MERCK

chromolith® HPLC columns

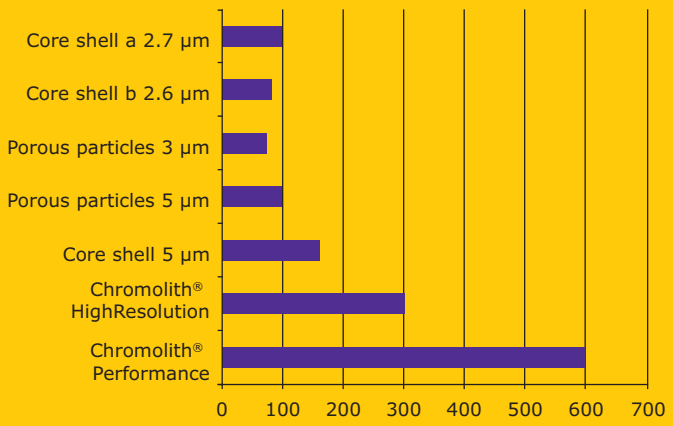
Race through separations with revolutionary technology

Tired of congestion?

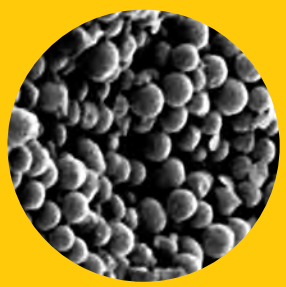
It goes without question that the development of faster separation processes is one of the most important issues in HPLC. Particularly in industry, chromatographers wish to speed up separations, and analyze more samples with the limited financial and human resources available.

One of the main issues preventing speed is congestion. With conventional particle-packed HPLC columns, higher efficiency always comes at the expense of higher back pressure. Even core-shell particle columns, which are designed for lower resistance, still exhibit unacceptable back pressure. Hence, the task is to minimize back pressure in order to maximize speed.

Plates per pressure (N/bar)



All columns are C18e modified, 100-4.6 mm. Sample: anthracene, eluted isocratically using acetonitrile/water (60/40) at 2 mL/min flow rate. Injection volume: 5 µL, detection at 254 nm UV. All analyses performed at room temperature.



A column packed with tiny particles is relatively easy to block since the space between particles is directly proportional to particle size (approx. one sixth of particle diameter). The smaller space leads to higher back pressure and blockage.

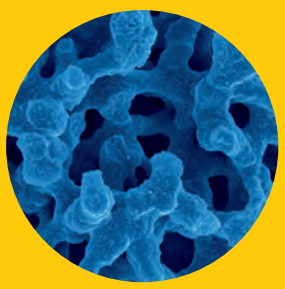
SEM image: Silica particles.

Speed up with Chromolith® columns

To truly accelerate chromatographic separations, there's no better choice than our Chromolith® HPLC columns. Due to their revolutionary monolithic technology, Chromolith® columns provide excellent separations in a fraction of the time required by conventional particulate columns.

The secret to the speed of Chromolith® columns is their exceptionally low back pressure. Produced from a continuous piece of porous silica using a sol-gel process, Chromolith® columns possess a defined bimodal pore structure with macro and mesopores in the micro and nanometer range. The high permeability and porosity of the silica skeleton, and the resulting low back pressure allow for more flexible flow rates than particle-packed columns. As a result, Chromolith® HPLC columns enable high-throughput analysis without loss of separation efficiency or peak capacity.

SigmaAldrich.com/chromolith



The thin skeleton ensures fast mass transfer, while the large channels allow unobstructed flow through the column.

SEM image: Cross section of a silica monolith

Total porosity > 80%.

Content

Why choose a Chromolith® column?

- High flow rates at low pressure
- Flat Van Deemter curve allows flow rate flexibility and flow gradients
- Improved HPLC system security: robustness, reliability, versatility
- Substantially longer column lifetime
- Compatible with various organic solvents, e.g. methanol, ethanol, isopropanol
- Column length no longer pressure limited
- Cost savings from higher sample throughput offsets expense of method revalidation in one month
- Suitable for use with standard HPLC/UHPLC instruments
- Available in various sizes and modifications
- Higher porosity allows fast adsorption and desorption kinetics
- Compatible with all mass spectrometers

Column selection guide

Compound Class	Chromolith® NH2	Chromolith® Si	Chromolith® DIOL	Chromolith® CN	Chromolith® Propyl Phenyl	Chromolith® RP-4	Chromolith® RP-8e	Chromolith® RP-18e	Chromolith® WP 300 Prot A
Aflatoxins		●					●	○	
Alcohols	●		●	●				●	
Aldehydes					●		●	○	
Alkaloids					●		●	○	
Aliphatic amines								●	
Amino Acids			○				○	●	
Antibiotics					●		●	○	
Aromatic amines					○			●	
Carboxylic acids	○								
Carotinoids		○	○				●	●	
Catecholamines								○	
Explosives								●	
Oils		○	●	●				●	
Oligonucleotides								○	
Esters							○	○	
Fat soluble vitamins	○	○			●			○	
Lipids		○						○	
Fatty acids		○					●	○	
Flavonoids		●		○	○			○	
Glycans		●						○	
Glycols		●	●	●					
Immunoglobulins									○
Inorganic ions	●								
Ketones		●			●		●	○	
Nitrosamines							●	○	
Nucleosides							●	○	
Nucleotides	○	●			●			●	
PAH					○			○	
PCB					●		●	○	
Peptides					○		●	○	
Pesticides								○	
Phenols				○			●	○	
Phospholipids		●	●					●	
Phthalates				○				○	
Preservatives			○		○			○	
Proteins			○			○	○	●	
Organic phosphates								●	
Steroids	○	●		○	●		●	○	
Metabolized steroids					●		●	○	
Sugars	○		○						
Sugar Alcohols	●								
Sulfonamides			●	●				○	
Sweeteners	○				●			○	
Water soluble vitamins	○		○					○	

For molecules smaller than 10 kDa use Chromolith® with 150Å mesopores

For molecules larger than 10 kDa and up to ~100 kDa use Chromolith® WP 300 with 300Å mesopores

○ Most commonly used column ● Column with some successful separation cases

Note: As chromatographic separation depends on many physical and chemical parameters, we cannot guarantee the success of a separation based on the recommended column modification.

Chromolith® columns at a glance

	Capillary columns			Analytical columns			Preparative columns	
								
	Column internal diameter							
	0.05 mm	0.1 mm	0.2 mm	2 mm	3 mm	4.6 mm	10 mm	25 mm
Chromolith® RP-18 e	Page 12-15, 49	Page 12-15, 49	Page 12-15, 49	Page 9, 11, 18-19, 47-48, 49	Page 20, 47-48, 49	Page 7-8, 10, 18, 20, 21, 47-48, 49	Page 30, 51	Page 32, 51
Chromolith® HighResolution RP-18 e		Page 14, 49	Page 12, 49			Page 11, 21-23, 47-48		
Chromolith® WP300 RP-18						Page 32-34, 50		
Chromolith® RP-8 e						Page 24, 47-48, 49		
Chromolith® HighResolution RP-8						Page 24, 47-48		
Chromolith® WP300 RP-8						Page 34, 47-48, 50		
Chromolith® WP300 RP-4						Page 23, 50		
Chromolith® Phenyl						Page 25, 47-48, 49-50		
Chromolith® CN						Page 26, 47-48, 50		
Chromolith® DIOL						Page 26, 47-48, 50		
Chromolith® Si (silica)						Page 27, 47-48, 50	Page 44, 47-48, 51	Page 46-48, 51
Chromolith® NH_2						Page 28, 47-48, 50		
Chromolith® WP 300 Epoxy						Page 37-41, 47-48, 50		
Chromolith® WP 300 Protein A						Page 35-37, 50		
	-			Loadability				+
	+			Sensitivity				-
	+			Solvent saving				-

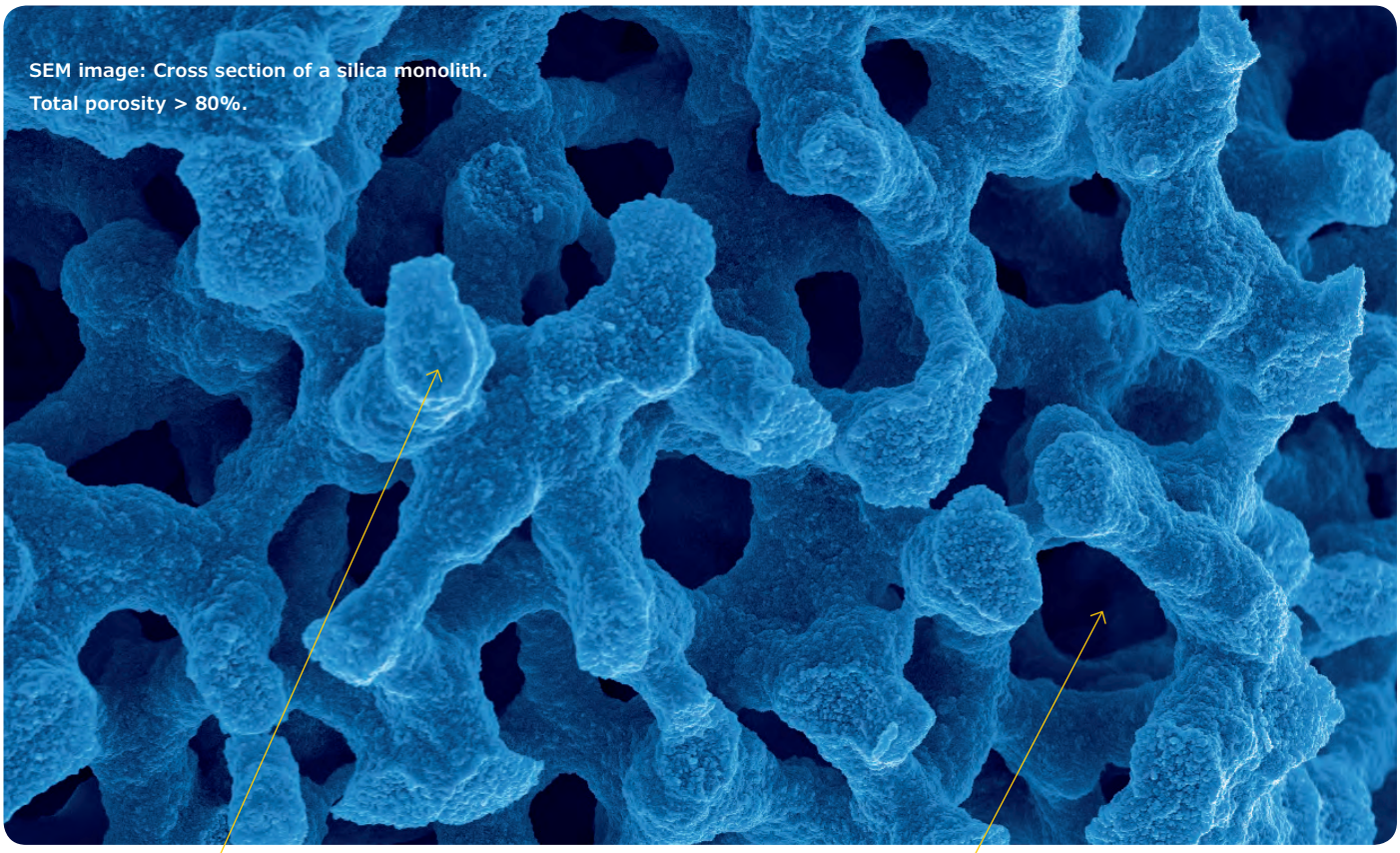
Chromolith® columns are available in various lengths:				
25 mm	50 mm	100 mm	150 mm	300 mm
+		Speed		-
-		Resolution		+

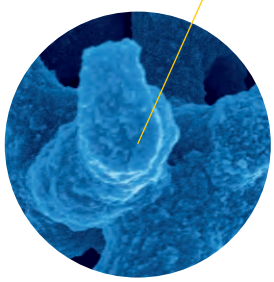
Chromolith® HPLC columns

Revolutionary monolithic silica replaces particles

Thanks to their patented monolithic silica technology, Chromolith® HPLC columns allow you to race through separations with maximum robustness and selectivity—at minimal back pressure.

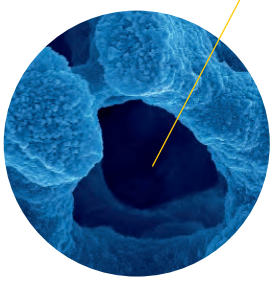
The revolutionary bimodal pore structure of Chromolith® columns provides a unique combination of macropores and mesopores.





Mesopores: A verage pore size is 13 nm for Chromolith®, 15 nm for Chromolith®HR, and 30 nm for Chromolith®WP 300.

Forms a fine porous structure with a large uniform surface area on which adsorption takes place, thus enabling high-performance chromatographic separation.



Macropores: Average pore size is 1,5µm for Chromolith® 2 mm i.d. , 1,15 µm for Chromolith® HR, and 2µm for all others.

Allows rapid flow of the mobile phase at low back pressure.

Characterization of Chromolith® HPLC columns

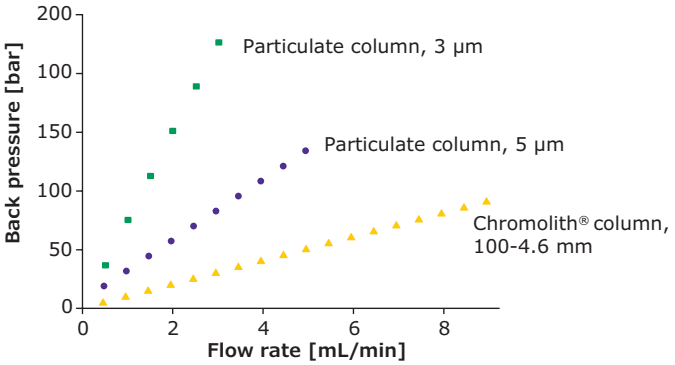
The use of conventional HPLC columns containing sub 2, 2, 3 or 5 µm silica particles often results in high back pressure (the smaller the particles the higher the pressure). This reduces column lifetime, system robustness and the operational range of flow rates. As a result, these columns are limited in length and in their number of theoretical plates. Attempts have been made to increase plate count by decreasing particle size, but this further raises back pressure, and restricts the variety of separations that can be satisfactorily achieved.

Another means of accelerating chromatographic analysis is through laboratory automation of HPLC systems. This method has come a long way toward improving sample throughput by enabling 24-hour operation. However, systems are still dependent on the separation technology itself, that is, the separation columns available.

The optimal solution is to use a column that offers faster throughput without the risk of blockage the Chromolith® column. In contrast to conventional HPLC columns, Chromolith® columns are not packed with small silica particles. Instead, each column consists of a single rod of high-purity polymeric silica gel with a bimodal pore structure of macro and mesopores. This unique construction enables highly efficient separations at unbeatable speeds.

Analysis speed

Chromolith® columns owe their rapid separation speed to their unique bimodal pore structure of macro and mesopores. The macropores reduce column back pressure and allow the use of faster flow rates, thereby considerably reducing analysis time. The mesopores form a fine porous structure, which creates a very large active surface area for high-efficiency separations.

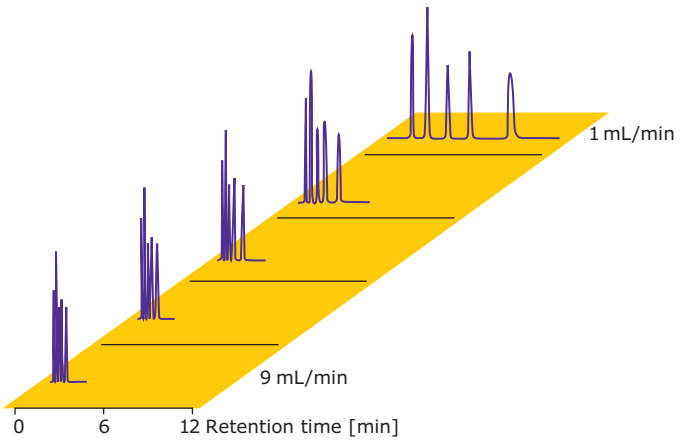


Column back pressure at different flow rates. Comparison of a Chromolith® Performance column, 100-4.6 mm vs. equivalent classical particulate HPLC columns.

With Chromolith® columns, flow rates can now easily be varied from 1 mL up to 9 mL per minute with the same high quality resolution.
A mixture of five beta-blocker drugs was analyzed to demonstrate 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 was possible, even at high flow rates. The beta-blockers were well separated with excellent peak symmetry. At 9 mL/min, analysis time was less than 1 minute, and the column back pressure was only 153 bar.

Chromolith® Performance RP-18e 100-4.6 mm

Column	Chromolith® Performance RP-18 endcapped 100-4.6 mm	
Mobile phase	Isocratic acetonitrile / 0.1 % trifluoroacetic acid in water, 20/80 (v/v)	
Pressure	Total pressure (including HPLC system) 25°C	
Detection	UV 220 nm	
Injection volume	5 µL	
Sample	Atenolol	63 µg/mL
	Pindolol	29 µg/mL
	Metoprolol	108 µg/mL
	Celiprolol	104 µg/mL
	Bisoprolol	208 µg/mL

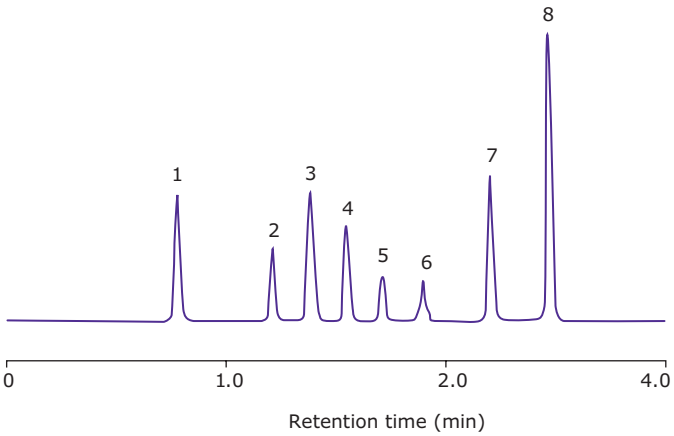


Flow programming

Chromolith® columns respond very quickly to changes in flow rate, giving you maximum flexibility in flow programming. Rates can be adjusted in mid-flow either to enhance the peak definition of the target compound, or to shorten the total separation time once the compound has successfully eluted. This enables clearer separation of two closely eluting peaks, without significantly affecting the total run time. A mid-flow change in rate can also reduce the total run time when certain compounds elute much later than others.

Chromolith® Performance RP-18e 100-4.6 mm

Column	Chromolith® Performance RP-18 endcapped 100-4.6 mm			
Mobile phase	A: Acetonitrile			
	B: 0.1 % Phosphoric acid in water			
Double gradient	**Time**	**% A**	**% B**	**Flow rate**
	0 min	35	65	3 mL/min
	1.8 min	46	54	3 mL/min
	2.2 min	80	20	5 mL/min
	3 min	80	20	5 mL/min
Pressure	90 bar maximum total pressure			
Detection	UV 254 nm			
Temperature	22 °C			
Injection volume	10 µL			
Sample	1. Phenol			
	2. 2-Chlorophenol			
	3. 2-Nitrophenol			
	4. 2,4-Dinitrophenol			
	5. Chloro-3-methylphenol			
	6. 2,4-Dinitro-6-methylphenol			
	7. 2,4,6-Trichlorophenol			
	8. Pentachlorphenol			

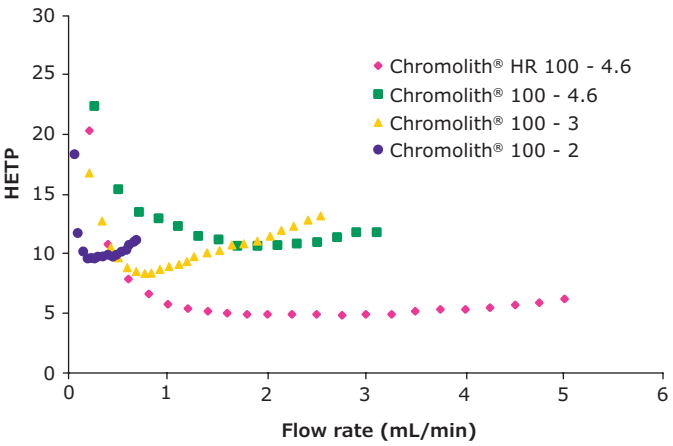


High separation efficiency

The traditional plate-count method of measuring quality shows that the separation efficiency of Chromolith® columns is better than standard 5 µm particulate columns, and just as good as 3.5 µm columns, but with the ability to continue up to 9 mL/min without reaching HPLC system pressure limits.

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

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 high plate count at low back pressure. (Please see: Chromolith® column coupler). With particulate columns, further column length is prevented by excessive back pressure.



Van Deemter plot of the height equivalent to a theoretical plate (HETP) vs. flow rate for Chromolith® columns.

Long-term stability

Besides lower back pressure and greater flow rate flexibility, Chromolith® columns also achieve faster equilibration after gradient elution than particle-packed columns of similar dimensions. These features allow high-throughput analysis without loss of separation efficiency or peak capacity.

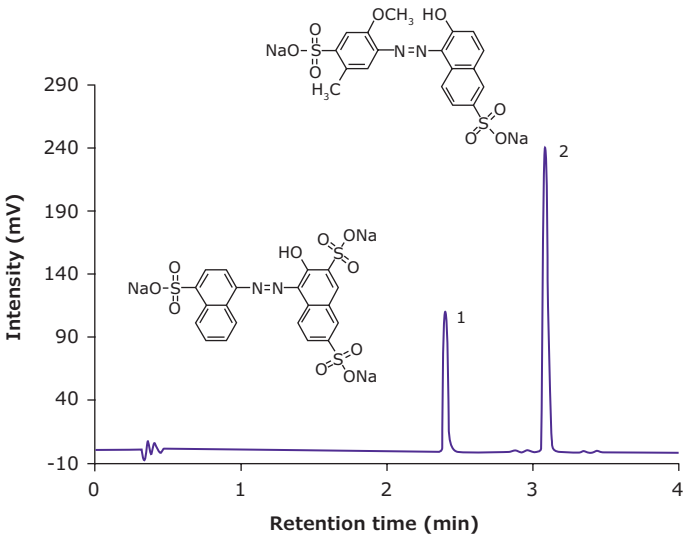
Column robustness

Chromolith® columns offer excellent robustness and unsurpassed column lifetime. This not only ensures maximum reliability and versatility, but also minimizes maintenance on the HPLC system. As a result, Chromolith® columns reduce costs per analysis while enhancing data integrity.

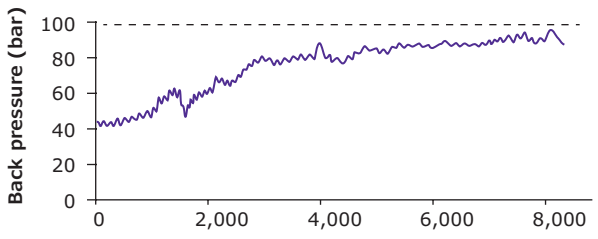
Food colorants in an alcoholic beverage

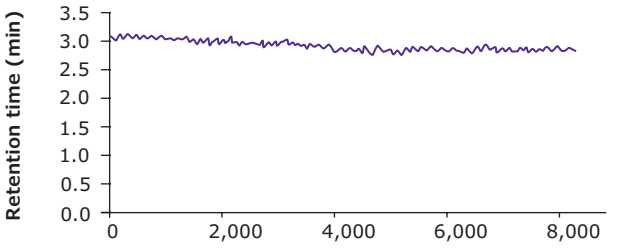
Chromolith® Performance RP-18e

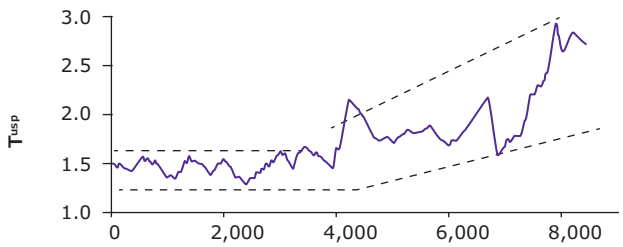
Column	Chromolith® Performance RP-18 endcapped 50 x 2.0 mm		
Mobile phase (v/v)	A: Acetonitrile B: 0.1 % Phosphoric acid in water		
Gradient Program	**Time**	**% A**	**% B**
	0.00 – 0.50 min	0	100
	0.50 – 4.50 min	0 → 50	100 → 50
	4.50 – 5.00 min	50 → 95	50 → 5
	5.00 – 6.00 min	95	5
	6.00 – 7.00 min	95 → 0	5 → 100
Pressure Drop	45 – 40 bar (648 – 556 psi)		
Detection	UV 500 nm		
Cell	1.4 µL		
Flow Rate	0.4 mL/min		
Temperature	25 °C		
Injection volume	2 µL		
Sample	Prior to analysis, the sample was filtered using a syringe equipped with a 0.45 µm filter disc.		

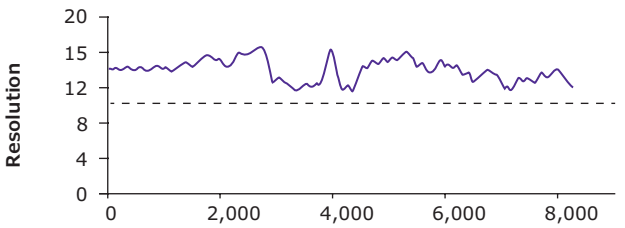


Analysis of colorants in rum. Two food colorants in rum, E 123 (Amaranth) and E 129 (Allura Red AC), were analyzed to illustrate the long-term performance and method robustness of Chromolith® columns. More than 8,000 samples (total volume of injected sample: 16 mL + 30 L mobile phase) were analyzed on a 50 x 2.0 mm Chromolith® RP-18 endcapped column.









The figures above illustrate how column back pressure, peak shape, and the chromatographic resolution between E129 (Allura Red AC) and E123 (Amaranth) are affected with time; 8,300 samples were analyzed. The largest effects are seen on peak shape and back pressure. A similar TUSP value is obtained over the first 4,000 injections, after which some deterioration is observed. Despite aging of the column, peak integration and thereby accurate quantitation of the two analytes is achieved. The column back pressure increases with time as sample matrix is accumulated in the column but never reaches over 100 bar (1,450 psi). The chromatographic resolution between the analytes is substantial (Rs >10) with good overall retentivity and no additional disturbing peaks are found in the chromatogram (UV detection at 500 nm).

Make your column longer for extra high resolution

The Chromolith® HPLC column coupler is designed for linking several monolithic columns together in order to further increase separation efficiency and column performance. The combination results in a theoretical plate count that is significantly higher than any particulate column available. At the same time, pressure is kept well below the HPLC system limit.

The superior column performance achieved by using the Chromolith® column coupler allows you to solve highly critical separation problems in which resolution is a limiting factor. This makes column coupling perfect for chromatographic separations of typically non-separable, complex mixtures.

The table shows a comparison between Chromolith® HPLC columns and particulate columns. The coupling of just two Chromolith® Performance RP-18 endcapped columns yields a separation efficiency of 19,000 theoretical plates per column, which is usually the maximum for particulate columns.

Typical column efficiency using the Chromolith® column coupler

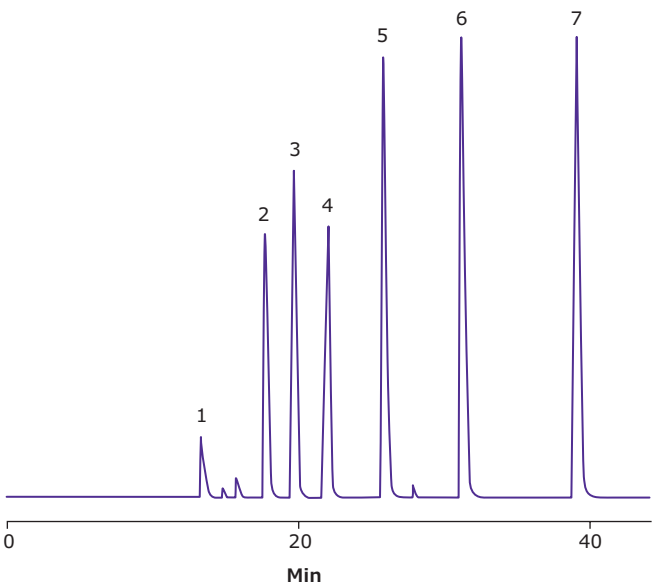
Column	Length (mm)	Pressure * (bar)	Plate number per column (Anthracene)
Chromolith® Performance 1x	100	30	10,000
Chromolith® Performance 2x	200	60	19,000
Chromolith® Performance 3x	300	90	27,000
Chromolith® Performance 4x	400	120	35,000
Chromolith® Performance 5x	500	150	41,000
Particulate column (5 µm)	250	220	18,500
Particulate column (3.5 µm)	150	400	19,000

Pressure * = 3 mL/min 75% acetonitrile, 25% water

Application of Chromolith® column coupler

81,000 plates at 85 bar pressure

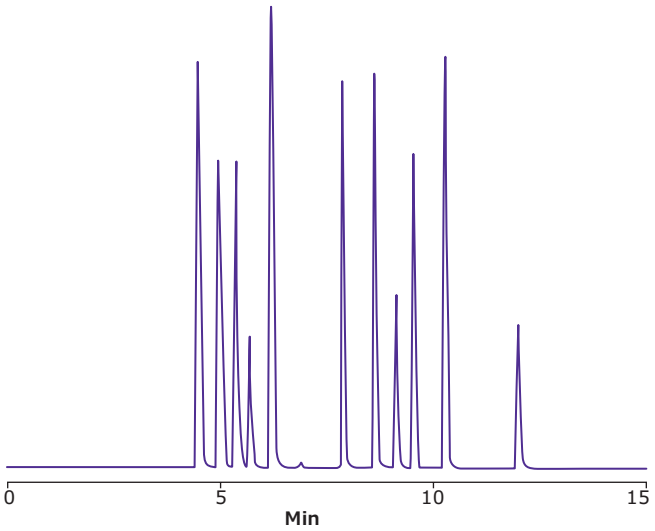
Column	10 columns of Chromolith® Performance RP-18 endcapped 100-4.6 mm
Mobile phase	80 /20 Acetonitrile / water
Flow rate	1 mL/min
Detection	UV 254 nm
Temperature	ambient
Injection volume	10 µL
Sample	1. Thiourea 2. Benzene 3. Toluene 4. Ethylbenzene 5. Propylbenzene 6. Butylbenzene 7. Pentylbenzene



Column coupling: 11 steroids

Chromolith® HighResolution RP-18e
2 x 100-4.6 mm / 1 x 50-4.6 mm

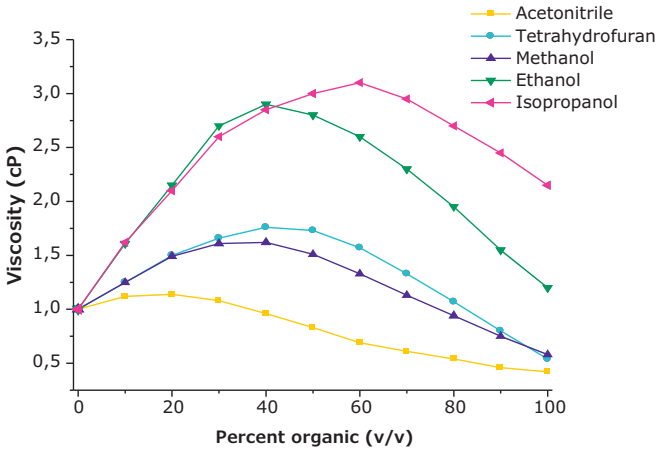
Column	Chromolith® HighResolution RP-18 endcapped 2 x 100-4.6 mm / 1 x 50-4.6 mm		
Mobile phase	ACN / water		
Gradient	**t (min)**	**ACN (%)**	**Water (%)**
	0	55	45
	2	95	5
	15	95	5
Flow rate	1 mL/ min		
Column pressure	30 – 68 bar		
LC system	LaChrom® L7000		
Detection	UV = 240 nm		
Vol. detector cell	16 µL		
Temperature	ambient		
Injection volume	10 µL		
Sample	1. Fluoxymesterone 2. Boldenone 3. Methandrostenolone 4. Testosterone 5. Methyltestosterone 6. Boldenone-Acetate 7. Testosterone-Acetate 8. Nandrolone-Propionate 9. Testosterone-Propionate 10. Nandrolone-Phenylpropionate 11. Testosterone-Isocaproate		



Full flexibility with mobile phases

The most common organic solvent in HPLC/UHPLC is acetonitrile because of low UV absorbance >200nm, and low viscosity what helps to minimize column back pressure. When UV detection is set to above 220nm, methanol also could be successfully used. However all organic solvents used in HPLC /UHPLC have higher viscosities than acetonitrile when mixed with water. Therefore column back pressure could be up to 1.7 times higher, what could breach max instrument back pressure limit as well as shorten column life time.

Since Chromolith® columns have such a low resistance to the flow, and high mechanical stability, if the detection method is UV then any HPLC solvent with sufficient UV transparency can be used e.g. methanol, ethanol or even isopropanol. The possibility to use other organic solvents (not only acetonitrile) could help to optimize the separation method to obtain better resolution, as well as providing other benefits. For example, methanol is more basic but less dipolar then acetonitrile; isopropanol tends to be less denaturing to biomolecules than methanol; ethanol is “greener” and less dangerous to chromatographers; buffer solubility is much better in methanol containing mobile phases compared to acetonitrile.

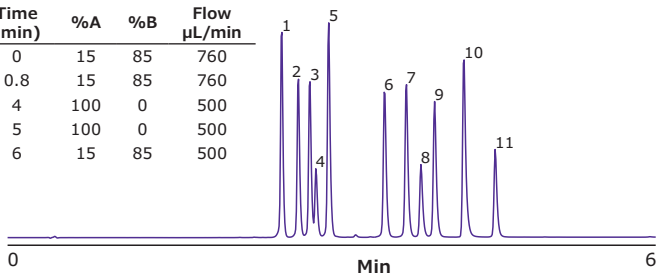


Doping drug separation using ACN and MeOH

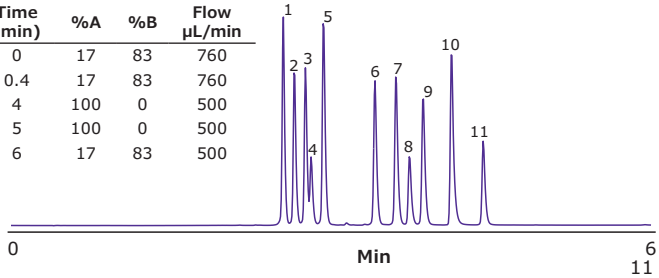
Chromolith® Performance RP-18e 100-2mm

Sample	Fluoxymesterone, Boldenone, Methandrostenolone, Testosterone, Methyltestosterone, Boldenone Acetate, Testosterone Acetate, Nandrolone Propionate, Testosterone Propionate, Nandrolone Phenylpropionate, Testosterone Isocaproate (dissolved in ACN/water 60/40 (v/v))
Mobile phase	A - ACN or MeOH, B – water
Injection	0.2 µL, Detection: UV 240 nm

Time (min)	%A	%B	Flow µL/min
0	15	85	760
0.8	15	85	760
4	100	0	500
5	100	0	500
6	15	85	500



Time (min)	%A	%B	Flow µL/min
0	17	83	760
0.4	17	83	760
4	100	0	500
5	100	0	500
6	17	83	500



Chromolith® Capillary columns

0.05 mm / 0.1 mm / 0.2 mm i.d.

Monolithic capillary columns have become increasingly important in the separation of biomolecules, especially in combination with mass spectrometry. In contrast to particulate columns, monolithic capillaries do not require frits, and have a much lower tendency to clog. This allows higher flow rates, improving the speed and quality of biomolecule characterization. To answer the growing interest in micro and nano-HPLC, we offer a wide range of outstanding monolithic silica capillaries with a variety of internal diameters, bonded phases, pore structures, and lengths.

Why choose capillary Chromolith® columns?

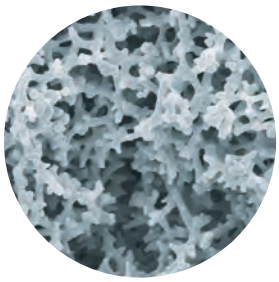
- Higher flow rates than particle-packed capillary columns at low pressure
- Long column lifetime
- Robust and easy handling
- Flow rates from 0.1–200 µL/min ensure ideal compatibility with LC/MS systems, with both ESI and APCI interfaces

Chromolith® CapRod®

Chromolith® CapRod® analytical capillary columns are supplied complete with sleeves and standard 1/16" PEEK fittings to allow for direct coupling to a UV detector or mass spectrometer.

A Chromolith® CapRod® capillary column combines the speed of monolithic silica technology with the sensitivity of nano-LC. This enables superior productivity for high throughput, highly sensitive proteomics-LC applications. Compared to particulate capillary columns, Chromolith® CapRod® capillaries demonstrate better performance with optimal resolution (narrow peak widths), increased productivity (higher sample throughput), and extended column lifetime. Furthermore, column length is less limited than with any other type of column. The capillaries can even be slightly bent to fit any LC configuration or instrument. Chromolith® CapRod® columns are designed to work with various nano or capillary-LC systems. This provides the highest efficiency and performance when coupled to mass spectrometers, both on-line (ESI, nanospray) and off-line (MALDI).

Compared to classical micro-particulate sorbents, Chromolith® CapRod® column can be operated at higher flow rates—without loss of performance, resolution, or limitations due to column back pressure. Separations can be achieved at 1–3 µL/min, compared to 200–400 nL/min for conventional media on a standard 100 µm LC capillary column. For more complex biological samples, a trapping capillary can be used to protect the separation column, and optimize separation efficiency.



Cross section of the bimodal pore structure of CapRod® column with macropores at approx. 2 µm (1 µm for Chromolith® HighResolution columns) and mesopores of 13 nm. The outer diameter of the capillary is 360 nm.

Recommended use and flow rate ranges

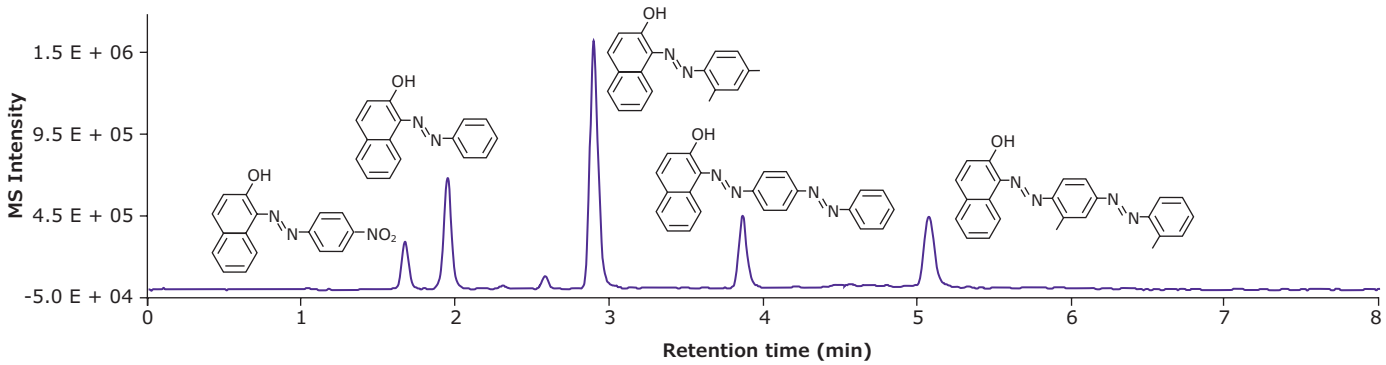
Recommended use	RP-18e 150 x 0.05 mm	RP-8e 150 x 0.1 mm	RP-18e 50 x 0.1 mm Trap	RP-18e 150 x 0.1 mm	RP-18e 300 x 0.1 mm	RP-18e 150 x 0.1 mm HR	RP-18e 50 x 0.2 mm Trap	RP-18e 150 x 0.2 mm	RP-18e 150 x 0.2 mm HR
Separation of small molecules	●		●	●	●	●	●	●	●
– of peptides	●	●	●	●	●	●	●	●	●
– of proteins		●							
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 back pressure (bar)	200	200	200	200	200	218	218	218	218

Separation example: Sudan dyes

Chromolith® CapRod® RP-18e 150-0.1 mm

Column	Chromolith® CapRod® RP-18 endcapped 150 x 0.1 mm
Mobile phase	A: Water + 0.1 % formic acid B: Acetonitrile + 0.1 % formic acid
Gradient	70 % B to 95 % B in 5 min
Flow rate	1.24 µL/min
Pressure Drop	76 bar (1,100 psi)
Detection	nano-ESI(+) 240 – 390 m/z
Temperature	ambient
Diluent	Acetonitrile
Injection volume	2.5 nL

Sample	Compound	Retention Time (min)	Monoisotopic mass (g/mol)	(M+H)+ (m/z)
	1. Para red	1.67	293.08	294.01
	2. Sudan I	1.95	248.09	249.01
	3. Sudan II	2.9	276.13	277.09
	4. Sudan III	3.87	352.13	353.08
	5. Sudan IV	5.08	380.16	381.11

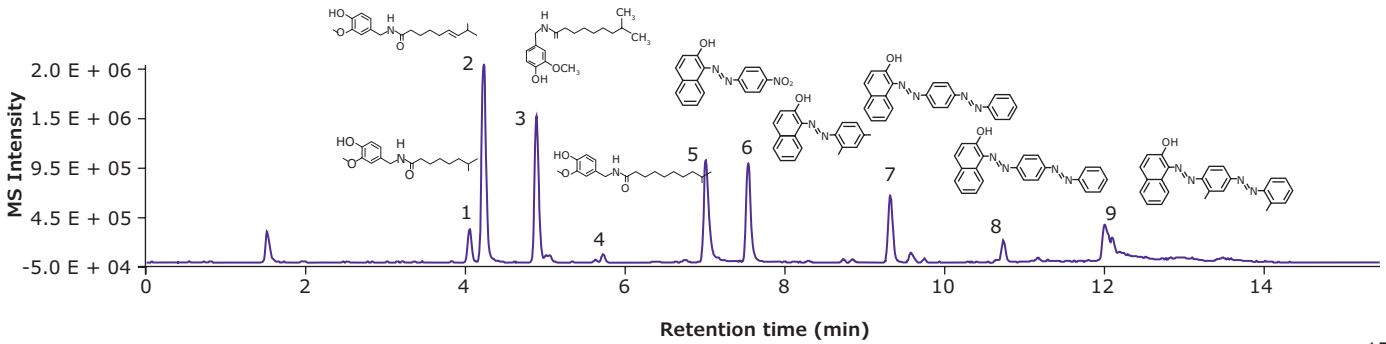


Separation example: Sudan dyes and capsaicinoids in hot chili sauce

Chromolith® CapRod® RP-18e 150-0.1 mm

Column	Chromolith® CapRod® RP-18 endcapped 150 x 0.1 mm
Mobile phase	A: Water + 0.1 % formic acid B: Acetonitrile + 0.1 % formic acid
Gradient	35 % B to 95 % B in 12 min
Flow rate	1.24 µL/min
Pressure Drop	80 bar (1,160 psi)
Detection	nano-ESI(+) 100 – 600 m/z
Temperature	ambient
Diluent	Acetonitrile
Injection volume	2.5 nL

Sample	Compound	Retention Time (min)	Monoiso-topic mass (g/mol)	(M+H)+ (m/z)
	1. Nordihydrocapsaicin	4.05	293.2	294.11
	2. Capsaicin	4.23	305.2	306.18
	3. Dihydrocapsaicin	4.9	307.21	308.19
	4. Homodihydrocapsaicin	5.73	321.23	322.11
	5. Para red	7.02	293.08	294.05
	6. Sudan I	7.55	248.09	249.05
	7. Sudan II	9.33	276.13	277.1
	8. Sudan III	10.74	352.13	353.11
	9. Sudan IV	12.11	380.16	381.21



Chromolith® Analytical columns, 130/150Å mesopores

2 mm / 3 mm / 4.6 mm i.d.

Standard HPLC columns with 3 or 5 µm silica particles often suffer from high back pressure. Hence, they are limited in length, and have a lower number of theoretical plates.

Chromolith® HPLC columns are not packed with small particles. Instead, each column consists of a single monolithic rod of high-purity polymeric silica gel with a revolutionary bimodal pore structure. This allows excellent separations in a fraction of the time that a standard particulate column takes.

Why choose analytical Chromolith® columns?
Fast, high-performance results
Substantially longer column lifetime
High resistance to column blockage
Cost savings from higher sample throughput and column durability
Compatible with all low dead volume LC instruments (UHPLC, UPLC, HPLC)
Possibility of flow gradients
Increased column performance by column coupling

Chromolith® RP-18 endcapped

The chemical basis of Chromolith® RP-18 endcapped columns–from starting materials to surface modifications—is the same as high-end particulate columns. Thus, their selectivity is comparable to high-quality C18 endcapped reversed-phase packed columns. This allows the use of standard methods when developing new protocols. The columns are based on high-purity silica, hence they minimize the negative effect of trace metals. Furthermore, they are chemically modified with n-alkyl chains that possess a high ligand density, and are fully endcapped to reduce the effect of unmodified silanol groups.

Chromolith® RP-18 endcapped 2 mm i.d. columns: Ultra-high performance on any instrument

Ultra-high performance and extremely low operating pressure make Chromolith® 2 mm columns truly unique. Excellent, ultra-fast results are obtained, not only in the new UHPLC and UPLC® instruments, but equally well in all standard HPLC systems with low dead volume. Chromolith® 2 mm columns have macropores of 1.5 µm in diameter, resulting in a column efficiency that exceeds 100,000 plates/meter. The mesopores are 13 nm (130 Å) in diameter, and the surface modification is octadecylsilane with full endcapping.

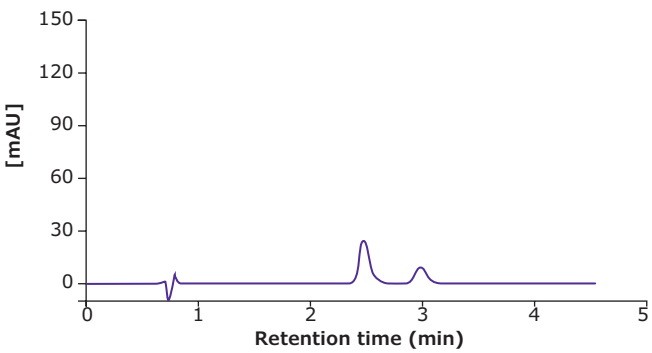
Increase sensitivity and save solvents with 2 mm i.d. Chromolith® RP-18 endcapped columns

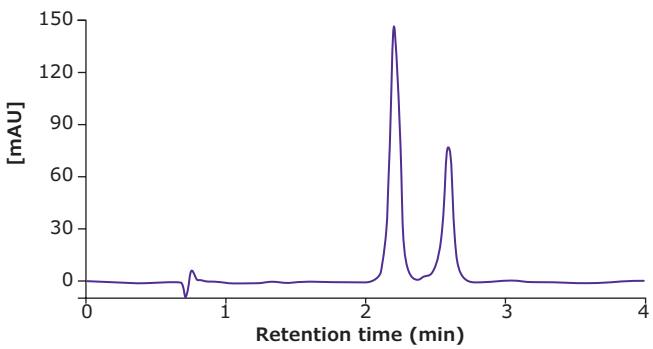
Chromolith® Performance RP-18e 100-4.6 mm

Column	Chromolith® Performance RP-18 endcapped 100-4.6 mm
Mobile phase	A: 100 % Acetonitrile B: 100 % Water + 0.1 % TFA (v/v) C: 100 % Methanol
Isocratic	Initial composition: A/B/C 30/60/10 (v/v/v)
Flow rate	2 mL/min
Pressure	45 bar (4.5 MPa, 65.3 psi)
Detection	Dionex Ultimate 3000 VWD-3400, 2.5 Hz, Response time 0.1 s, UV = 210 nm
Vol. detector cell	11 µL
Temperature	ambient
Injection volume	1 µL
Sample	Bimatoprost Bimatoprost free acid

Chromolith® Performance RP-18e 100-2 mm

Column	Chromolith® Performance RP-18 endcapped 100-2 mm
Mobile phase	A: 100 % Acetonitrile B: 100 % Water + 0.05 % TFA (v/v) C: 100 % Methanol
Isocratic	Initial composition: A/B/C 30/60/10 (v/v/v)
Flow rate	380 µL/min
Pressure	48 bar (4.8 MPa, 70 psi)
Detection	Dionex Ultimate 3000 VWD-3400, 2.5 Hz, Response time 0.1 s, UV = 210 nm
Vol. detector cell	1.4 µL
Temperature	ambient
Injection volume	1 µL
Sample	Bimatoprost Bimatoprost free acid



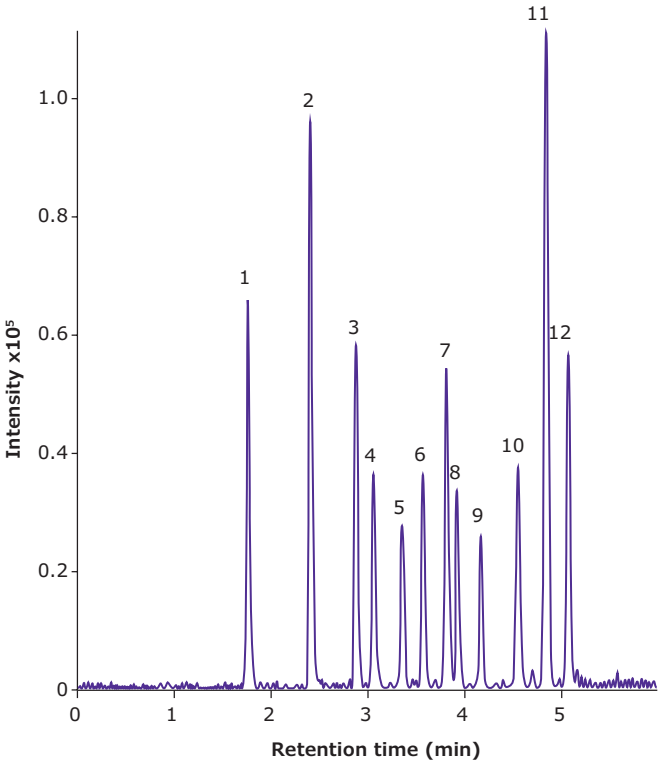


The same separation on a Chromolith® 2 mm i.d. column demonstrates improved sensitivity and solvent savings of 81 %.

Separation of Steroids & Metabolites

Chromolith® FastGradient RP-18e 50-2 mm

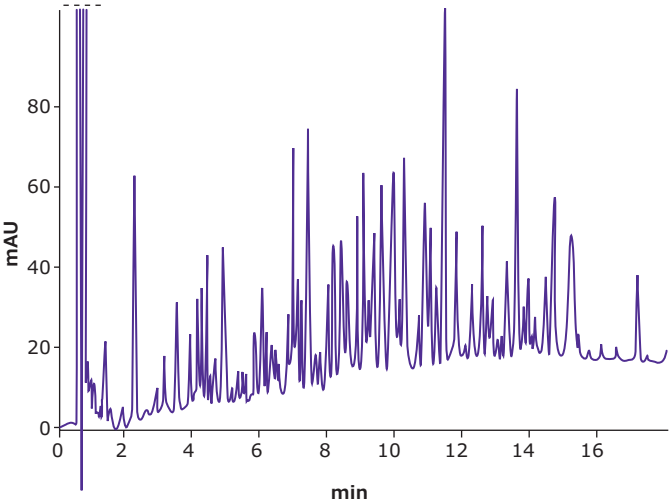
Column	Chromolith® FastGradient RP-18 endcapped 50-2 mm		
Mobile phase	A: ACN + 0.1 % HCOOH B: Water + 0.1 % HCOOH		
Gradient	**Time**	**% A**	**% B**
	0	15	85
	4.5	70	30
	6	70	30
Flow rate	0.5 mL/min		
Pressure	55 – 85 bar		
Detection	MS; Ion Source: ESI; Ion Trap		
Sample	1. Metabolite of Fluoxymesterone		353 m/z
	2. Metabolite of Stanozolol		345 m/z
	3. Metabolite of Danazol		343 m/z
	4. Testosterone		289 m/z
	5. Epitestosterone		289 m/z
	6. Metabolite of Methyltestosterone		271 m/z
	7. Metabolite of Calusterone		285 m/z
	8. Metabolite of Clostebol		305 m/z
	9. Boldenone-acetate		329 m/z
	10. Testosterone-acetate		331 m/z
	11. Nandrolone-17-Propionate		331 m/z
	12. Testosterone-Propionate		345 m/z



Separation example: Proteomics

Chromolith® Performance RP-18e 100-2 mm

Column	Chromolith® Performance RP-18 endcapped 100-2 mm
Mobile phase	A: 95 % H_2O/5 % ACN/0.1 % TFA (v/v/v) B: 5 % H_2O/95 % ACN/0.085 % TFA (v/v/v)
Gradient	from 5 % B to 50 % B in 20 min
Flow rate	0.3 mL/min
Detection	UV 214 nm
Sample	1 µL BSA digest (1 mg/mL)



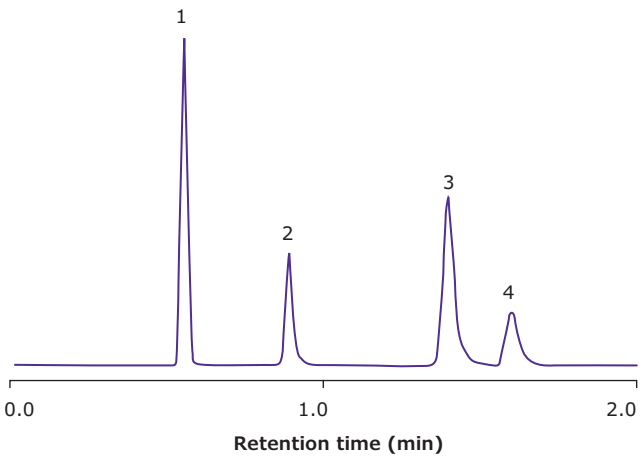
Chromolith® RP-18 endcapped 3 mm i.d. columns: Fast and solvent saving separations at lower flow rates

Chromolith® Performance RP-18 endcapped 100-3 mm column is the ideal alternative to conventional particulate columns with internal diameters of 3, 4 or 4.6 mm. Even difficult separations, which often take 15 to 30 minutes on particulate columns, typically only require 5 to 10 minutes on Chromolith® 3 mm column. Furthermore, the columns can be easily linked using the column coupler to produce columns of 20 cm or more. As shown below, this results in very high peak resolution at moderate pressure, with flow rates between 1 to 1.5 mL/min.

Chromolith® Performance RP-18e 100-4.6 mm

Column	Chromolith® Performance RP-18 endcapped 100-4.6 mm
Mobile phase	Acetonitrile / water 40/60
Flow rate	4.0 mL/min
Pressure	137 bar
Detection	UV 254 nm 2.4 µL flow cell*
Temperature	ambient
Injection volume	1 µL*
Sample	1. Biphenyl-4,4'-ol 2. Biphenyl-2,2'-ol 3. Biphenyl-4-ol 4. Biphenyl-2-ol

* For optimum results with 3 mm columns, extra-column volume must be small.

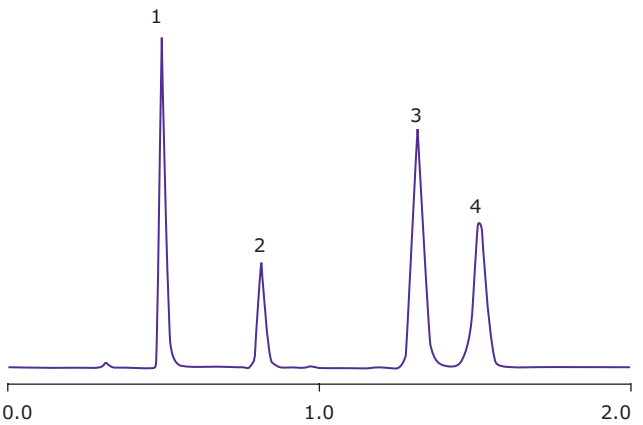


A typical fast separation of four compounds in less than two minutes using a Chromolith® 4.6 mm i.d. column at 4 mL/min.

Chromolith® Performance RP-18e 100-3 mm

Column	Chromolith® Performance RP-18 endcapped 100-3 mm
Mobile phase	Acetonitrile / water 40/60
Flow rate	1.7 mL/min
Pressure	100 bar
Detection	UV 254 nm 2.4 µL flow cell*
Temperature	ambient
Injection volume	1 µL*
Sample	1. Biphenyl-4,4'-ol 2. Biphenyl-2,2'-ol 3. Biphenyl-4-ol 4. Biphenyl-2-ol

* For optimum results with 3 mm columns, extra-column volume must be small.



The same separation on a Chromolith® 3 mm i.d. column demonstrates improved sensitivity at just 1.7 mL/min. This equates to solvent savings of 57 %.

Chromolith® HighResolution RP-18 endcapped

Chromolith® 4.6 mm i.d. RP-18 endcapped columns: The faster way to trouble-free separations

Chromolith® 4.6 mm i.d. columns represent the most commonly used column dimension. They are compatible with all standard HPLC instruments, and allow a wide range of flow rates, from 0.6 to 4.5 mL/min. These columns are available in two versions: standard columns with 2 µm macropores, and HighResolution columns with 1.15 µm macropores.

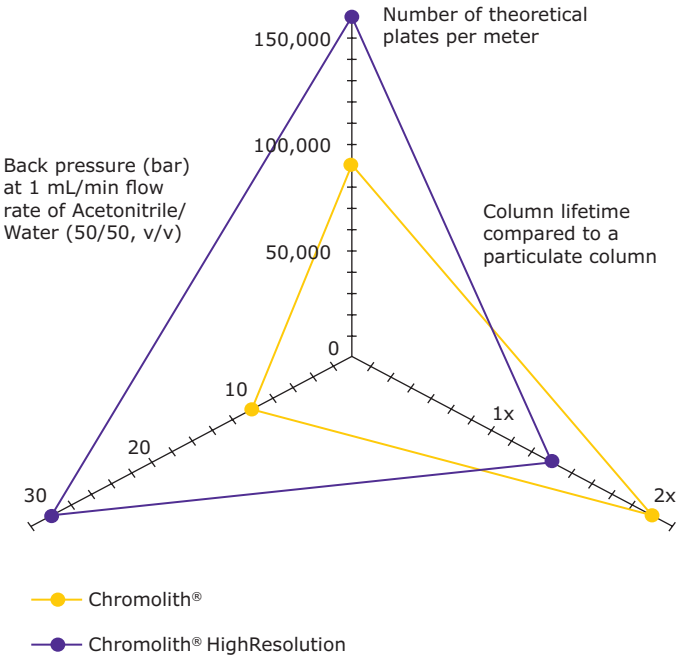
Standard Chromolith® columns have 2 µm macropores, and an efficiency equal to 4.5 µm particulate columns. They allow very high flow rates, extreme throughputs, and the analysis of relatively dirty samples. The lifetime of these columns is particularly long. If necessary, up to ten Chromolith® columns can be coupled in a row to enhance efficiency and resolution.

In contrast, Chromolith® HighResolution (HR) columns possess 1.15 µm macropores, which results in higher efficiency and improved peak shape. Although this causes higher back pressure, it is still less than half that of any particulate column of the similar efficiency.

Chromolith® HighResolution RP-18 endcapped

Comparison: Chromolith® and Chromolith® HighResolution

Chromolith® HighResolution columns have around 50% higher efficiency, excellent peak symmetry and still more than 30 % longer lifetime compared with particulate columns. Two Chromolith® HighResolution columns could be easily coupled in order to achieve even higher resolution. The completely endcapped stationary phase enables peak-tailing free elution of basic compounds.

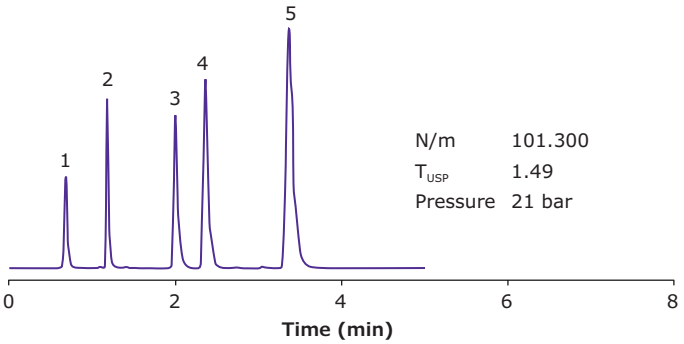


Our classical Chromolith® columns are recommended for analyzing matrix-rich samples, as this type of column will have a longer lifetime. Also, lower back pressure would allow column coupling if necessary.

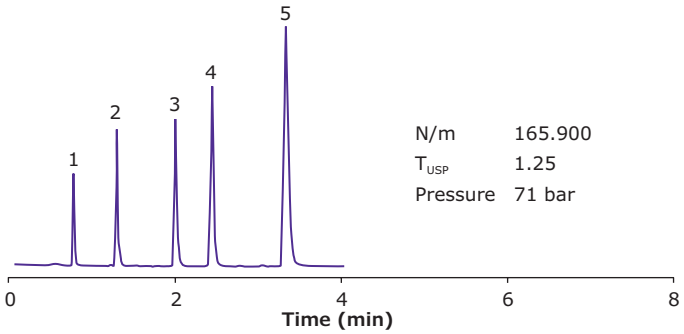
Higher efficiency, symmetrical peaks

Mobile phase	Acetonitrile / water 60/40
Flow rate	2 mL/min
Detection	UV 254 nm
Temperature	ambient
Injection volume	5 µL
Sample	1. Urea 2. Biphenyl-2-ol 3. Progesterone 4. Hexanophenon 5. Anthracene

Chromolith® Performance RP-18e 100-4.6 mm



Chromolith® HR RP-18e 100-4.6 mm

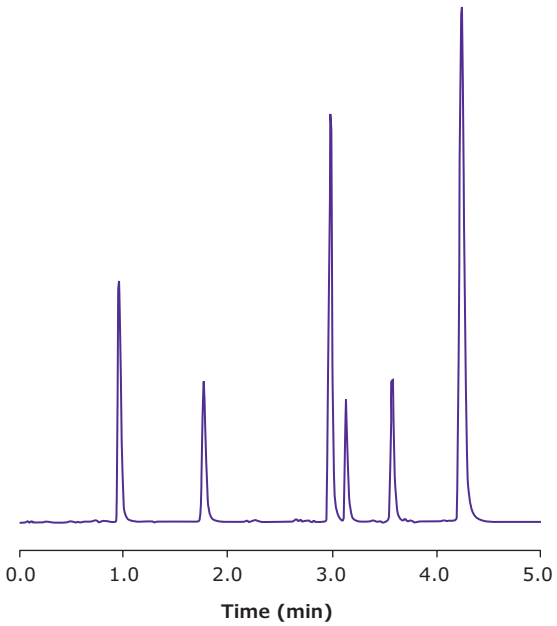


Improved peak shape for basic compounds

The completely endcapped stationary phase enables the elution of basic compounds with significantly less tailing.

Chromolith® HighResolution RP-18e 100-4.6 mm

Column	Chromolith® HighResolution RP-18 endcapped 100-4.6 mm
Mobile phase	A: ACN B: 20 mM NaH_2PO_4 buffer pH 7.6
Gradient	0 min 20% A 0.5 min 45% A
Flow rate	2 mL/min
Column pressure	63 – 69 bar
Detection	UV 254 nm
Vol. detector cell	16 µL
Temperature	ambient
Injection volume	1 µL
Sample	1. Caffeine 2. Aniline 3. N-Methylaniline 4. 2-Ethylaniline 5. 4-Nitranisole 6. N,N-Dimethylaniline

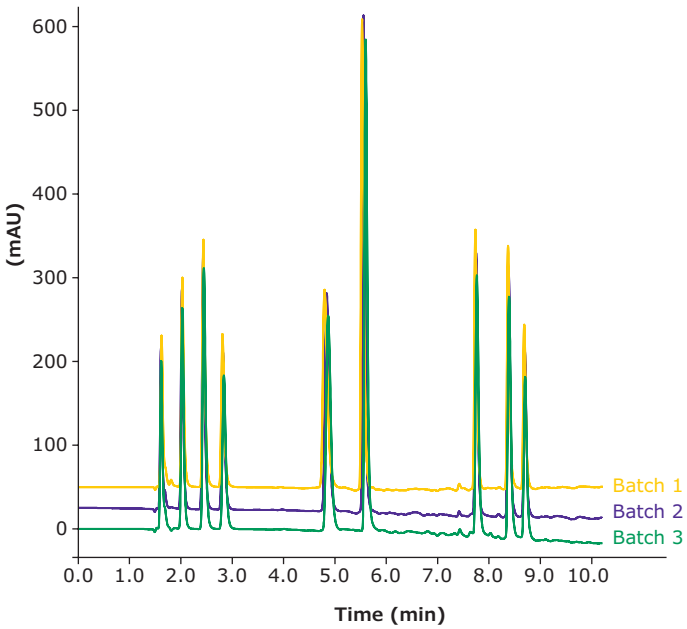


Excellent batch-to-batch reproducibility

The batch-to-batch reproducibility of Chromolith® HPLC columns is strictly controlled and fulfills the requirements of QA and QC laboratories.

Chromolith® HighResolution RP-18e 100-4.6 mm

Column	Chromolith® HighResolution RP-18 endcapped 100-4.6 mm
Mobile phase	A: Acetonitrile + 0.1 % TFA B: Water + 0.1 % TFA
Gradient	2 min 0% A 10 min 30% A
Flow rate	1 mL/min
Detection	UV 210 nm
Temperature	25°C
Injection volume	2 µL
Sample	1. Norepinephrine 2. Octopamine 3. Epinephrine tartrate 4. Dopamine 5. DOPA 6. Norephedrine 7. Ephedrine 8. N-Methylephedrine



The ideal alternative to sub-3 µm particulate columns

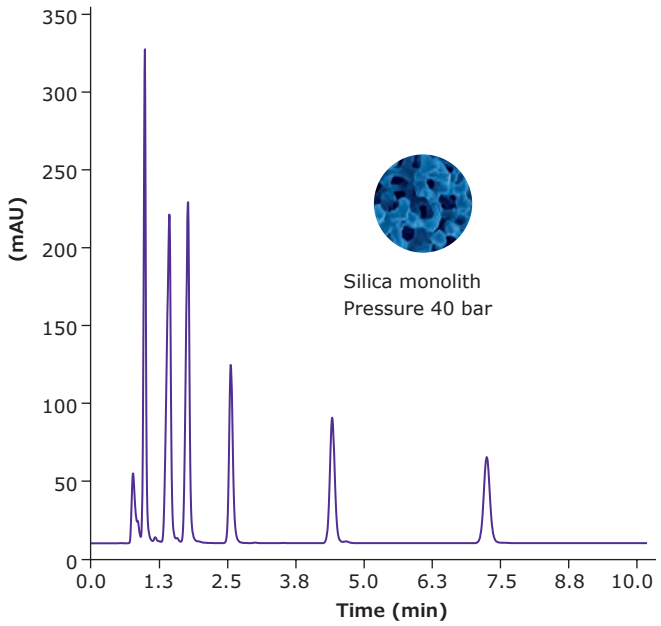
At a flow rate of 1 mL/min, a chromatogram run on a Chromolith® HR column is almost identical to one run on a particulate column with sub-3 µm particles. Chromolith® HR column also delivers similar results to a column packed with 2.6 µm i.d. core-shell particles – however at much lower back pressures.

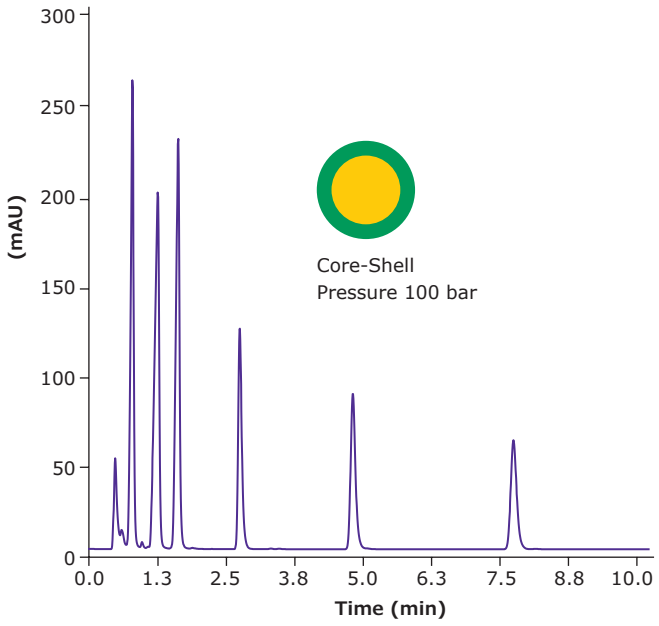
Chromolith® HR Silica monolith

Column	Chromolith® HighResolution RP-18 endcapped 50-4.6 mm, Silica monolith		
Mobile phase	A: Acetonitrile B: 20 mM Phosphate buffer pH 4.5		
Gradient	**Time/min**	**% A**	**% B**
	0	20	80
	12	40	60
Flow rate	1.0 mL/min		
Pressure	40 bar		
Detection	UV 230 nm		
Temperature	22°C		
Injection volume	2 µL		
Sample	1. Ascorbic acid 2. 4-Hydroxybenzoic acid 3. Benzoic acid 4. Sorbic acid 5. Methyl 4-hydroxybenzoate 6. Ethyl 4-hydroxybenzoate 7. Propyl 4-hydroxybenzoate		

Core-shell, 2.6 µm particles

Column	Core-shell RP-18 endcapped 50-4.6 mm, 2.6 µm particles		
Mobile phase	A: Acetonitrile B: 20 mM Phosphate buffer pH 4.5		
Gradient	**Time/min**	**% A**	**% B**
	0.0	20	80
	12	40	60
Flow rate	1.0 mL/min		
Pressure	100 bar		
Detection	UV 230 nm		
Temperature	22°C		
Injection volume	2 µL		
Sample	1. Ascorbic acid 2. 4-Hydroxybenzoic acid 3. Benzoic acid 4. Sorbic acid 5. Methyl 4-hydroxybenzoate 6. Ethyl 4-hydroxybenzoate 7. Propyl 4-hydroxybenzoate		





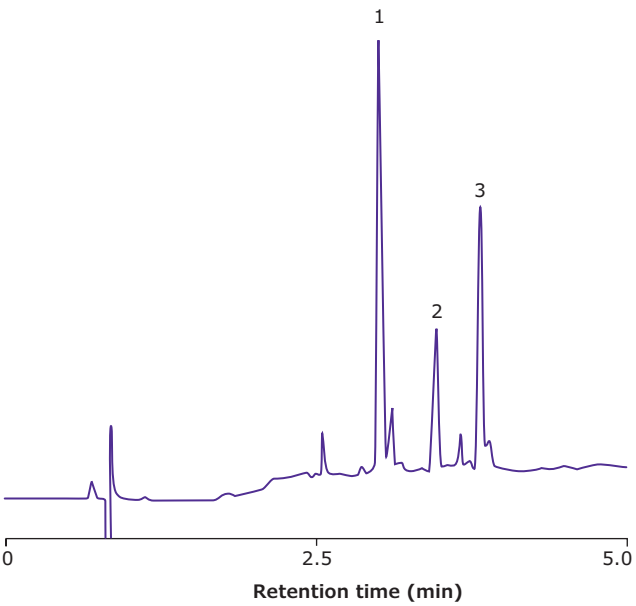
Chromolith® & Chromolith® HR RP-8 endcapped

With its shorter alkyl chain, Chromolith® & Chromolith® HR RP-8 endcapped columns offer less retention and slightly different selectivity than Chromolith® RP-18 endcapped columns. Thus, it is possible to achieve a baseline separation on the RP-8 endcapped bonded column, whereas no separation at all is observed under identical elution conditions on a RP-18 endcapped bonded silica column. Chromolith® RP-8 endcapped HPLC columns offer all the benefits of monolithic silica technology for reversed-phase chromatography.

Separation examples

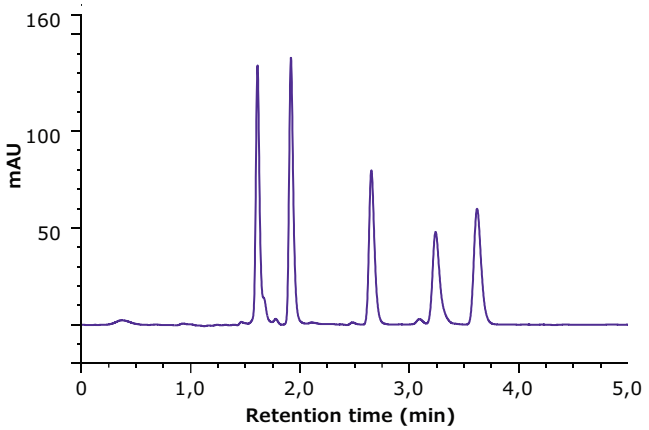
Chromolith® Performance RP-8e 100-4.6 mm

Column	Chromolith® Performance RP-8 endcapped 100x4.6 mm		
Mobile phase	A: Acetonitrile / water 90/10 + 0.1% TFA B: 0.1% TFA in water		
Gradient	**Time/min**	**% A**	**% B**
	0	45	55
	1	90	10
	3	90	10
Flow rate	2 mL/min		
Pressure	30 – 40 bar		
Detection	214 nm		
Temperature	ambient		
Injection volume	30 µL		
Sample	1. (Sar1, Ala8)-Angiotensine II		87 µg/mL
	2. (Sar1, Ile8)-Angiotensine II		87 µg/mL
	3. Angiotensine I		47 µg/mL



Chromolith® HighResolution RP-8e 100-4.6 mm

Column	Chromolith® HighResolution RP-8 endcapped 100x4.6 mm
Mobile phase	ACN/ Water 25/75 + 0,04% TFA Isocratic
Pressure	49 bar
Detection	UV 220 nm
Temperature	ambient
Injection volume	0,5 µL
Flow	1 mL/min
Sample	Gly-Tyr, Val-Tyr-Val, Met enkephalin, Leu enkephalin, Angiotensin II 0,5 mg/mL each



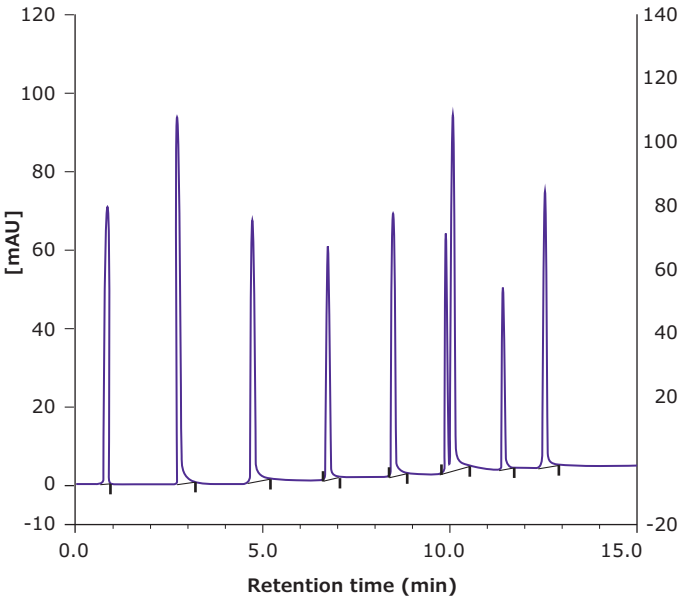
Chromolith® Phenyl

Due to their π-π interactions, Chromolith® Phenyl HPLC columns offer greater selectivity towards aromatic ring-containing compounds than standard alkyl phases. These columns are ideal for the separation of aromatic compounds, flavonoids, fatty acids, PAH, preservatives, purines and pyrimidines.

Separation examples

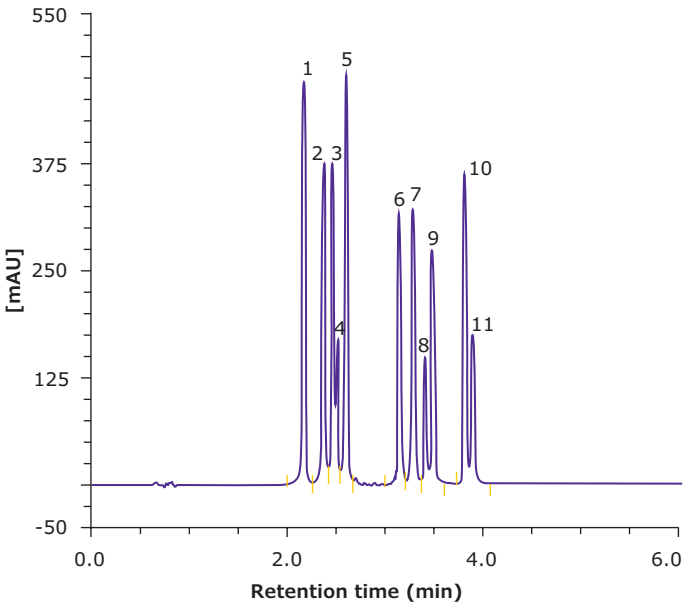
Chromolith® Phenyl 100-4.6 mm

Column	Chromolith® Phenyl 100x4.6 mm
Mobile phase	A: ACN / B: H2O
Gradient	0 min 22% A 15 min 85% A 5 min 95% A
Flow rate	2 mL/min
Detection	UV 254 nm, response time 0.1 s
Injection volume	2 µL
Sample	1. Thiourea 2. Acetanillide 3. Acetophenone 4. Propionphenone 5. Butyrophenone 6. Benzophenone 7. Valerophenone 8. Hexanophenone 9. Heptanophenone dissolved in 100 mL Methanol/Water 90/10



Chromolith® Phenyl 100-4.6 mm

Column	Chromolith® Phenyl 100x4.6 mm		
Injection	1 µL		
Detection	5Hz, Response Time 0.1s, UV=260nm		
Cell	11 µL		
Flow Rate	1 mL/min		
Mobile Phase	A: 100% 10mM Phosphate buffer pH 3.0 B: 100% Acetonitrile		
Gradient	**Time/min**	**% A**	**% B**
	0-10	95	5
	10-15	95≥5	5≥95
Temperature	25°C		
Diluent	Standard: 100% Ethanol		
Sample	Mandelic acid 2,9 mg/mL Day-Cream 50 mg		
Sample prep.	50 mg Day-Cream weighed into a 25 mL volumetric flask and filled up with Ethanol. The suspension was sonicated for 20 min. The solution was filtered through a 0.45 µm filter directly into the vial.		
Pressure Drop	21 - 28 Bar (305- 406 psi)		



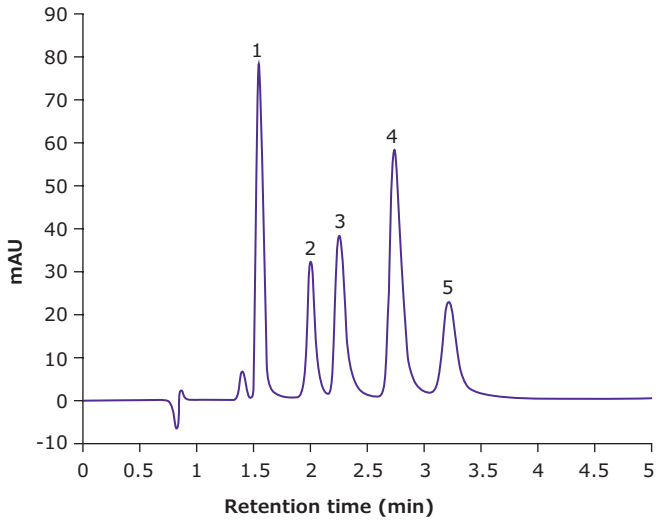
Chromolith® CN

Cyano columns are generally more polar than traditional alkyl silica columns. The functional groups are highly ordered, reducing steric hindrance for the solute. The modification also allows cation exchange activity, which is higher at neutral pH than in acidic conditions. Chromolith® CN columns are suitable for the separation of alkaloids, oils, flavonoids, glycols, phenols, phthalates, steroids and sulfonamides.

Separation example: Five estrogens

Chromolith® CN 100-4.6 mm

Column	Chromolith® CN 100-4.6 mm	
Mobile phase	Methanol/0.1% TFA 30/70 v/v	
Flow rate	2.0 mL/min	
Detection	220 nm	
Cell volume	11 µL	
Temperature	ambient	
Injection volume	5 µL	
Sample	1. Estriol	9.4 mg/mL
	2. Estradiol	8.7 mg/mL
	3. Testosterone	11.6 mg/mL
	4. Ethynylestradiol	7.9 mg/mL
	5. Estrone	13.3 mg/mL
	Solved in 100 mL ACN/$H_2$0 5/5	



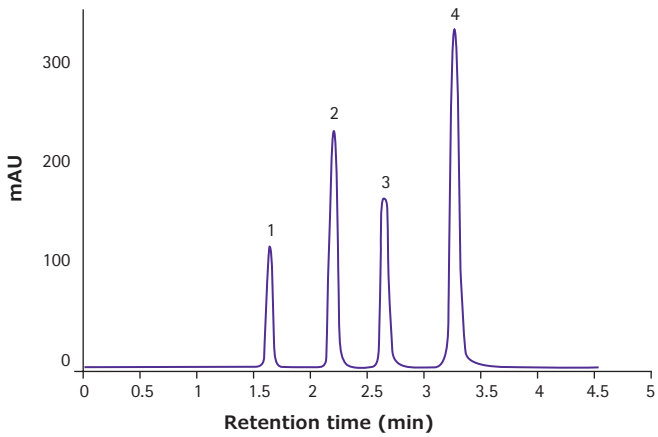
Chromolith® Diol

Chromolith® Diol columns are more versatile than bare silica columns, and often offer improved reproducibility. The bonded phase's hydroxyl groups provide good selectivity without excessive retention. This is due to weaker hydrogen bonding with diol groups than with silanols on a bare silica surface. In aqueous phases, the diol phase can effectively shield the silica surface from interacting with proteins. Diol columns are commonly used for the separation of steroids and sterols under normal-phase conditions. Chromolith® Diol columns are suitable for the separation of alcohols, amino acids, carotinoids, oils, glycols, preservatives, proteins, sugars, sulfonamides, and water-soluble vitamins.

Separation example: Anisole

Chromolith® Diol 100-4.6 mm

Column	Chromolith® Diol 100-4.6 mm	
Mobile phase	n-Heptane / Dioxane 95/5 v/v	
Flow rate	1.3 mL/min	
Detection	254 nm	
Cell volume	11 µL	
Temperature	ambient	
Injection volume	5 µL	
Sample	1. Anisole	390 µg/mL
	2. 3-Nitroanisole	70 µg/mL
	3. 4-Nitroanisole	260 µg/mL
	4. 2-Nitroanisole	180 µg/mL



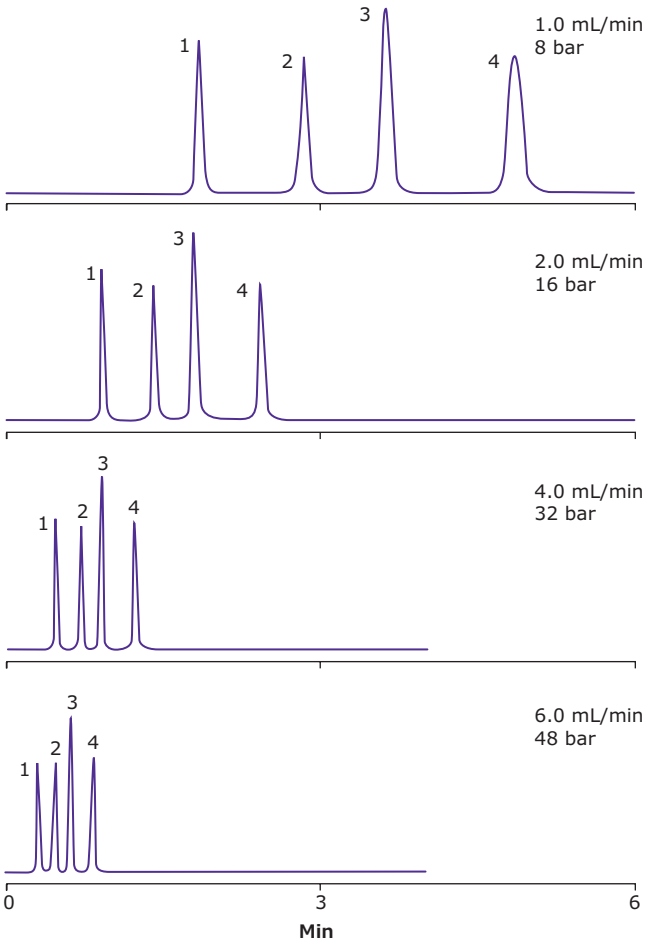
Chromolith® Si

Based on high-purity silica, Chromolith® Si column is designed for normal-phase separations of polar non-ionic organic compounds. The column offers all of the benefits of monolithic silica technology.

Separation examples

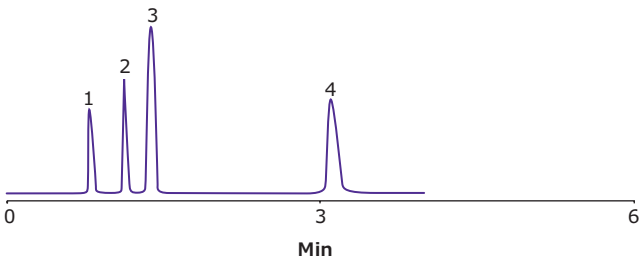
Chromolith® Performance Si 100-4.6 mm

Column	Chromolith® Performance Si 100-4.6 mm	
Mobile phase	n-Heptane / Dioxane 95/5 v/v	
Flow rate	2 mL/min	
Pressure	14 bar	
Detection	254 nm	
Temperature	ambient	
Injection volume	5 µL	
Sample	1. Anisole	0.39 mg/mL
	2. 3-Nitroanisole	0.07 mg/mL
	3. 4-Nitroanisole	0.26 mg/mL
	4. 2-Nitroanisole	0.18 mg/mL



Chromolith® Performance Si 100-4.6 mm

Column	Chromolith® Performance Si 100-4.6 mm	
Mobile phase	n-Heptane / Dioxane 95/5 v/v	
Flow rate	2 mL/min	
Pressure	14 bar	
Detection	254 nm	
Temperature	ambient	
Injection volume	10 µL	
Sample	1. Toluene	0.16 mg/mL
	2. Nitrobenzene	0.02 mg/mL
	3. 2,3-Dimethylanthraquinone	0.02 mg/mL
	4. 2-Nitroacetanilide	0.10 mg/mL



Chromolith® NH_2

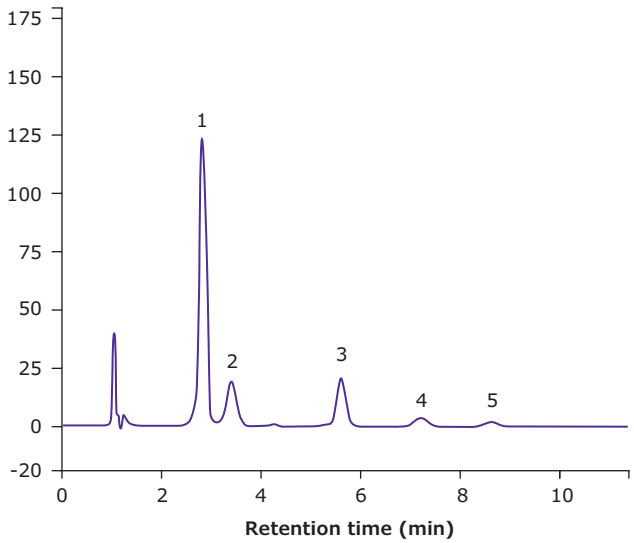
Chromolith® aminopropyl-modified columns possess medium polarity, between those of bare (normal-phase) silica and reversed-phase silica. Consequently, they display hydrophilic as well as hydrophobic properties, and can be used under both reversed-phase and normal-phase conditions. However, retention is weaker than on silica or RP supports. In acidic solutions, the NH_2 groups are protonated ($^{-}NH_3^{+}X^{-}$) and display the characteristics of a weak anion exchanger. Hence, the columns can also be used as ion exchangers.

Chromolith® NH_2 columns offer high matrix tolerance and analysis speed, as well as an extended lifetime within the pH range of 2.5 to 7.5. These columns are suitable for the separation of anions, organic acids, and carbohydrates (mono and disaccharides, such as fructose, glucose, sucrose, maltose and lactose).

Separation example

Chromolith® Performance NH_2 100-4.6 mm

Column	Chromolith® Performance NH2 100-4.6 mm	
Mobile phase	Acetonitrile / Water 80/20	
Flow rate	1.5 mL/min	
Pressure	9 bar	
Detection	190 nm	
Detector cell volume	16 µL	
Temperature	23 °C	
Injection volume	10 µL	
Sample	1. Fructose	53.71 mg
	2. Glucose	46.38 mg
	3. Sucrose	68.75 mg
	4. Maltose	15.71 mg
	5. Lactose	62.05 mg



Chromolith® WP 300 Analytical columns for bioapplications, 300 Å mesopores

4.6 mm i.d.

Biotherapeutics, for example bio-engineered drugs and peptide therapeutics, represent the promise of new medical treatments for the future. Production costs have been falling leading to an extremely high demand for suitable analytical methods for process monitoring and quality control of these biomolecules. HPLC is the preferred method of analysis, and it is therefore important to use the right column for these larger molecules.

Accurate analysis of proteins, antibodies and large peptides requires columns with good permeability, along with better mass transfer and selectivity. In order for size-exclusion not to influence the separation, the pore size should be approximately ten-times larger than the molecule being analyzed.

Chromolith® columns have shown great potential and superiority in comparison to standard silica. In contrast to conventional packed-particle columns, wide pore (300 Å) monolithic silica columns are made of a single continuous bed of high purity porous silica that is then bonded with C18, C8, C4 and Protein A depending on the use of the column.

Monolithic columns remove back pressure as the primary consideration in method development and allow flow rate flexibility for much higher throughput, a choice of column lengths for superior resolution, and more solvent options for optimum selectivity.

With no individual particles to shift or break, column performance is consistent over a much longer lifetime, making them ideal for relatively ”dirty or matrix rich” sample analysis. High permeability also makes them very forgiving of less rigorously prepared samples, in addition to making it easier to aggressively flush out for reequilibration.

Why choose Chromolith® WP 300 columns

- Completely bioinert column hardware
- High biorecovery
- Special selectivities for biomolecules
- Very low column back pressure
- High-speed separation possible
- Substantially longer column lifetime
- High resistance to column blockage
- Cost savings from higher sample throughput and column durability
- Possibility of flow gradients

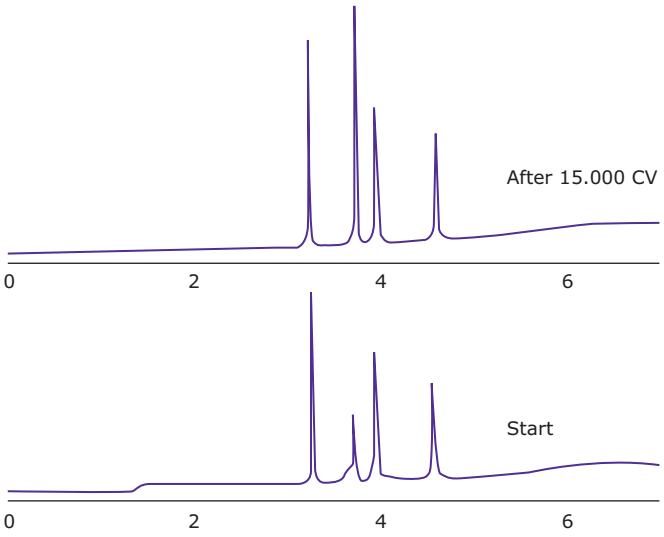
Analytical columns for bioapplications 4.6 mm i.d.

Column Stability & Reproducibility

Chromolith® WP columns were burdened with 15.000 CV of water and acetonitrile containing 0.1% TFA. The chromatographic change in separation behavior is shown below.

Separation of four proteins on a Chromolith® WP 300 RP-8, 100-4.6mm

Mobile phase	A 0.1% TFA in water B 0.1% TFA in acetonitrile
Detection	220 nm
Peak Identification	Ribonuclease Cytochrome C Holo-Transferrin Apomyoglobin
Gradient	1 min 4% B; 10 min 4 – 60% B; 5 min 60% B

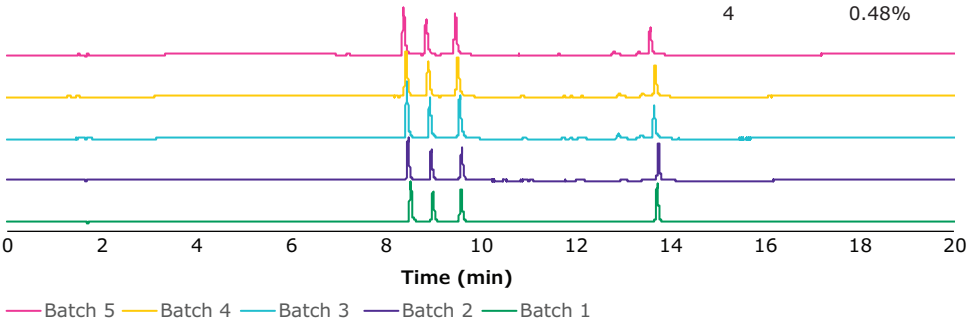


Separation of peptide/protein mixture using Chromolith® WP 300 RP-18

Separation conditions

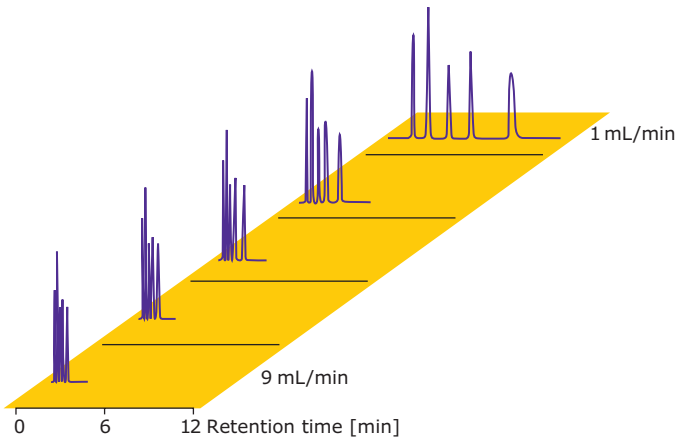
Eluent A	Water + 0.1% TFA		
Eluent B	ACN + 0.1% TFA		
Flow rate	1.0 mL/min		
Detection	UV 220 nm		
Temperature	60°C		
Injection	1.0 µL		
Cell volume	11 µL		
Gradient	**Time/ min**	**% A**	**% B**
	0.00	96%	4%
	1.00	96%	4%
	16.00	40%	60%
	21.00	40%	60%

Peak No.	RSD Ret. Time
1	0.66%
2	0.62%
3	0.58%
4	0.48%



Fast chromatography with low column back pressure columns

Owing to the very high porosity of the Chromolith® Widepore 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.

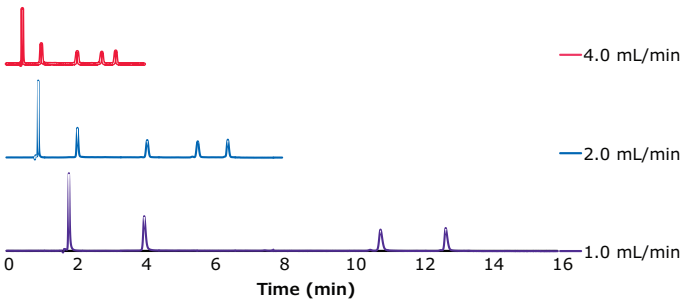


Column back pressure at different flow rates. Comparison of a Chromolith® Widepore column vs. equivalent classical particulate HPLC columns.

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-speed separation is possible even at high flow rate.

Separation of five peptides on a Chromolith® WP 300 RP-18, 100-4.6mm at various flow rates

Mobile phase	A 0.1% TFA in water B 0.1% TFA in acetonitrile
Detection	220 nm
Peak Identification	1. Gly-Tyr 2. Val-Tyr-Val 3. Met enkephalin 4. Leu enkephalin 5. Angiotensin II
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

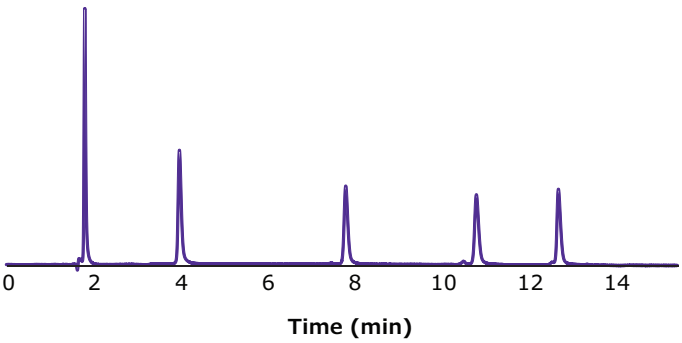


Chromolith® WP 300 RP-18, RP-8 and RP-4: reversed-phase HPLC columns for bioapplications

Reverse-phase chromatography is often used for protein and peptide separations. The longer octadecyl (C18) chains can efficiently separate complex peptide mixtures; shorter C8 modified columns are used for small, less hydrophobic proteins; C4 is mainly applied for separation of hydrophobic proteins.

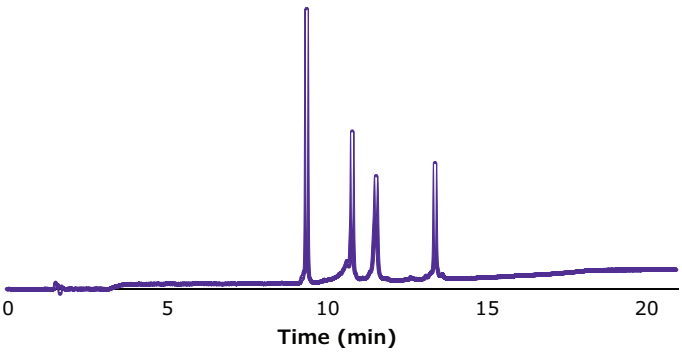
Separation of five peptides on a Chromolith® WP 300 RP-18, 100-4.6mm

Mobile phase	A 0.1% TFA in water B 0.1% TFA in acetonitrile
Flow rate	1.0 ml/min
Detection	220 nm
Peak Identification	1. Gly-Tyr 2. Val-Tyr-Val 3. Met enkephalin 4. Leu enkephalin 5. Angiotensin II
Gradient	1 min 10% B 10 min 10 – 20% B 5 min 20% B



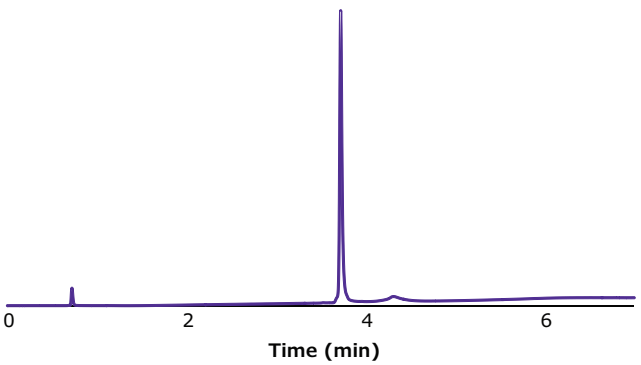
Separation of peptide/protein mixture on a Chromolith® WP 300 RP-8, 100-4.6mm

Mobile phase	A 0.1% TFA in water B 0.1% TFA in acetonitrile
Flow rate	1.0 ml/min
Detection	220 nm
Peak Identification	1. Angiotensin II 2. Neurotensin 3. Ribonuclease 4. Myoglobin
Gradient	1 min 4% B 15 min 4 – 60% B 5 min 60% B



Analysis of Cetuximab® sample on a Chromolith® WP 300 RP-4, 100-4.6mm

Mobile phase	A 0.1% TFA in water B 0.1% TFA in acetonitrile
Flow rate	2.2 ml/min
Detection	220 nm
Sample	5 mg/mL Cetuximab®
Gradient	0.1 min 4% B; 4.9 min 4 – 60% B 2 min 60% B

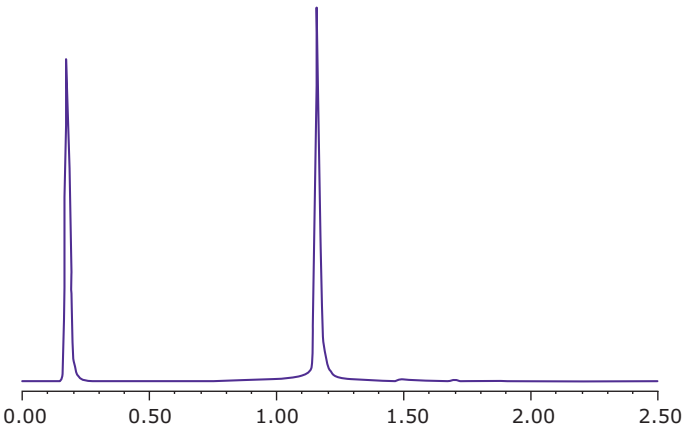


Chromolith® WP 300 Protein A – Fast monoclonal antibody quantitation

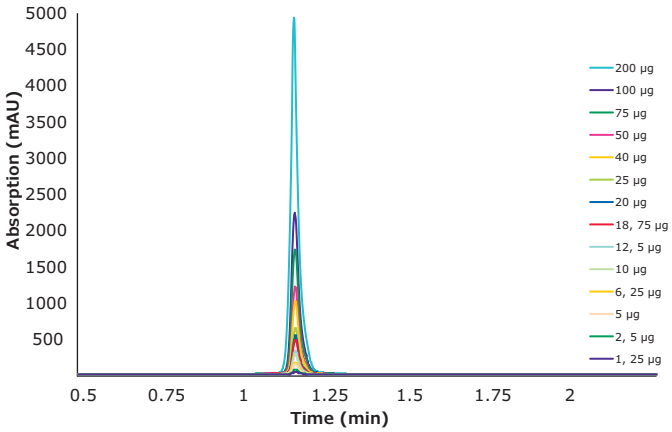
Affinity chromatography is a most selective technique which takes advantage of very specific molecular interactions, for example antigen and antibody. The Chromolith® WP 300 Protein A HPLC column is designed to monitor monoclonal antibody titer and yield determination from cell-culture supernatants. Analytical scale procedure helps to optimize the titer of monoclonal antibody for the optimal time for harvest of the monoclonal antibody products. Chromolith® WP 300 Protein A column could be used for separation of all IgGs (except class 3). Columns provide extremely fast separations and could be used longer; minimizing analysis costs.

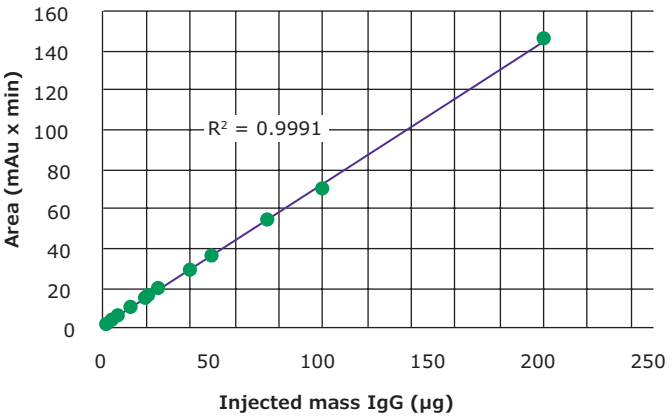
Separation of monoclonal antibodies

Eluent A	100mM sodium phosphate pH7.4		
Eluent B	100mM sodium phosphate pH2.5		
Flow rate	2.0 mL/min		
Detection	280 nm		
Temperature	25°C		
Injection volume	10µl		
Gradient	Time	%A	%B
	0.00	100	0
	0.25	100	0
	0.26	0	100
	1.25	0	100
	1.26	100	0
	2.50	100	0



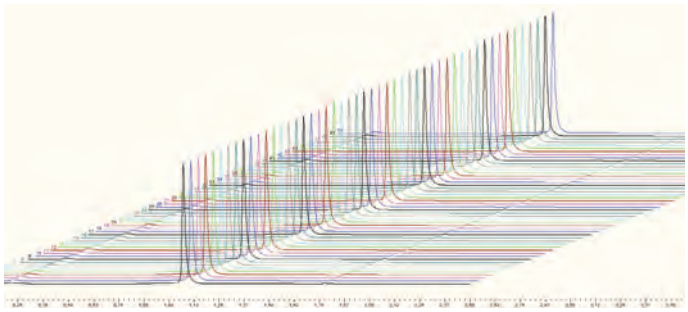
Linearity





Constant binding efficiency at any flow rate

- High-speed separation at high flow rate due to excellent mass transfer properties of the monolithic skeleton
- Separation of IgG demonstrates the extreme time savings and high separation efficiency made possible with Chromolith® protein A columns.
- IgG was well separated with excellent peak symmetry
- At 5 mL/min the total analysis time is less than 1 minute and the net column backpressure is only 21 bar
- Antibody binding is not affected by flow rate

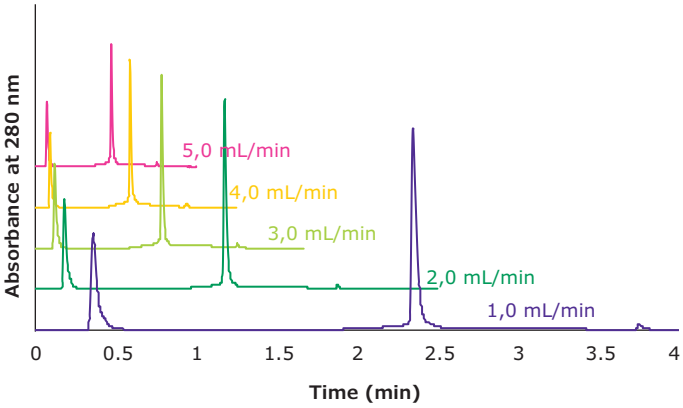


Results

Parameter	RSD
Retention time	< 0,1%
Peak Height	< 0.9%
Peak area	< 0.5%

Monolithic column provides low column backpressure at high flow rates

Flow rate	Unbound area	IgG area	Pressure (bar)
1,0 mL/min	39%	61%	3
2,0 mL/min	39%	61%	6
3,0 mL/min	39%	61%	10
4,0 mL/min	39%	61%	13
5,0 mL/min	39%	61%	21



Stability test

Chromolith® WP 300 Protein A column offers high stability and reproducibility

More than 53.000 column volumes of 100 mM sodium phosphate buffer including 10.000 pH shifts were applied to the column

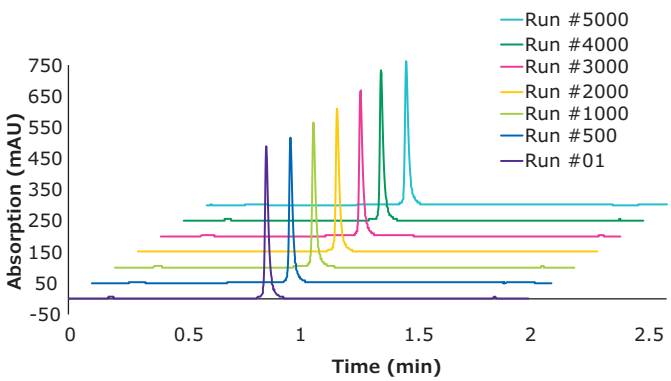
Stability test	2.0 mL/min 280 nm 25°C 10 µL IgG (1 mg/mL)		
Ret. time IgG	RSD < 0.5%		
Peak area IgG	RSD < 1.1%		
Buffer A	100 mM sodium phosphate pH7.4		
Buffer B	100 mM sodium phosphate pH2.5		
Gradient	Time	%A	%B
	0.00	100	0
	0.05	100	0
	0.06	0	100
	1.10	0	100
	1.15	100	0
	2.00	100	0

Column separation reproducibility and life time

Reproducibility

Chromatographic overlay of 50 injections

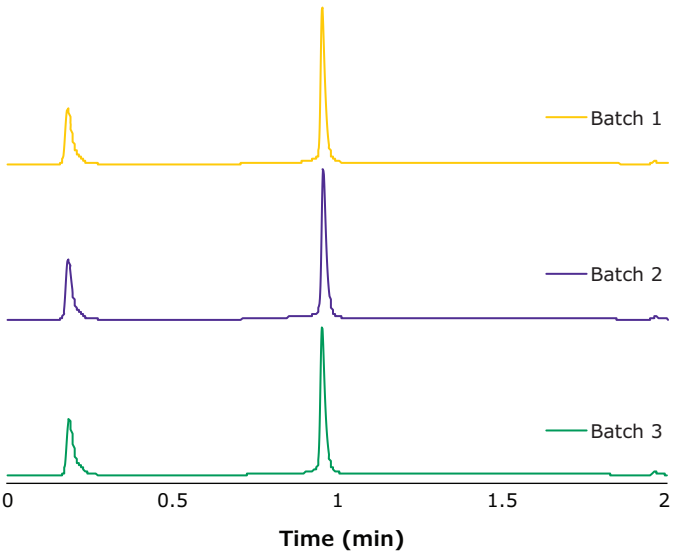
For mAb titer determination, it is necessary to use a column offering a high reproducibility. The Chromolith® protein A column offers highly reproducible elution of bound antibodies with constant retention time and low change in peak area.



Batch-to-batch reproducibility

Comparison data

Batch	IgG			Pressure
	Retention Time (Min)	Peak Width (Min)	Peak Symmetry	
1	0.952	0.034	1.56	10
2	0.957	0.033	1.39	10
3	0.953	0.031	1.47	10



Chromolith® WP 300 Epoxy

Chromolith® WP 300 Epoxy columns are specially designed for the user-specific immobilization of ligands and their later application in HPLC. The unique bimodal pore structure of silica monoliths allows efficient coupling independent of molecule size. The wider mesopores also enable the use of proteins and antibodies as both ligand immobilized on the column, and later analyte separated by an immobilized column.

Potential applications: attach Trypsin to obtain HPLC column-protein digestion reactor; attach protein and measure other protein interaction with the attached one; attach any chiral selector to obtain a chiral column attach any affinity ligand to obtain custom made affinity column etc.

Preparing the column

The Chromolith® WP 300 Epoxy column is shipped in 100% 2-Propanol. The column has to be washed with 20 CV deionized water before immobilization.

Immobilization via epoxide functions

The reaction mechanism for a direct immobilization using the epoxide groups is shown in **Figure 2.**

The epoxy ring system enables a nucleophilic attack through a ring opening process leading to a covalent bond between the nucleophilic functional group and the primary carbon atom. At the adjacent carbon atom, a hydroxyl group is formed. Epoxides can react with carboxyl, thiol, amine and hydroxyl groups depending on the pH of the medium. It is very common for the reaction between epoxides and amines to form a secondary amine bond between the support and ligand. The use of lyotropic salts in the reaction media enhances the coupling yield. The use of lyotropic salts drives the soluble ligand toward the surface of the support by a salting out effect enhancing the covalent reaction of epoxide and amine groups at moderate pH.

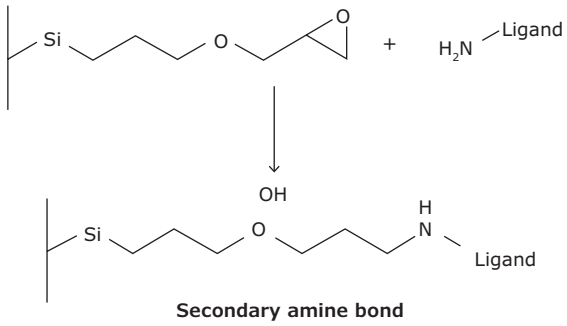


Figure 2. Reaction between epoxide functions on monolithic surface with amines forming a secondary amine bond

Additionally, other parameters influence the coupling yield of epoxide reaction such as the ligand size and concentration, reaction time and temperature. Generally, a higher reaction time will lead to a higher surface coverage and coupling yield. Smaller molecules need higher concentrations than larger molecules (e.g. proteins) to achieve the same degree of surface coverage.

After coupling of the ligand, residual epoxide groups on monolithic surface has to be quenched to avoid undesired backbone interactions with the analytes. Suitable reagents for quenching are 1M glycine or 1M urea. If the used ligand is stable at low pH, it is possible to use 150mM phosphoric acid to hydrolyze remaining epoxide groups. All quenching reactions are finished after at least 30 minutes.

See below for an example immobilization protocol using the epoxide ring system for direct immobilization.

- Connect the column to an HPLC pump (stand-alone-system is recommended) and equilibrate the column with 30 CV of 50mM sodium phosphate + 1.9M ammonium sulfate pH8.0 using a flow rate of 2.0 mL/min. The column end is connected directly to the waste. The equilibration step is performed at room temperature.

- Dissolve the desired amount (1–10 mg/ml) of ligand in 25 ml 50mM sodium phosphate + 1.9M ammonium sulfate pH 8.0 buffer and recheck the pH value of the ligand solution. Subsequently, connect the solution to the HPLC pump.
- Connect the column end also to the ligand solution and immobilize the ligand to the column and circulate for a maximum of 24 hours at a flow rate of 0.2 mL/min at room temperature.
- Quench the remaining epoxide groups with 1M glycine for 2 hours at a flow rate of 1.0 mL/min at room temperature. The column end is directly connected to the waste.
- Finally, equilibrate the column with your working solvent.

Immobilization via Schiff base mechanism

The immobilization via a Schiff base mechanism requires reaction of the epoxide group to form an aldehyde. The aldehydes react with amines forming a Schiff base linkage, which is enhanced under alkaline conditions. The Schiff base linkage is susceptible to hydrolysis and can reform the carbonyl and amine groups. The linkage of both can be stabilized by reduction to a secondary amine bond. As mild reductant, sodium cyanoborohydride can drive the immobilization to completion at neutral pH. Furthermore, it can be used in acidic conditions to quench the residual carbonyl functions at the monolithic surface.

The Schiff base mechanism is much more reactive compared to the described epoxy reaction. Nevertheless, the reaction is affected by several parameters including reaction time, temperature, ligand size and concentration.

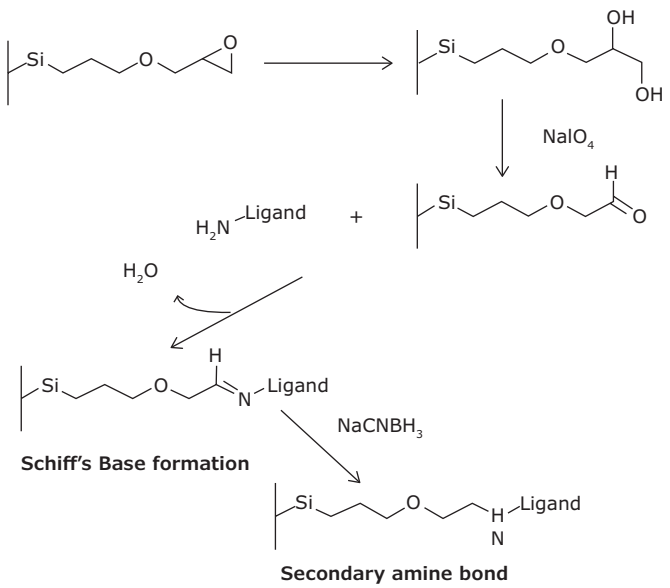


Figure 3. Scheme of immobilization via Schiff base mechanism.

See below for an example immobilization protocol using the Schiff base mechanism for immobilization of amines.

- Connect the column to an HPLC pump (stand-alone-system is best) and hydrolyze the epoxide functional groups to diols with 70 CV of 2% sulfuric acid using a flow rate of 2.0 mL/min at room temperature. The column end is connected directly to the waste.
- Wash the column with at least 15 CV deionized water at a flow rate of 2.0 mL/min. The column end is still connected to the waste.
- The arisen diol groups are oxidized by 100 CV 100mM sodium periodate in water/methanol 4:1 (v/v) to carbonyls at 2.0 mL/min and room temperature. The column end is still connected to the waste.
- Again, wash the column with at least 15 CV deionized water at a flow rate of 2.0 mL/min. The column end is still connected to the waste.
- Dissolve the desired amount (1–10 mg/mL) of ligand and 5mM sodium cyanoborohydride in 25 mL 50 mM sodium phosphate + 1.9 M ammonium sulfate pH 8.0 buffer and connect the solution to the HPLC pump.
- Connect the column end also to the ligand solution to immobilize the ligand to the column by circulating for a maximum of 24 hours at a flow rate of 0.2 mL/min and room temperature.
- Reduce the remaining carbonyl groups with 20mM sodium cyanoborohydride, dissolved in 50mM sodium phosphate pH 3.0, for 60 CV at 2.0 mL/min and room temperature. The column end is directly connected to the waste.
- Finally, equilibrate the column with your working solvent.

Generally, it is possible to perform all activation or immobilization steps at lower temperatures. However, please be aware that lower temperatures prolong the immobilization time.

Use as HPLC column

After immobilization, the column is ready to use for the desired purpose of the immobilized ligand. The type of required solvent or buffers depends on the type of ligand used.

Chromolith® WP Epoxy 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 (e.g. 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. Nevertheless, be careful not to expose the column to conditions which could cause denaturation of your ligand.

Do not exceed the pH range from 1.5 to 7.5 with Chromolith® Widepore columns during analysis. Higher pH 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 a loss of resolution.

Column lifetime is highly dependent on the sample and conditions, and cannot be generalized.

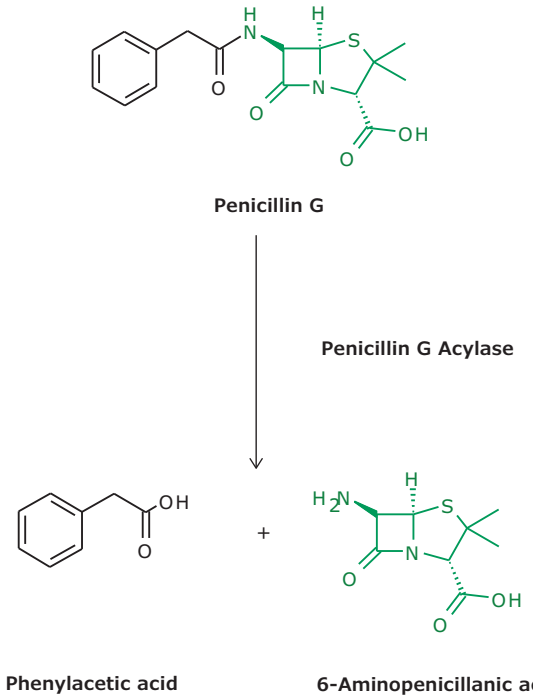
For samples with large quantities of contaminants, we recommend to apply one or more sample preparation methods prior to separation (e.g. solid phase extraction, filtration, centrifugation, etc.). Make sure that your samples and the mobile phases are clean and particulate free by using HPLC grade solvents and reagents.

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

Example immobilization

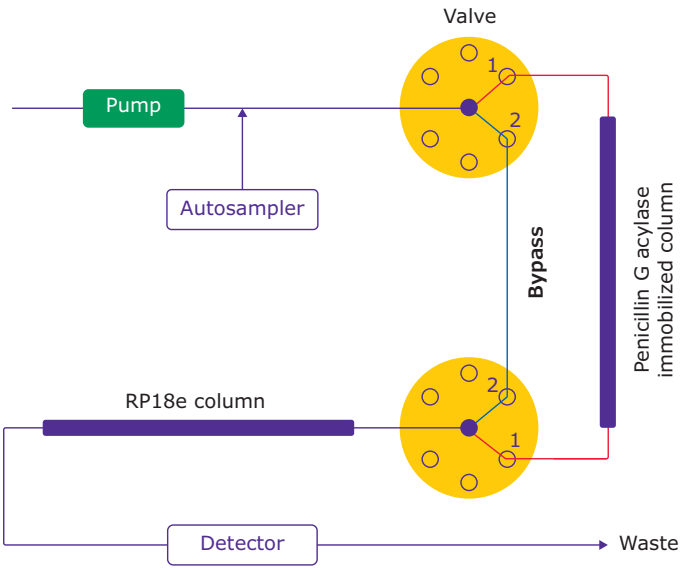
Immobilization of penicillin acylase

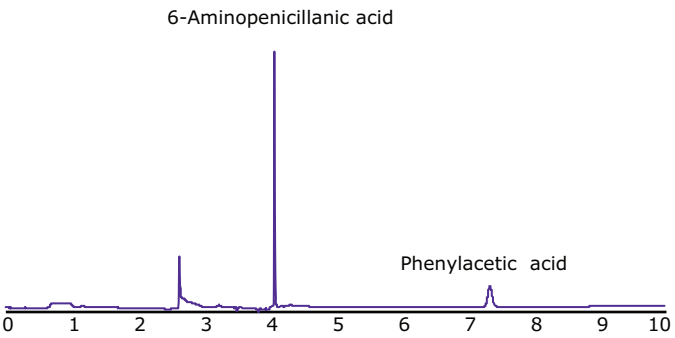
- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-4.6 mm column
- 80 mg penicillin acylase dissolved in 25 mL 50 mM sodium phosphate + 1.9M ammonium sulfate pH8.0
- Immobilization for 24 hours at 0.2 mL/min
- Quenching of remaining epoxide groups with glycine



Immobilization of penicillin acylase – Enzymatic bioreactor

Flow rate	1.0 mL/min					
Temperature	23°C					
Detection	UV 225nm					
Eluent A	10 mM sodium phosphate pH7,0					
Eluent B	10 mM sodium phosphate pH3,0					
Eluent C	Acetonitrile					
Sample	1.0 µL Penicillin G (3.5 mg/mL)					
Gradient	**Time**	**Valve**	**A**	**B**	**C**	
	0	1	100	0	0	
	2	1	100	0	0	
	2	2	0	80	20	
	4	2	0	80	20	
	9	2	0	50	50	
	9.5	2	0	50	50	
	9.6	2	0	80	20	
	15	2	0	80	20	



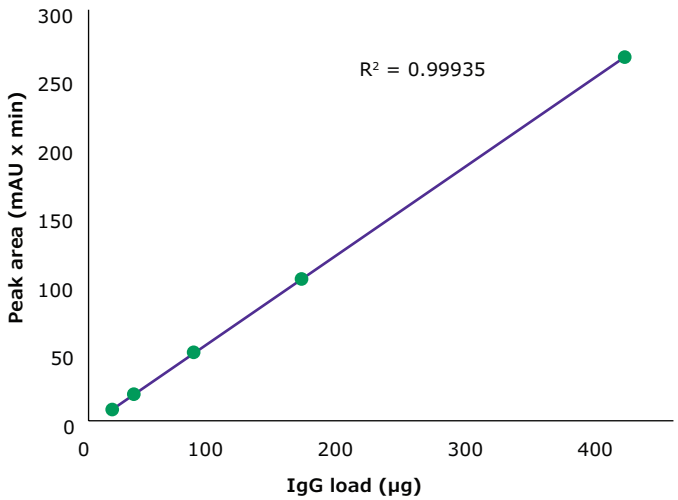


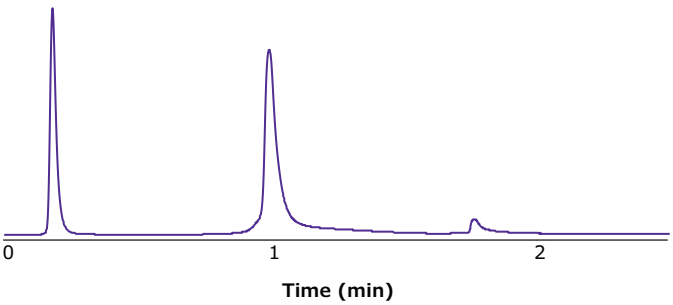
Immobilization of protein G

- According to Epoxy method
- Chromolith® WP 300 Epoxy 25-4.6 mm column
- 12.5 mg protein G dissolved in 6.25 mL 50 mM sodium phosphate + 1.9 M ammonium sulfate pH 8.0
- Immobilization for 4 hours at 0.2 mL/min
- Quenching of remaining epoxide functions with glycine

Immobilization of protein G – Affinity chromatography

Eluent A	100 mM sodium phosphate pH7.4		
Eluent B	100 mM sodium phosphate pH2.5		
Flow rate	2.0 mL/min		
Detection	280 nm		
Temperature	25°C		
Injection volume	10 µL		
Gradient	**Time**	**%A**	**%B**
	0	100	0
	0.5	0	100
	0.6	0	100
	1.2	100	0



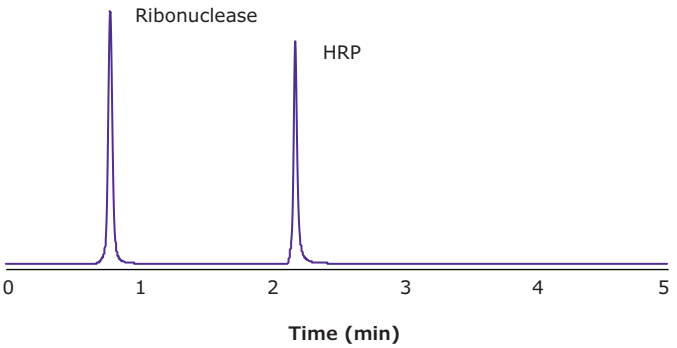


Immobilization of concanavalin A

- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-4.6 mm column
- 50 mg concanavalin A from Jack bean dissolved in 25 mL 50mM Na_2HPO_4, 1mM $CaCl_2$ + 1,9M $(NH_4)_2SO_4$ pH 8,0
- Immobilization for 4 hours at 0.2 mL/min
- Quenching of remaining epoxide functions with glycine

Immobilization of concanavalin A – Affinity chromatography

Eluent A	50mM sodium acetate, 200mM sodium chloride, 1mM calcium chloride pH5.3		
Eluent B	Eluent A + 100mM Methyl-α-D-mannopyranoside		
Flow rate	2.0 mL/min		
Detection	214 nm		
Temperature	25°C		
Injection volume	5 µL		
Gradient	**Time**	**%A**	**%B**
	0	100	0
	1	100	0
	1.25	0	100
	3.5	0	100
	3.6	100	0
	5	100	0

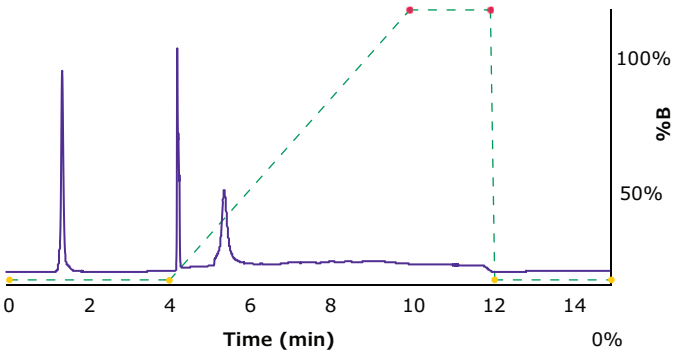


Immobilization of iminodiacetic acid

- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-4.6 mm column
- 1 g imidodiacetic acid dissolved in 25 mL 50 mM Na_2HPO_4 + 1,9M $(NH_4)_2SO_4$ pH 8,0
- Immobilization for 72 hours at 0.2 ml/min
- No quenching of remaining epoxide functions
- Column was flushed with $CuSO_4$ solution before separation

Immobilization of imidodiacetic acid– Affinity chromatography

Eluent A	20mM sodium phosphate + 100mM sodium chloride pH7,0
Eluent B	Eluent A + 200mM imidazole
Flow rate	1.0 mL/min
Detection	280 nm
Temperature	25°C
Injection volume	20 µL



Chromolith® Preparative columns

10 mm / 25 mm i.d.

Offering faster sample throughput at lower pressure, Chromolith® Prep and SemiPrep HPLC columns are ideal for direct scale-up from analytical to prep and semi-prep. The excellent accessibility of the mesopores (total porosity > 80 %), and the short diffusion length inside the pores ensure fast adsorption and desorption kinetics. This leads to faster separations and higher productivity. The monolithic structure of Chromolith® SemiPrep and Prep columns also eliminates inlet bed settling or bed splitting under high pressure. Column reliability, reproducibility and extended lifetime are assured

Why choose preparative Chromolith® columns
Direct scale-up from analytical to semi-prep or prep columns
Faster sample throughput at lower operating pressure than semi/prep columns packed with 5 µm particles
Sharp separations, even with high sample loading
Excellent column lifetime due to rugged monolithic silica structure
Higher porosity allows fast adsorption and desorption kinetics
Compared to particulate sorbents, monolithic columns ensure shorter separation times and less solvent consumption
Higher productivity and greater efficiency than particulate sorbents

Chromolith® SemiPrep

Perfect scale-up from analytical to preparative LC

Ready-to-use Chromolith® SemiPrep column.

Optimum separation at flow rates exceeding 40 mL/min

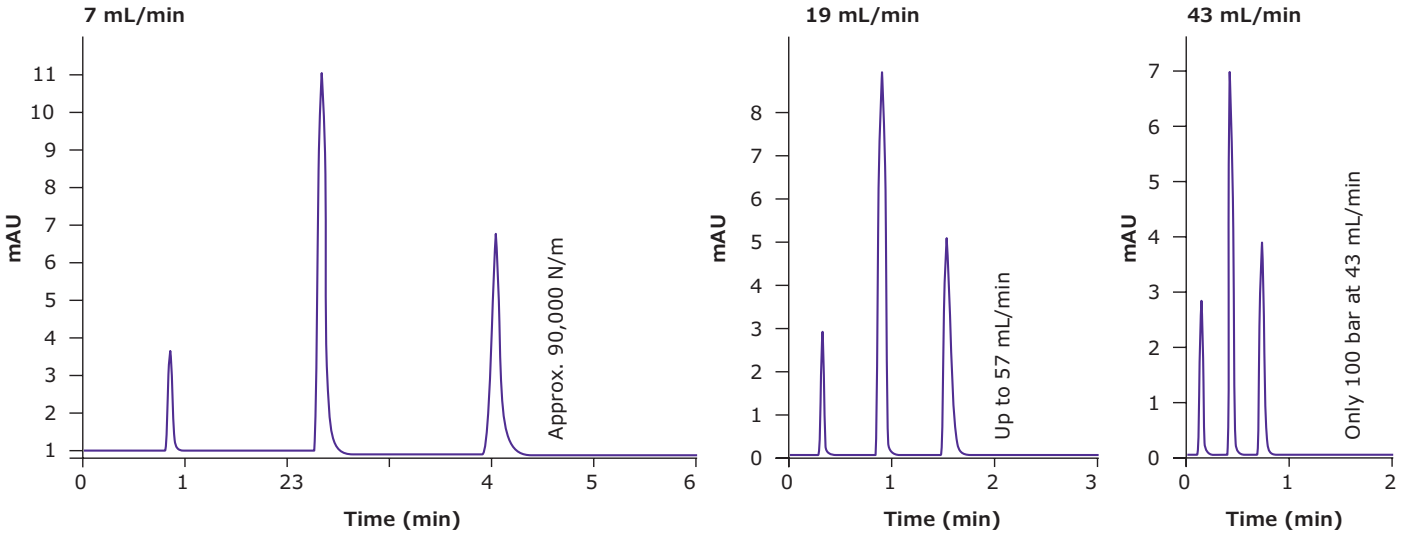
Chromolith® SemiPrep HPLC columns are ideally suited for direct scale-up from analytical to semi-prep. This is because they offer faster sample throughput at a lower operating pressure compared to semi-prep columns packed with 5 µm particles.

Chromolith® SemiPrep 10 mm i.d. columns combine high separation speed with excellent performance. This makes them the perfect alternative to particulate columns of 10 mm i.d. (and even 21.2 mm i.d.). They have the same bimodal porous silica rod structure as Chromolith® analytical columns with an internal diameter of 4.6 mm. Their macropores are 2 µm in diameter and the mesopores are 13 nm. This combination dramatically reduces separation time while increasing efficiency.

Separation of a standard mixture

Chromolith® SemiPrep C18e, 100-10 mm

Mobile phase	Acetonitrile/water 60/40
Flow rate	2 mL/min
Detection	UV 254 nm
Temperature	ambient
Injection volume	5 µL
Sample	1. Thiourea 2. Progesterone 3. Anthracene



Acetonitrile/water 60/40, Data for anthracene (3rd peak)

Chromolith® Prep

Higher speed, efficiency and productivity

Ready-to-use Chromolith® Prep column.

Preparative HPLC involves much higher sample volumes than analytical chromatography. Consequently, greater sample throughput and separation speed are essential for optimal productivity. These criteria are best fulfilled by Chromolith® Prep columns. The combination of macro and mesopores maximizes separation efficiency and flow rate, while minimizing resistance.

The formula for direct scale-up

Analytical separations can be easily transferred to Chromolith® SemiPrep and Prep columns by linear transfer of methods. The objective of any preparative separation strategy is high sample throughput per unit of time. Therefore columns are often run under concentration and/or volume overload conditions. However, the maximum load on the column is dependent on the complexity of the separation and the nature of the sample. Whether working in a linear or non-linear mode, the flow rate or injection volume is calculated according to the equation below.

$$\frac{X_{an}}{\Pi r^2_{an}} = \frac{X_{pr}}{\Pi r^2_{pr}} \cdot \frac{1}{C_L}$$

X_{an}	Flow rate in the analytical system	
X_{pr}	Flow rate in the preparative system	$X_{pr} = X_{an} \cdot r^2_{pr} \cdot c_L / r^2_{an}$
r_{an}	Radius of analytical column	
r_{pr}	Radius of preparative column	
c_L	Length of the preparative column to length of the analytical column	
M	Substance mass	$M_{pr} = M_{an} \cdot r^2_{pr} \cdot c_L / r^2_{an}$

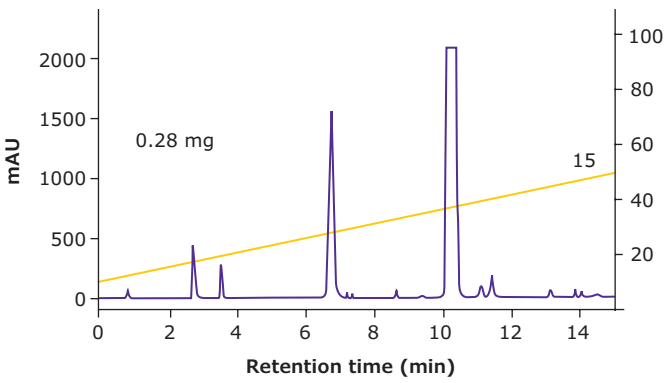
Guide values of typical flow rates and loading capacities for transfer from an analytical to a preparative column

Columns	Column dimension (L/D)	Typical flow rate	Loading capacity	Loading volume
Analytical column	100 – 4.6 mm	2 mL/min	5 mg	5 – 50 µL
Preparative column	100 – 25 mm	60 mL/min	150 – 370 mg	100 – 1,500 µL

Analytical separation

Chromolith® Performance RP-18e 100-4.6 mm

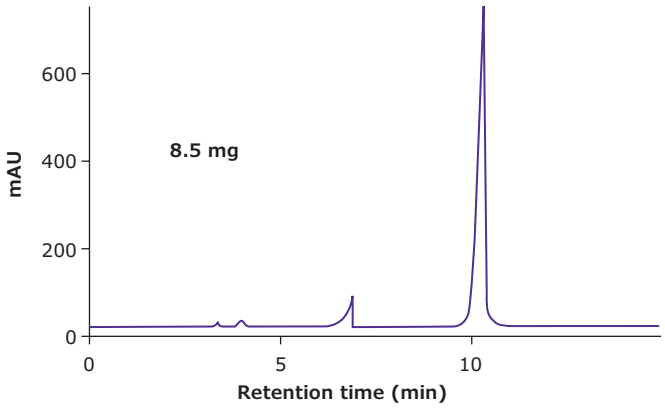
Column	Chromolith® Performance RP-18 endcapped 100-4.6 mm
Mobile phase	A: Water + 0.1% formic acid B: Acetonitrile
Gradient	linear gradient from 10% B to 40% in 14 min
Flow rate	2 mL/min
Detection	UV 254 nm
Sample	0.28 mg Heterocyclic racemate (EMD 53986) in 10 µL DMSO



Preparative separation

Chromolith® Prep RP-18e 100-25 mm

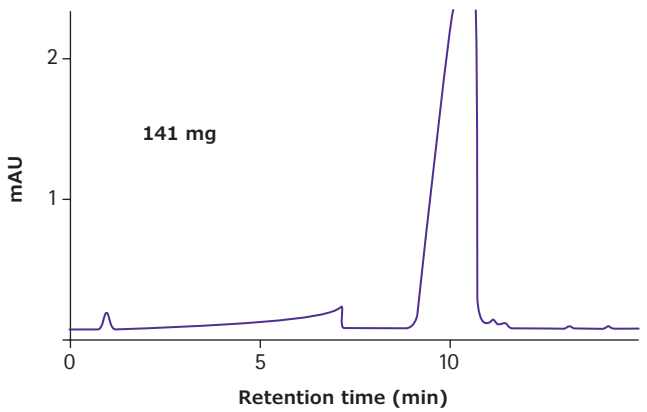
Column	Chromolith® Prep RP-18 endcapped 100-25 mm
Mobile phase	A: Water + 0.1% formic acid B: Acetonitrile
Gradient	linear gradient from 10% B to 40% in 14 min
Flow rate	60 mL/min
Detection	UV 254 nm
Sample	8.46 mg Heterocyclic racemate (EMD 53986) in 300 µL DMSO



Preparative separation

Chromolith® Prep RP-18e 100-25 mm

Column	Chromolith® Prep RP-18 endcapped 100-25 mm
Mobile phase	A: Water + 0.1 formic acid B: Acetonitrile
Gradient	linear gradient from 10% B to 40% in 14 min
Flow rate	60 mL/min
Detection	UV 254 nm
Sample	141 mg Heterocyclic racemate (EMD 53986) in 300 µL DMSO



Various applications with Chromolith® Prep monolithic columns

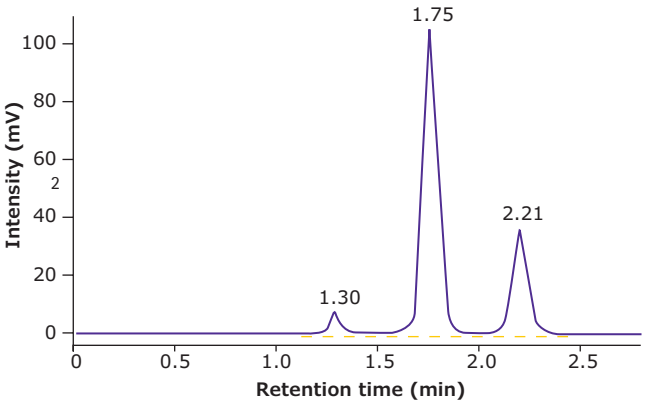
Comparison of flow rates

Chromolith® Prep columns can be operated at a flow rate of up to 400 mL/min, and pressures of up to 100 bar. This is a tenfold increase in flow rate compared to particulate columns of an equivalent size.

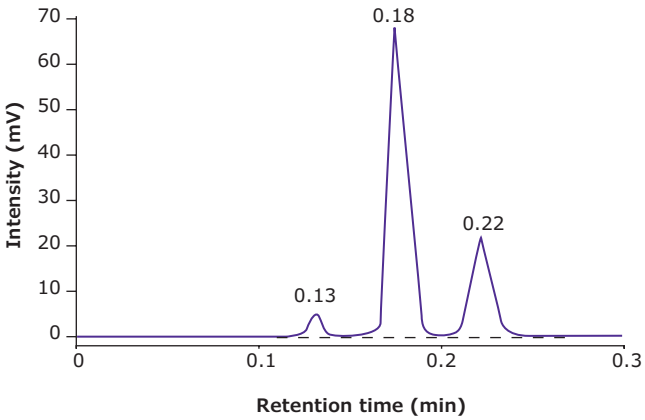
Separation at different flow rates 40 and 390 mL/min

Chromolith® Prep Si 100-25 mm

Column	Chromolith® Prep Si 100-25 mm
Solvent	n-Heptane/Dioxane (80/20 v/v)
Flow rate	40 mL/min
Sample	1. Toluene 2. Dimethylphthalate 3. Dibutylphthalate



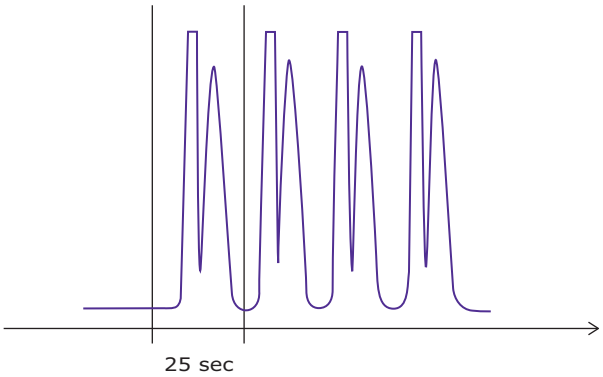
Column	Chromolith® Prep Si 100-25 mm
Solvent	n-Heptane / Dioxane (80/20 v/v)
Flow rate	390 mL/min
Sample	1. Toluene 2. Dimethylphthalate 3. Dibutylphthalate



Separation of diastereomers with a productivity of 861 g/d

Chromolith® Prep Si 100-25 mm

Column	Chromolith® Prep Si 100-25 mm
Solvent	n-Heptane / Dioxane (80/20 v/v)
Flow rate	140 mL/min
Injection	249 mg
Cycle time	25 sec
Sample	Fluoro-dihydro-oxyranyl-benzopyran



Chromolith®

HPLC guard cartridges and kits

Although monolithic columns are well known for their robustness and longevity, our Chromolith® guard cartridges, cartridge holders and kits further enhance these advantages.

Guard cartridges

Chromolith® HPLC guard cartridges are extremely easy to use. They are simply added directly in front of the main column to protect it from chemical or mechanical contamination. Due to the benefits of monolithic technology, and the convenience of Chromolith® guard columns, they are also popular for use with classical particulate columns. Moreover, guard columns can be used as trap columns when large sample volumes are to be injected. Guard columns should be changed frequently in order to avoid excessive accumulation of impurities.

Guard cartridge starter kit for 2 and 3mm i.d. columns

The Chromolith® guard cartridge kit includes everything needed to significantly enhance the lifetime of monolithic columns: a guard cartridge holder, and three guard cartridges.

Guard cartridge holders

Depending on your needs, we offer several different guard cartridge holders: made out of PEEK for 2 and 3 mm i.d. cartridges; bioinert PEEK lined stainless steel holder and standard stainless steel holder for 4.6 mm i.d. cartridges and holders for 10 and 25 mm i.d. cartriges.

Guard cartridge holder type	Material holder is made of	Max. back pressure	How to tighten holder	Guard cartridge i.d.	Guard cartridge length
a)	PEEK	200 bar (2,940 psi)	Finger-tight	2, 3 mm,	5 mm
b)	PEEK lined SS	400 bar (5,880 psi)	Finger-tight + tool (not included)	4.6 mm	5 mm, 10 mm
c)	SS	400 bar (5,880 psi)	Finger-tight + tool (not included)	4.6 mm	5 mm, 10 mm
d)	PEEK / SS	150 bar (2,205 psi)	Finger-tight + tool (not included)	10 mm	10 mm
e)	PEEK	100 bar (1,470 psi)	Finger-tight + tool (included)	25 mm	10 mm

For maximum convenience and flexibility, guard cartridges are available in five dimensions with corresponding holders made of PEEK, PEEK lined stainless steel, having different maximum back pressures.

PEEK = Poly Ether Ether Ketone, SS = Stainless Steel

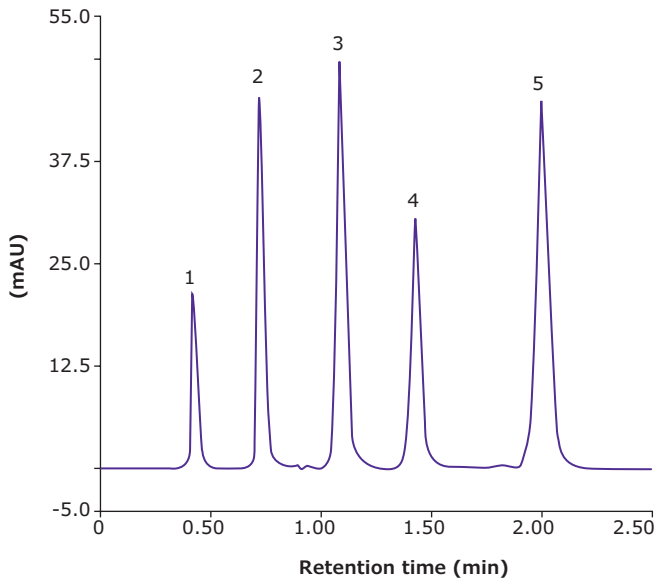
Separation examples with and without a pre-column

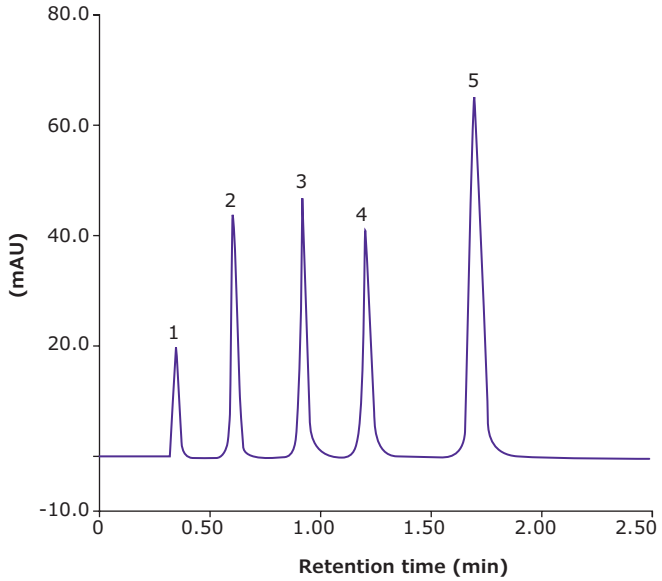
Chromolith® Performance RP-18e 100-2 mm with a Chromolith® RP-18e 5-2 mm pre-column

Column	Chromolith® Performance RP-18e 100-2 mm with pre-column Chromolith® RP-18e 5-2 mm	
Mobile phase	Acetonitrile / water 60/40	
Flow rate	0.38 mL/min	
Pressure	20 bar	
Detection	UV 254 nm	
Anthracene	N/m	113540
	TUSP	1.14
	K´-value	3.79
Sample	1. Thiourea	
	2. Biphenyl-2-ol	
	3. Progesterone	
	4. Hexanophenone	
	5. Anthracene	

Chromolith® Performance RP-18e 100-2 mm without a pre-column

Column	Chromolith® Performance RP-18e 100-2 mm without pre-column	
Mobile phase	Acetonitrile / water 60/40	
Flow rate	0.38 mL/min	
Pressure	20 bar	
Detection	UV 254 nm	
Anthracene	N/m	115460
	TUSP	1.07
	K´-value	3.90
Sample	1. Thiourea	
	2. Biphenyl-2-ol	
	3. Progesterone	
	4. Hexanophenone	
	5. Anthracene	





As seen in the examples, guard columns have very little negative effects on the separation. These may include a slight shift in elution time, or a minimal loss in efficiency.

Ordering information

Capillary columns

Product	Modification	I.d.	Length	Type	Content	Cat. No.
Chromolith® CapRod®	RP-18e	0.05 mm	150 mm		1 analytical column	**1.50403.0001**
Chromolith® CapRod®	RP-8e	0.1 mm	150 mm		1 analytical column	**1.50400.0001**
Chromolith® CapRod®	RP-18e Trap	0.1 mm	50 mm		1 trapping column	**1.50426.0001**
Chromolith® CapRod®	RP-18e	0.1 mm	150 mm		1 analytical column	**1.50402.0001**
Chromolith® CapRod®	RP-18e	0.1 mm	300 mm		1 analytical column	**1.50424.0001**
Chromolith® CapRod® HR	RP-18e	0.1 mm	150 mm		1 analytical column	**1.50404.0001**
Chromolith® CapRod®	RP-18e Trap	0.2 mm	50 mm		1 trapping column	**1.50409.0001**
Chromolith® CapRod®	RP-18e	0.2 mm	150 mm		1 analytical column	**1.50405.0001**
Chromolith® CapRod® HR	RP-18e	0.2 mm	150 mm		1 analytical column	**1.50407.0001**

Analytical columns

Product	Modification	I.d.	Length	Type	Content	Cat. No.
Chromolith® Validation Kit	RP-18e	2 mm	100 mm		3 columns from 3 different batches	**1.52062.0001**
Chromolith® Performance	RP-18e	2 mm	100 mm		1 HPLC column	**1.52006.0001**
Chromolith® FastGradient	RP-18e	2 mm	50 mm		1 HPLC column	**1.52007.0001**
Chromolith® Flash	RP-18e	2 mm	25 mm		1 HPLC column	**1.52014.0001**
Chromolith® Guard Cartridge Kit	RP-18e	2 mm	5 mm	a*	3 guard cartridges, 1 cartridge holder	**1.52008.0001**
Chromolith® Guard Cartridge	RP-18e	2 mm	5 mm	a*	3 guard cartridges	**1.52009.0001**
Chromolith® Validation Kit	RP-18e	3 mm	100 mm		3 columns from 3 different batches	**1.52063.0001**
Chromolith® Performance	RP-18e	3 mm	100 mm		1 HPLC column	**1.52001.0001**
Chromolith® FastGradient	RP-18e	3 mm	50 mm		1 HPLC column	**1.52002.0001**
Chromolith® Flash	RP-18e	3 mm	25 mm		1 HPLC column	**1.52003.0001**
Chromolith® Guard Cartridge Kit	RP-18e	3 mm	5 mm	a*	3 guard cartridges, 1 cartridge holder	**1.52004.0001**
Chromolith® Guard Cartridge	RP-18e	3 mm	5 mm	a*	3 guard cartridges	**1.52005.0001**
Chromolith® Performance	RP-18e	4.6 mm	100 mm		1 HPLC column	**1.02129.0001**
Chromolith® Validation Kit	RP-18e	4.6 mm	100 mm		3 columns from 3 different batches	**1.51466.0001**
Chromolith® SpeedROD	RP-18e	4.6 mm	50 mm		1 HPLC column	**1.51450.0001**
Chromolith® Flash	RP-18e	4.6 mm	25 mm		1 HPLC column	**1.51463.0001**
Chromolith® Guard Cartridge	RP-18e	4.6 mm	10 mm	b/c*	3 guard cartridges	**1.51452.0001**
Chromolith® Guard Cartridge	RP-18e	4.6 mm	5 mm	b/c*	3 guard cartridges	**1.51451.0001**
Chromolith® Validation Kit	RP-18e	4.6 mm	100 mm		3 columns from 3 different batches	**1.52019.0001**
Chromolith® HR	RP-18e	4.6 mm	100 mm		1 HPLC column	**1.52022.0001**
Chromolith® HR	RP-18e	4.6 mm	50 mm		1 HPLC column	**1.52021.0001**
Chromolith® HR	RP-18e	4.6 mm	25 mm		1 HPLC column	**1.52020.0001**
Chromolith® HR Guard Cartridge	RP-18e	4.6 mm	5 mm	b/c*	3 guard cartridges	**1.52025.0001**
Chromolith® Performance	RP-8e	4.6 mm	100 mm		1 HPLC column	**1.51468.0001**
Chromolith® HR	RP-8e	4.6 mm	100 mm		1 HPLC column	**1.52064.0001**
Chromolith® Guard Cartridge	RP-8e	4.6 mm	5 mm	b/c*	3 guard cartridges	**1.52013.0001**

* Guard column type examples and detailed information please find on page 47.

Product	Modification	I.d.	Length	Type	Content	Cat. No.
Chromolith®	Phenyl	4.6 mm	25 mm		1 HPLC column	**1.52056.0001**
Chromolith®	Phenyl	4.6 mm	50 mm		1 HPLC column	**1.52057.0001**
Chromolith®	Phenyl	4.6 mm	100 mm		1 HPLC column	**1.52058.0001**
Chromolith® Guard Cartridge	Phenyl	4.6 mm	5 mm	b/c*	3 guard cartridges	**1.52059.0001**
Chromolith®	CN	4.6 mm	25 mm		1 HPLC column	**1.52046.0001**
Chromolith®	CN	4.6 mm	50 mm		1 HPLC column	**1.52047.0001**
Chromolith®	CN	4.6 mm	100 mm		1 HPLC column	**1.52048.0001**
Chromolith® Guard Cartridge	CN	4.6 mm	5 mm	b/c*	3 guard cartridges	**1.52050.0001**
Chromolith®	DIOL	4.6 mm	25 mm		1 HPLC column	**1.53170.0001**
Chromolith®	DIOL	4.6 mm	50 mm		1 HPLC column	**1.53171.0001**
Chromolith®	DIOL	4.6 mm	100 mm		1 HPLC column	**1.53172.0001**
Chromolith® Guard Cartridge	DIOL	4.6 mm	5 mm	b/c*	3 guard cartridges	**1.53175.0001**
Chromolith® Performance	Si	4.6 mm	100 mm		1 HPLC column	**1.51465.0001**
Chromolith® Guard Cartridge	Si	4.6 mm	5 mm	b/c*	3 guard cartridges	**1.52011.0001**
Chromolith® Performance	NH2	4.6 mm	100 mm		1 HPLC column	**1.52028.0001**
Chromolith® SpeedROD	NH2	4.6 mm	50 mm		1 HPLC column	**1.52027.0001**
Chromolith® Flash	NH2	4.6 mm	25 mm		1 HPLC column	**1.52026.0001**
Chromolith® Guard Cartridge	NH2	4.6 mm	5 mm	b/c*	3 guard cartridges	**1.52030.0001**
Chromolith® Guard Cartridge Holder	-	4.6 mm	5 mm	c*	1 cartridge holder	**1.52032.0001**
Chromolith® Guard Cartridge Holder	-	4.6 mm	10 mm	c*	1 cartridge holder	**1.52033.0001**
Chromolith® Column Coupler	-	-	-		1 column coupler	**1.51467.0001**

Chromolith® WP 300 columns

Product	Modification	I.d.	Length	Type	Content	Cat. No.
Chromolith® WP 300 Protein A	Protein A	4.6 mm	25 mm		1 HPLC column	**1.52258.0001**
Chromolith® WP 300 RP18	RP-18	4.6 mm	100 mm		1 HPLC column	**1.52270.0001**
Chromolith® WP 300 RP18	RP-18	4.6 mm	50 mm		1 HPLC column	**1.52271.0001**
Chromolith® WP 300 RP18 Guard Cartridge	RP-18	4.6 mm	10 mm	b*	3 guard cartridges	**1.52272.0001**
Chromolith® WP 300 RP18 Guard Cartridge	RP-18	4.6 mm	5 mm	b*	3 guard cartridges	**1.52273.0001**
Chromolith® WP 300 RP8	RP-8	4.6 mm	100 mm		1 HPLC column	**1.52265.0001**
Chromolith® WP 300 RP8	RP-8	4.6 mm	50 mm		1 HPLC column	**1.52266.0001**
Chromolith® WP 300 RP8 Guard Cartridge	RP-8	4.6 mm	10 mm	b*	3 guard cartridges	**1.52267.0001**
Chromolith® WP 300 RP8 Guard Cartridge	RP-8	4.6 mm	5 mm	b*	3 guard cartridges	**1.52268.0001**
Chromolith® WP 300 RP4	RP-4	4.6 mm	100 mm		1 HPLC column	**1.52260.0001**
Chromolith® WP 300 RP4	RP-4	4.6 mm	50 mm		1 HPLC column	**1.52261.0001**
Chromolith® WP 300 RP4 Guard Cartridge	RP-4	4.6 mm	10 mm	b*	3 guard cartridges	**1.52262.0001**
Chromolith® WP 300 RP4 Guard Cartridge	RP-4	4.6 mm	5 mm	b*	3 guard cartridges	**1.52263.0001**
Chromolith® WP 300 Epoxy	Epoxy	4.6 mm	100 mm		1 HPLC column	**1.52250.0001**
Chromolith® WP 300 Epoxy	Epoxy	4.6 mm	50 mm		1 HPLC column	**1.52251.0001**
Chromolith® WP 300 Epoxy	Epoxy	4.6 mm	25 mm		1 HPLC column	**1.52252.0001**
Chromolith® WP 300 Epoxy Guard Cartridge	Epoxy	4.6 mm	10 mm	b*	3 guard cartridges	**1.52253.0001**
Chromolith® WP 300 Epoxy Guard Cartridge	Epoxy	4.6 mm	5 mm	b*	3 guard cartridges	**1.52254.0001**
Chromolith® Guard Cartridge Holder Bioinert	-	4.6 mm	5 mm	b*	1 cartridge holder	**1.52255.0001**
Chromolith® Guard Cartridge Holder Bioinert	-	4.6 mm	10 mm	b*	1 cartridge holder	**1.52256.0001**

* Guard column type examples and detailed information please find on page 47.

Semi-preparative and preparative columns

Product	Modification	I.d.	Length	Type	Content	Cat. No.
Chromolith® SemiPrep	RP-18e	10 mm	100 mm		1 HPLC column	**1.52016.0001**
Chromolith® SemiPrep Guard Cartridge	RP-18e	10 mm	10 mm	d*	3 guard cartridges	**1.52036.0001**
Chromolith® SemiPrep	Si	10 mm	100 mm		1 HPLC column	**1.52015.0001**
Chromolith® SemiPrep Guard Cartridge	Si	10 mm	10 mm	d*	3 guard cartridges	**1.52035.0001**
Chromolith® SemiPrep Guard Cartridge holder	-	10 mm	10 mm	d*	1 cartridge holder	**1.52037.0001**
Chromolith® Column Coupler	-	-	-		1 column coupler	**1.51467.0001**
Chromolith® Prep	Si	25 mm	100 mm		1 HPLC column, 2 connectors (1/8" and 1/16")	**1.25251.0001**
Chromolith® Prep	RP-18e	25 mm	100 mm		1 HPLC column, 2 connectors (1/8" and 1/16")	**1.25252.0001**
Chromolith® Prep guard cartridge	Si	25 mm	10 mm	e*	1 guard cartridge	**1.25260.0001**
Chromolith® Prep guard cartridge	RP-18e	25 mm	10 mm	e*	1 guard cartridge	**1.25261.0001**
Chromolith® Prep sealing set		25 mm			2 O-rings	**1.25254.0001**
Chromolith® Prep tool set		25 mm			1 mounting tool filter, 1 mounting tool, 1 hook wrench	**1.25255.0001**
Chromolith® Prep end cap set		25 mm			1 inlet cap complete, 1 outlet cap	**1.25256.0001**
Chromolith® Prep frit set		25 mm			10 frits	**1.25257.0001**
Chromolith® Prep 25 mm guard cartridge holder		25 mm	10 mm	e*	1 cartridge holder	**1.25262.0001**
Chromolith® Prep 25 mm column coupler		25 mm			1 column coupler	**1.25259.0001**

* Guard column type examples and detailed information please find on page 34.

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