethylanilin

8. Toluene

9. Ethylbenzene

Chromolith® SpeedROD 50 – 4.6 mm

Chromolith® Flash 25 – 4.6 mm

- ultra-fast separations
- ideal for DMPK and fast LC/MS



SpeedROD Column

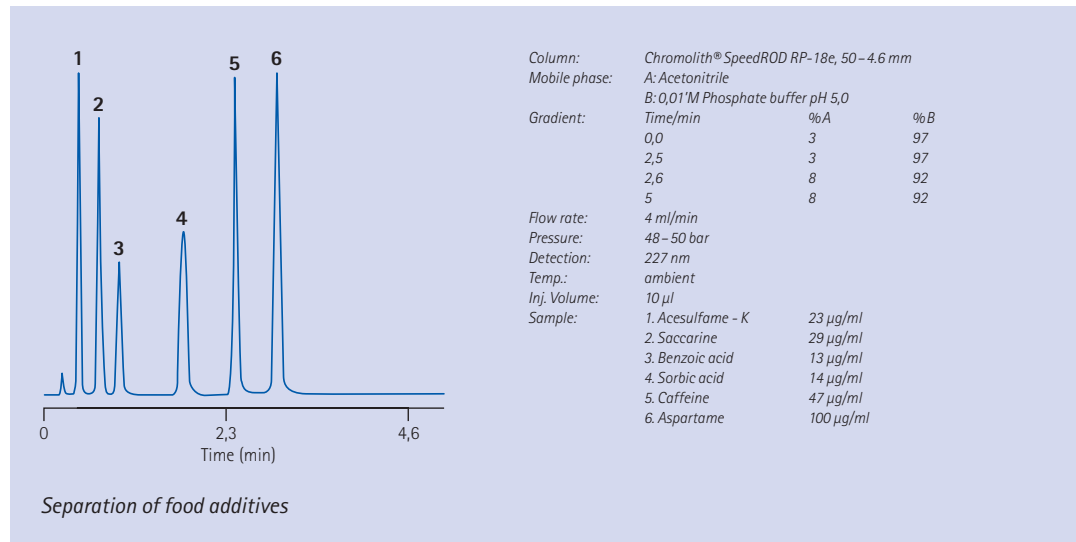
Chromolith® SpeedROD and Flash are the ideal alternatives to short high performance 3 µm particulate HPLC columns

The plate count can exceed 100,000 plates/meter, yet the column back-pressure is typically 8 times less than a 3 µm column!

Many publications demonstrate the use of these columns in very fast gradient applications, where Chromolith® columns typically have much longer lifetimes than particulate columns.



Flash Column

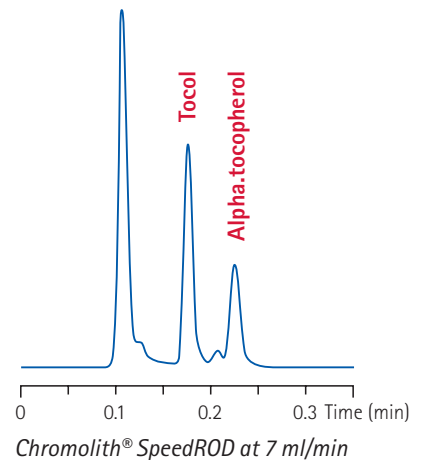


Sometimes the time per analysis can be under one minute!

The benefits of obtaining accurate quantitative results fast cannot be underestimated and can lead to major cost savings.

The chromatogram here shows the rapid analysis of tocopherols in biological matrix. (chromatogram courtesy of AS Vitas, Norway; www.vitas.no)

Column:	Chromolith® SpeedRod RP-18 endcapped	Injection volume:	1 µL
Mobile phase:	Water: Methanol (2:98, v/v)	Column temp:	40 °C
Detection:	Fluorescence detection	Sample:	plasma precipitated with 3 volumes of 2-propanol
	Ex: 295, Em: 330		



Rugged, reliable and long-lasting

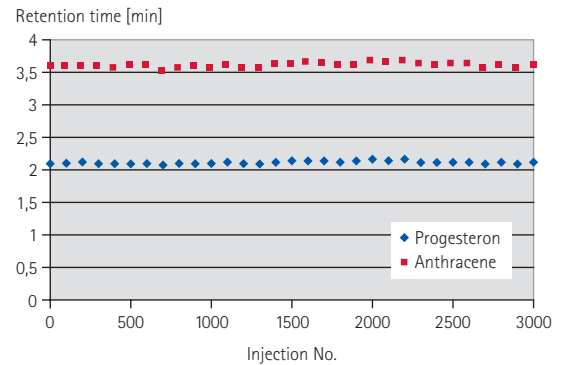
a characteristic of all Chromolith® columns

Long column lifetime and high resistance to column blockage reduce costs per analysis and enhance data integrity



Chromolith® HPLC columns have demonstrated immense robustness and set a new standard for long column lifetime. The rigid monolithic silica skeleton with 2 µm macropores is the reason for this improved performance.

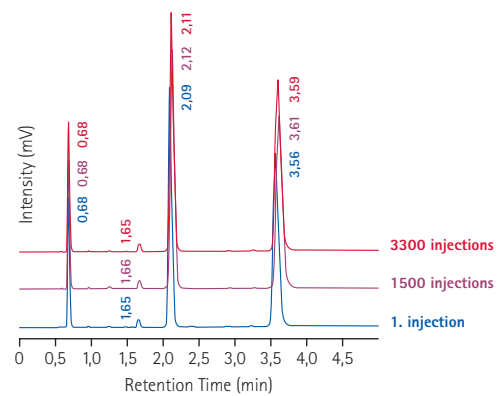
The diagrams show the results of a stability test with 3300 injections and 50,000 column volumes of mobile phase.



The three superimposed chromatograms show the reproducibility of the first, the 1500th and the 3000th injections.

Chromatographic conditions

Column: Chromolith® Performance RP-18e, 100 – 4.6 mm
Mobile phase: Acetonitrile/water 60/40 (v/v)
Detection: UV@ 254 nm
Sample: Thiourea 10 µg/mL
Progesteron 100 µg/mL
Anthracene 10 µg/mL



Literature references demonstrating Chromolith® HPLC column stability include:

- 1) V. Borges et al. *J. Chromatography B*, 804 (2004) 277–287,
- 2) J-T Wu et al. *Rapid Commun. Mass Spectrom.* 15 (2001) 1113–1119



Chromolith® Guard Columns

Chromolith® Guard Columns further extend the column lifetime and further protect the column from both particulate and chemical contamination.

Guard columns are available in 5 mm or 10 mm lengths and in kits complete with guard column holder.

Perfect scale-up from analytical to preparative LC

Chromolith® SemiPrep 100-10 mm RP-18 endcapped

Optimum separation at flow rates exceeding 40 mL/min

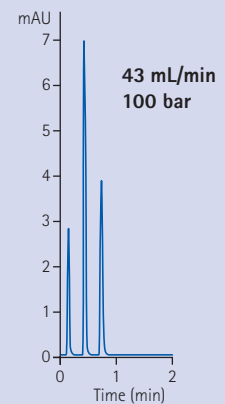
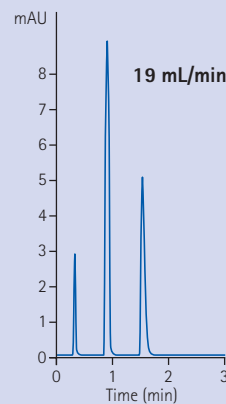
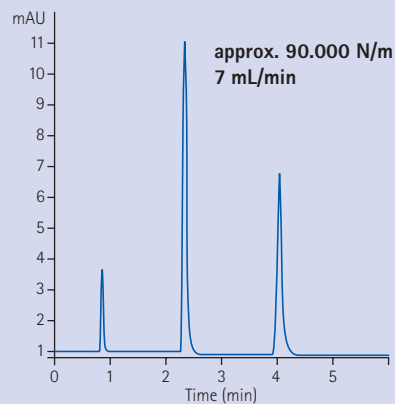
Chromolith® SemiPrep 10 mm i.d. columns combine high separation speed with very high separation performance. They are the ideal alternative to particulate columns with 10 mm i.d. (and even 21.2 mm).



Separation of a standard mixture

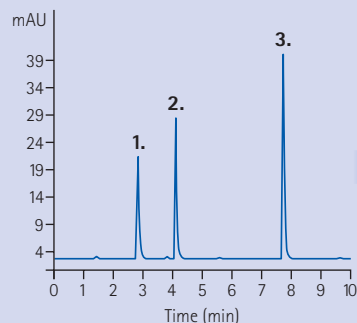
Acetonitrile/water 60/40

Data for anthracene (3rd peak)



Accurate scale-up from analytical to preparative columns

25 mg injected onto a Chromolith® SemiPrep RP-18 endcapped column show the same excellent separation when compared with the corresponding analytical column.

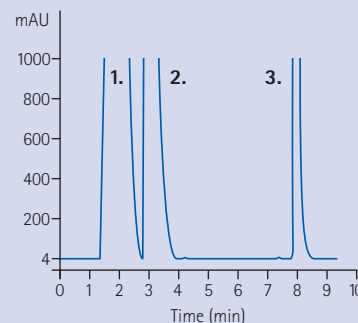


Performance 100-4,6 mm

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

Chromatographic conditions

Column: Chromolith® RP-18e

Gradient: A: Acetonitrile with 0,1% TFA,
B: Water with 0,1% TFA,
0-10 min 15-80% A

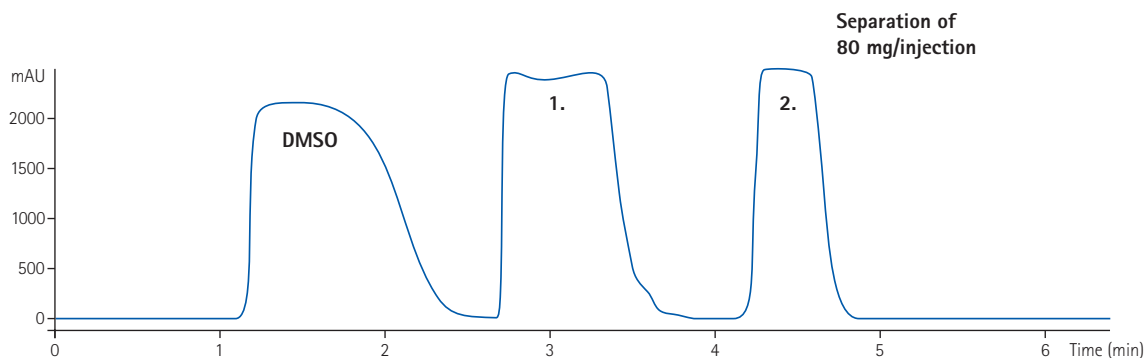
Detection: UV @ 270 nm

Chromolith® SemiPrep 100–10 mm

for high throughput purification in Drug Discovery

Sample Loadability

The sample loadability depends on many factors including the solubility of the sample in the mobile phase. The following example shows that the sample loadability on the Chromolith® SemiPrep column can exceed 80 mg. Here DMSO/Methanol (1:1) is used as solvent.



Chromatographic conditions

Column: Chromolith® SemiPrep RP-18e, 100–10 mm
Mobile phase: A: Acetonitrile with 0,05% TFA B: Water with 0,05% TFA
Gradient: 0–1 min 5% A; 1–5 min 5–90% A; 5–5.2 min 95% A; 5.2–6.2 min 95% A
Flow rate: 8 mL/min
Detection: UV @ 214 nm
Injection volume: 400 µL
Sample: 1. Propranolol 200 mg/mL
2. Nifedipine 200 mg/mL dissolved in DMSO/Methanol 1/1

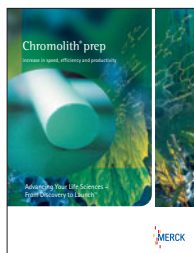
By courtesy of Dr. A. Espada and C. Anta, Lilly Spain

LC/MS Compatibility

Chromolith® SemiPrep columns are optimized for LC/MS by a surface modification process minimizing column bleed.

Chromolith® prep 100–25 mm

for High Speed Preparative Chromatography



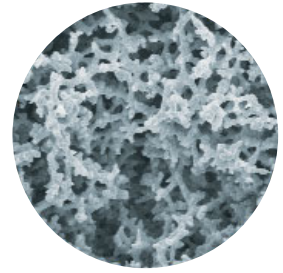
Highest Productivity – Chromolith® prep 25 mm i. d. column

The aim of a chromatographic separation at production scale is to optimize sample throughput as a function of time. The Chromolith® 25 mm i.d. prep column increases the flow rate enormously, compared to particulate preparative columns, and enables optimum process productivity at production scale.

More information? Ask for the Chromolith® prep brochure, which may also be download from www.merck-lsp.de/servlet/PB/menu/1107830/index.html

Chromolith® CapRod™ 0.1 mm capillary column –

fast nano-LC for proteomics and small molecules



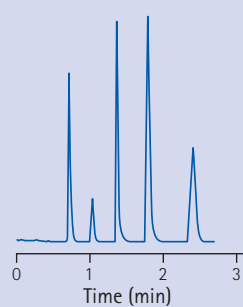
REM of column cross-section showing monolithic structure

Chromolith® CapRod™ capillary columns combine the speed of monolithic silica technology with the sensitivity of nano-LC, thus enabling high-speed high sensitivity proteomics LC and LC/MS measurements.



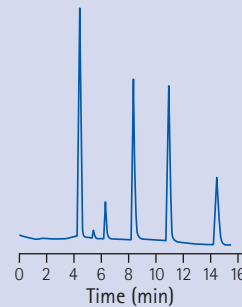
CapROD™
column

Separation of low molecular weight analytes



3 µL/min, 130 bar

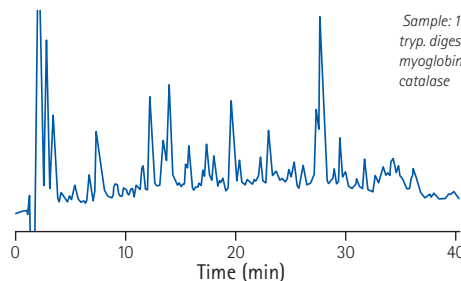
Column: Chromolith® CapRod™
RP-18 endcapped,
150 mm x 100 µm
Eluent: ACN/H₂O = 50/50 (v/v)
Inj. Vol.: 0.05 µL
Temp.: R. T.
Detection: UV 210 nm



0.5 µL/min, 21 bar

Sample:
1. Thiouracil
2. Acetophenone
3. Benzene
4. Toluene
5. Naphthalene

LC/MS analysis of 4 digested proteins



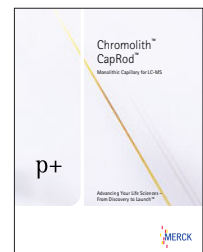
Sample: 100 ng protein mix
tryp. digested cytochrome C,
myoglobin, carbonic anhydrase,
catalase

Mobile Phase: A: 2% Acetonitrile in 0.1%
formic acid
B: 80% Acetonitrile in
0.08% formic acid
Gradient: 2% B to 40% B in 35 min.
Flow rate: 3 µL/min
Pressure: 85 bar

More information:
ask for Chromolith®
CapRod™ brochure

Benefits of Chromolith® CapRod™ at a glance

- Flow rates many times faster than with 3 µm particulate columns (up to 4 µL/min)
- Excellent peak symmetry with formic acid
- High detector sensitivity
- Optimized for LC/MS



Chromolith® HPLC Columns –

an award winning innovation



Pittcon Gold Award 2001 – Best Product 2001

R&D 100 Award 2001 – One of the 100 most important innovations in 2001

German Industry Innovation Award 2001 – One of 5 most important innovations in 2001

Specifications:

Silica type:	high purity (99.999%)	Column cladding material:	PEEK
Particle size:	monolithic	Mobile phase compatibility:	
Macropore size:	2 µm	<i>all standard HPLC solvents may be used with the following restrictions:</i>	
Mesopore size:	13 nm (130 Å)	Max. dichloromethane conc.:	5%
Pore volume:	1.0 ml/g	Max. tetrahydrofuran conc.:	50%
Surface area:	300 m ² /g	Max. dimethylsulphoxide DMSO:	5% but OK as sample solvent
Total porosity:	> 80%	pH range:	2–7.5
Selectivity equivalent to:	L3 (USP)	Max. pressure:	200 bar for 4.6 mm columns
		Max. pressure:	150 bar for 10 mm columns
		Max. pressure:	100 bar for 25 mm columns
Surface modification:	RP-18 endcapped	Surface modification:	RP-8 endcapped
Selectivity equivalent to:	L1 (USP)	Selectivity equivalent to:	L7 (USP)
Carbon content:	18%	Carbon content:	11%
Surface coverage:	3,6 µmol/m ²		



Chromolith® HPLC columns – ordering information

Merck part number	Description	Length	Internal diameter	Items
Capillary column				
1.50402.0001	Chromolith® CapRod™ 150–0.1 mm RP-18 endcapped (with 1/16" PEEK connections)	150 mm	0.1 mm	1 column
Analytical columns				
1.02129.0001	Chromolith® Performance 100–4.6 mm RP-18 endcapped	100 mm	4.6 mm	1 column
1.51450.0001	Chromolith® SpeedROD 50–4.6 mm RP-18 endcapped	50 mm	4.6 mm	1 column
1.51463.0001	Chromolith® Flash 25–4.6 mm RP-18 endcapped	25 mm	4.6 mm	1 column
1.51468.0001	Chromolith® Performance 100–4.6 mm RP-8 endcapped	100 mm	4.6 mm	1 column
1.51465.0001	Chromolith® Performance 100–4.6 mm Si	100 mm	4.6 mm	1 column
1.51467.0001	Chromolith® Column Coupler			1 piece
1.51466.0001	Chromolith® Validation Kit RP-18E (3 columns from different batches)	100 mm	4.6 mm	3 columns
Guard Columns				
1.51451.0001	Chromolith® RP-18 endcapped Guard Column 5–4.6 mm 3 pieces	5 mm	4.6 mm	3 pieces
1.51452.0001	Chromolith® RP-18 endcapped Guard Column 10–4.6 mm 3 pieces	10 mm	4.6 mm	3 pieces
1.51470.0001	Chromolith® RP-18 endcapped Guard Column 5–4.6 mm kit	5 mm	4.6 mm	3 pieces + holder + tool
1.51471.0001	Chromolith® RP-18 endcapped Guard Column 10–4.6 mm kit	10 mm	4.6 mm	3 pieces + holder + tool
Semi-preparative and preparative columns				
1.52016.0001	Chromolith® SemiPrep 100–10 mm RP-18 endcapped	100 mm	10 mm	1 piece
1.25251.0001	Chromolith® prep 100–25 mm Si	100 mm	25 mm	1 piece
1.25252.0001	Chromolith® prep 100–25 mm RP-18 endcapped	100 mm	25 mm	1 piece

These products are not intended for use as in-vitro diagnostics in terms of European Directive 98/79/EC. They are for research purposes only, for investigating in-vitro samples without any medical objective.

Further Information on Merck
and our products:
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Life Science & Analytics
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Fax: +49-(0) 6151-72-60 80
eMail: chromatography@merck.de
www.chromatography.merck.de

We inform and advise our customers to the best of our knowledge and ability but without any engagement or liability on our part. Our customers must obey all existing laws and regulations. This also applies in respect of any protected rights of third parties. Our information and advice does not eliminate the need for our customers to check, on their own responsibility, that our products are suitable for the purpose envisaged.